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Pilchard *(Sardinops sagax)* nursery areas and recruitment process assessment between different regions in southern Western Australia

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Fisheries research in Western Australia
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The primary function of the Fisheries Research Division is to provide scientific advice to government in the formulation of management policies for developing and sustaining Western Australian fisheries.

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Abstract

Pilchards have been the primary target of purse seine fisheries along the south and lower west coasts of WA for nearly 15 years. Western Australia's pilchard population consists of separate west coast and south coast breeding stocks. The adult pilchards in the south coast stock can, in turn, be further divided into three separate assemblages. These adult assemblages correspond to the purse seine fisheries at Albany, Bremer Bay and Esperance. However, while the separateness of the adult assemblages is well established, the status of the juveniles within the context of these adult groups is not understood. For example, the habitats used as nursery areas by pilchards have not been discovered. This lack of knowledge represents a problem for managing the three south coast pilchard fisheries. There is thus a need to determine the relationship between juveniles from the different assemblages of adult pilchards amongst regions of southern WA. In particular, whether juveniles which originate in each region largely remain separate or mix together needs to be determined. Following this, knowledge on the rates of mixing of pre-recruits should be investigated so that the relative contribution from any one region to any other region can be estimated.

This project was designed as a pilot study with the aims of:

1. *determining if specific pilchard nursery areas exist or if juveniles are simply spread out along the south coast,*
2. *undertaking chemical analyses of the otoliths of juvenile pilchards from each fishing zone, including those from samples obtained in previous years, to determine if separate groups of pre-recruits can be identified consistently over several years.*
3. *determining if large numbers of pre-recruit pilchards can be tagged using chemical dyes to mark otoliths or other bones*
4. *assessing whether there is a potential to develop a fishery independent index of recruitment.*

Since industry infrastructure was a key part of the intended field program to catch juvenile pilchards, the post pilchard-mortality downturn, and subsequent cessation, of commercial pilchard-fishing activity at Albany and Bremer Bay seriously impacted the ability to search for juvenile pilchards in these two regions. Thus, effort was concentrated in the Esperance region, the only to retain a commercial pilchard fishery

during the project. Juvenile pilchards were successfully caught in research nets but the catch rates were low. Because fishing for juvenile pilchards was significantly more difficult for Fisheries WA staff than anticipated, only one site at Esperance could be sampled on a regular basis. This negated the opportunity to search for juvenile pilchards in different areas and therefore the existence, or not, of specific nursery areas could not be ascertained using direct capture techniques. Considering the high level of effort required for researchers to catch juvenile pilchards, sampling of the commercial catches by Fisheries WA staff continues to provides the best means of assessing recruitment levels each year.

Chemical analysis of the otoliths indicates that in some years juveniles that move in to each of the three regions had lived in similar habitats prior to recruitment but in other years had lived in quite different habitats. The similarity of habitats occupied by juveniles in some years supports a hypothesis of a large, single pool of recruits that supplies each adult assemblage. However, the differences in other years suggests that there can be up to three distinct groups of recruits which have utilized different nursery areas.

Pilchards caught in Esperance using a commercial vessel and held in a cage for several weeks were given a non-toxic chemical dye mixed into a high-energy food. Although the food was readily eaten by the pilchards, analyses in the laboratory could not detect any of the chemical on either the otoliths or other bones. This study was therefore able to show that chemical marking with dyes is not a useful tagging methods for pilchards in WA. Unless other methods of tagging can be developed, the origin(s) of juvenile pilchards within each of the three southern WA fishing zones cannot be resolved with such methods. Techniques which examine DNA may assist with determining the relationships between juveniles from the different zones.

1.0 Introduction

1.1 Background

Pilchards (*Sardinops sagax*) have been the primary target in purse seine fisheries along the southern coast of WA for nearly 15 years. Annual catches across this region have previously been around 10, 000 tonnes, with much of this being caught in the Albany region. More recently, stock assessments have indicated a decline in spawning biomass of pilchards in the Albany region and management has responded by reducing the TAC for this zone. Thus, whereas the annual catch at Albany during the early 1990s was over 5,500 tonnes, in 1997 and 1998 the TAC was only 3,030 tonnes.

Three separate management zones have been established for the pilchard fisheries on the southern coast of WA (Fig. 1); these are associated with the landing ports of Albany, Bremer Bay and Esperance. Several lines of evidence indicate that adult fish in each of these zones remain largely separate from those in other zones. That is, there are functionally separate adult stocks for each zone. This has been shown by significant differences in ratios of oxygen and carbon stable isotopes (Edmonds & Fletcher, 1997). Furthermore, peak spawning time differs between each region, and while Albany has two spawning periods per year, Bremer Bay and Esperance have only one. Finally, although patterns can be followed in successive annual catch-at-age-curves within each zone, these catch curves have consistently varied between the zones. Likewise, mean length, weight and age (otolith weight) has also differed between regions. These various data indicate that there is little mixing of adults between zones, which has justified each zone being treated as distinct for the purposes of management.

However, the above lines of evidence only apply to adult fish, typically those over 2 years of age. Thus, there is a considerable gap in our knowledge of the life history of pilchards in WA. This gap essentially applies to fish between the lengths of approximately 20 mm (i.e. larvae) and about 100 mm SL. This size range equates to those fish too big to have been sampled in the various plankton surveys undertaken in relation to the south coast pilchard fisheries (Fletcher *et al.*, 1996) and too small to be effectively captured by the typical commercial purse seine gear (Fletcher, 1995). This in turn means that between the ages of one to two months and two years, when pilchards begin recruiting to the fishing grounds (Fletcher, 1995) their movements are virtually unknown. Fletcher *et al.*, (1994) have shown that larvae arising from eggs spawned in the Albany region can be transported up to 120 km east, apparently under the influence of the Leeuwin Current. This would take the 'Albany' larvae as far east as Bremer Bay, so there is a good potential for a direct link between these two regions at the pre-juvenile stage. Similarly, it is possible that larvae which originate near some fishing grounds off southern WA may be transported as far east as the Great Australian Bight, with the potential for transport well into South Australian waters (Gaughan *et al.*, 2001).

Although there is sometimes westward transport of pilchard larvae in the Albany region, this is weaker and less regular than eastward transport under the influence of the Leeuwin Current (Fletcher *et al.*, 1996). Because there are not any major populations of pilchards in the western part of the Albany zone (i.e. Cape Leeuwin to Walpole), if larvae originating from each of the three fishing regions are predominantly transported east and these larvae are important for future recruitment, then it follows that juveniles recruiting to Albany would

have had further to swim back to the fishery since this region has no ‘upstream” stock to supply larvae. That is, while the adult stock at Albany may supply larvae to Bremer Bay, and Bremer Bay may supply larvae to Esperance, there is no region which supplies larvae to Albany.

Following on from this, the origins of recruits in any one zone are also unknown, as are the rates of potential mixing of pre-recruits which have originated in different management zones. Thus, the potential for a link between zones via the per-recruit stages is recognized and accepted, with the result that management has aimed to keep the exploitation rate of the entire south coast breeding stock within sustainable bounds when setting individual TACs for each zone.

Despite knowing that the south coast pilchard most likely constitute a single breeding stock with zones linked via a common pool of pre-recruits (the pool hypothesis), the degree to which they are linked needs to be resolved. An important aspect of the pool hypothesis which needs to be investigated is determining whether or not each zone contributes similar proportions of recruits to (a) the entire breeding stock and (b) individual zones. For example, one zone may contribute on average more recruits than other zones.

While the pilchard fisheries at Esperance and Bremer Bay appear to be relatively stable, the Albany fishery has recently undergone major changes following on from a reduced TAC, which in turn followed several years of very poor recruitment and evidence of a declining stock. In order to maintain the viability of the Albany fleet the number of boats has declined from 22 to 15 over the past year (i.e. in 1998). Despite this rationalization, the need to maintain at least short term economic viability of the industry at Albany has meant that the exploitation rate of pilchards in the Albany region has been considerably higher than in Bremer Bay and Esperance. Thus, although there has been a higher exploitation rate at Albany, this has been tempered by the fact that the entire breeding stock across the south coast has been subject to a relatively conservative exploitation rate due to the lower levels of exploitation at the other two regions.

The potential problem with this type of management of the south coast pilchard fisheries is that because levels of mixing of recruits between zones is not known, there is a risk that exploitation of pilchards in one zone could impact on recruitment in another zone. For example, if a large proportion of the recruits at Bremer Bay were actually spawned at Albany, then the reduced spawning biomass at Albany could impact the Bremer Bay stock. Alternatively, development and expansion of pilchard fisheries in Esperance and Bremer Bay may have impacted on the levels of recruitment, and thus stock size, at Albany.

The south coast pilchard fishery is currently undergoing management changes aimed at ensuring long term viability in each of the three regions. However, the problem addressed above is severely limiting the process of change in management because various parties within the industry disagree as to which “direction” potential (i.e. perceived) deleterious impacts may be flowing. A significant part of this argument relates to our lack of knowledge of the pre-recruit stages. Thus, one line of thought is that fishing in Esperance may have a negative affect on the adult stock off Albany, whereas the opposing suggestion is that there is no affect. There are currently no data to support either side of the argument.

1.2 Need

There is an urgent need to determine the relationship between pre-recruit stages from the different stocks of adult pilchards amongst regions of southern WA. In particular, whether pre-recruits which originate in each region largely remain separate or mix together needs to be determined. Following this, knowledge on the rates of mixing of pre-recruits should be investigated so that the relative contribution from any one region to any other region can be estimated.

The key issues that need to be addressed are:

1. Do specific pilchard nursery areas exist?
2. Do recruits to each region come from a common pool of pre-recruits (e.g. one year olds)?
3. Is there significant eastward and or westward movements of pre-recruits between zones?
4. Does each zone of the fishery contribute similar numbers of recruits or is one zone (or two) more important than the others.

1.3 Objectives

1. Ascertain if pre-recruit pilchards can be caught on a regular (or even semi-regular) basis at each of the south coast regions or if a major nursery area exists for the entire stock.
2. Undertake an analysis of the oxygen and carbon stable isotope ratios for otoliths of pre-recruit and young post-recruit pilchards from each fishing zone to determine if separate groups of pre-recruits can be identified.
3. Undertake an analysis of the oxygen and carbon stable isotope ratios for the central region of otoliths from fully recruited pilchards at each region caught over the past 8 years to determine if separate groups of pre-recruits can be identified consistently over several years.
4. Attempt to tag large numbers of pre-recruit pilchards using tetracycline, calcein and possibly other ‘dyes’ to mark otoliths and other calcium based structures such as fin rays to determine if this is a viable research tool for pilchards on the south coast of WA.
5. Assess whether there is a potential to develop a fishery independent index of recruitment.

2.0 Methods

2.1 Capture of the juvenile pilchards

Pre-recruit pilchards, i.e. aged up to approximately two years, are known to occasionally be present at commercial fishing grounds along the south coast of WA. However, the periodicity of these events has not been documented and appears to be irregular. Nor have small pilchards been caught in any of the beach seine sampling programs which have been undertaken at various locations along the south coast over recent years (e.g. Ayvazian *et al.*, 2000, FRDC99/153). Thus, there is little information on where to find small pilchards. Anecdotal evidence from the south coast purse seine industry suggests that small pilchards are most typically seen in the Esperance region, with observations for each zone also suggesting that schools of one year olds "move in" from the east. Therefore, the Esperance region would be a focus point for capturing small pilchards, particularly within the Recherche Archipelago east of Esperance.

Although small pilchards have not been recorded from research beach seine catches, they may have occasionally been caught by commercial beach seine fishers and sold as blue sprats (*Spratelloides robustus*). In Geographe Bay on the lower west coast of WA (Fig. 1), small pilchards are regularly caught, typically as by-catch, by beach seine fishers targeting whitebait (*Hyperlophus vittatus*) with small meshed nets. Thus, for this region at least there is confirmed evidence of small pilchards using shallow inshore waters as a nursery area. However, except for regions where the purse seine fleets operate on the south coast (King George Sound- Albany, Bremer Bay, Esperance Bay), there is no large expanse of relatively calm water on the southern coast of WA which would equate to Geographe Bay on the lower west coast. It is therefore possible that juvenile pilchards on the south coast are not using shallow inshore areas as nursery areas.

Due to the irregularity with which pre-recruits have previously been observed and the associated uncertainty with where and when they are likely to be found, the initial sampling was to entail exploration of various habitats (i.e. inshore, offshore and various depths) within different geographic areas using a variety of fishing techniques including purse seining and light/gillnet fishing. For this reason, sampling was to be conducted three times during the year, but with each trip lasting for the relatively long period of 19 days to allow sufficient time for intensive searching for pre-recruit pilchards. However, the use of the purse seine net required assistance from industry members with previous experience operating small nets of this type. This precluded use of the purse seine net in regions east of Esperance, as had been planned, because industry members could not spend time away from town. Further discussions with industry members at Esperance subsequently indicated that it would quite likely not be a fruitful approach, given the time-line of the project, for Fisheries WA staff to attempt capturing juvenile pilchards with a purse seine net because industry members would best be able to notice when there were any juveniles around; the anticipated method of searching for schools of juveniles using a sonar was thus terminated.

Effort was therefore directed towards using a combination of lights and gillnets to fish for pilchards at night. Fieldwork proposed for Bremer Bay and Albany was not conducted due to very poor commercial catches and general lack of commercial fishing activity following the 1998/1999 mass mortality of pilchards across southern Australia. Hence, light/gillnet fishing was only undertaken at Esperance. Furthermore, in order to optimise the efficiency

of the light/gillnet fishing technique, this work was undertaken during the dark phase of the moon. Finally, restriction of light/gillnet fishing to the Esperance region also allowed the maintenance (e.g. feeding) of the caged pilchards held in the Esperance harbour during February, March and April.

2.1.1 Location of sampling site

The majority of the light/gill net sampling was conducted on the NE corner of Cull Island (Figs. 2 and 7), approximately 5 nm SW of the Esperance port in the Recherche Archipelago. This location was chosen because it is sheltered from the prevailing southerly winds, which was particularly important given the small size of the boat and that sampling was done at night. Also, local commercial fishermen contend that the depth of water close to the island (20-40 m) is a suitable depth in which to encounter juvenile pilchards; large numbers of small pilchards have previously been observed in close proximity to Cull Island.

In addition to the monthly sampling at Cull Island, opportunistic sampling was also undertaken at Sandy Hook Island, Limpet rock and Black Island (Fig. 2). Sampling in these particular locations was in response to reports of small fish in these areas by commercial fishermen. However, particularly good weather conditions were required for Fisheries WA staff to sample at Limpet Rock and Black Island, and a commercial vessel was needed to sample at Sandy Hook Island due to the distance from port. As a consequence, the majority of fishing effort was undertaken at Cull Island.

2.1.2 Light/gillnet fishing

Gill net fishing was conducted monthly from December 1999 through to June 2000 to coincide with the new moon, thus optimising the effectiveness of the lights used to attract the fish toward the nets. Two types of lights were used to attract fish; an underwater light that was hung just below the surface from the stern of the boat, and a light that was mounted on the canopy of the vessel pointing beyond the stern (Fig. 3). As both lights used 1000 W incandescent globes and were powered by a 1500 W portable generator, the lights had to be used alternately. Pollard was used in addition to lights to help attract fish to the boat, and an echo sounder was used to monitor fish abundance around the boat.

Three commercial vessels (Amanda Rosa, Firebird and Jumbo II) and a Fisheries WA vessel were used during this project to collect juvenile pilchards using the light/gillnet method.

Three different types of nets were used to catch juvenile pilchards during this study:

1. floating gill net 67 m long with a 2 m drop made from 3 panels of monofilament net with two different mesh sizes in the following order; 13 mm, 19 mm and 13 mm (Fig. 3).
2. rectangular 13 mm panel of monofilament mesh that hangs from the gunwale of the boat to the ocean floor (up to 30 m) and is 2 m wide (Fig. 3).
3. a square dab (200 x 300 mm) with 3 mm mesh.

The floating gill net was set first and hung from the stern of the vessel out into deeper water (up to 40 m), attached to a dan buoy at the other end. This net was set in the direction of the prevailing wind, with the boat anchored ‘up wind’, usually in the lee of Cull Island when sampling from the Fisheries vessel. The floating gill net was set at least ½ an hour before dark so that we could ensure that it was hanging correctly and so we could be sure that our anchor was secure. This net was only set once and retrieved at the end of the sampling

session because it was too difficult to re-set the net and re-anchor in the dark. In contrast, the net that hung vertically from the gunwale of the vessel was set after dark, and was checked and re-set 3 to 4 times during a sampling session. Additionally, the dab net was used opportunistically to catch small fish that came up to the boat, in the event that one of these fish may be a pilchard.

2.2 Oxygen and carbon isotope analysis

In order to assess whether pre-recruit pilchards do in fact consist of a common pool, the isotopic ratios of oxygen and carbon were examined from the five major commercial fishing zones of Fremantle, Dunsborough, Albany, Bremer Bay and Esperance. Otoliths were extracted from the archives from samples collected over the past 10 years and chemically degraded until the central region of the otolith had a weight and dimension similar to that for pre-recruit pilchards. The isotopic ratios of oxygen and carbon have been widely used to delineate populations (Campana, 1999), therefore, these degraded otoliths should provide evidence of where pre-recruits have resided for the majority of their lives prior to capture.

2.2.1 Subsampling from the otolith archives

Selected pilchard otoliths were taken from archives between the years of 1990 and 1998 from Fremantle, Dunsborough, Albany, Bremer Bay and Esperance, with two outlying samples coming from South Australia and Victoria (Fig. 1). In order to gather sufficient otolith material for chemical analysis (at least 0.8 mg), 10 otoliths (preferably 5 male and 5 female) were collected for each age class (i.e. 1 - 8 year old fish) for each year at a given location. Otoliths were sorted into age classes by otolith weight (Table 1), based on the criteria described in Fletcher (1995).

When possible, otoliths were taken from samples collected in June and July, which are the two months where most fish are caught on the south coast. However, if samples were lacking for these months the selection was expanded until a suitable number of otoliths could found. This was particularly necessary for the west coast fisheries of Fremantle and Dunsborough, because their most significant catches come during the summer months as opposed to the winter months for the south coast fisheries. As a consequence, a large proportion of the archived otoliths from Fremantle and Dunsborough were taken from February through to May, rather than June or July.

Due to the infrequency of 1 year old fish being captured in any of the five major fisheries, these fish were included in the analysis regardless of what time of year they were collected. In addition, because some of these fish have particularly small otoliths it was often necessary to collect both otoliths from more than 10 individuals. As one otolith from a 1 year old fish normally weighs less than 0.8 mg, degradation was not required.

2.2.2 Chemical analysis

2.2.2.1 Otolith degradation

Otoliths were degraded down to a weight of 0.8 mg which, although at the upper weight range for 1 year old pilchards, was necessary as 8 mg of otolith material (i.e. 10 otoliths at 0.8 mg) was the preferred pooled sample weight. This is because up to 4 mg of otolith material can be lost during the deproteination process and a minimum of 4 mg is required for the isotope analysis.

Otoliths were degraded by adding a volume (depending on otolith weight) of 0.2 M HCl to individual otoliths that were immersed in 3.5 mL of distilled water. The water was added in order to slow down the rate of dissolution, thus helping the otolith degraded more evenly. The amount of acid required was calculated by the following formula:

Whole otolith weight - 0.8 mg * C (407.405)

The constant (C) was based on the linear relationship (R^2 = 0.9295) observed between the volume of acid added (V) and the amount of otolith material removed (O) for 1129 individual degradations (Fig. 4). The constant could then be derived from the mean V/O for all of the degradations. This constant was revised on three occasions with an increase in the number of samples being processed, as some otoliths (particularly the larger ones) were not being fully degraded down to the required weight.

After the HCl and distilled water were added, the otolith sample was shaken in a foam tray that was mounted on a vortex mixer at low speed for 20 minutes. This allowed the otoliths to move around freely in the dilute acid solution, ensuring that all surfaces of the otoliths were exposed to the acid and hence, degraded evenly. When the degradation process was completed the otoliths were rinsed with distilled water three times, dried, and reweighed. If individual otoliths were not degraded down to the required weight (0.8 $\pm$ 0.14 mg, i.e. up to 0.94 mg) they were put through the degradation process again, taking into account the new otolith weight. Visual tests using a compound microscope were undertaken before and during the study to ensure that otoliths were degraded down to the approximate size and shape of a one year old fish (Fig. 5).

2.2.2.2 Otolith carbonate deproteination

Individual degraded otoliths were pooled into one container and then crushed using an agate mortar and pestle. Approximately 0.6-0.7 mL of NaOCl (sodium hypochlorite) was added to the crushed otolith material (as opposed to the hydrogen peroxide/water bath method used by Edmonds and Fletcher (1997), and left to stand for a minimum of 2 hours. The otolith carbonate was then rinsed 4 times with distilled water, and dried overnight. In order to minimise the loss of otolith material, the otolith solution was centrifuged for 1 minute at 4000 rpm after each rinse.

The combination of bleaching without a water bath and centrifuging enabled more otolith material to be retained during the deproteination process than the hydrogen peroxide/water bath method used by Edmonds and Fletcher (1997). Thus, the mean loss of carbonate was less than 2 mg as opposed to 4 mg in the latter method, allowing us to include pooled otolith samples weighing as little as 6 mg in the analysis. This was particularly useful in cases where there were fewer than 10 otoliths (or less than 8 mg of 1 year old otoliths) available from the archives to make up a sample.

The ^{18}O:^{16}O and ^{13}C:^{12}C ratios for otolith carbonate were estimated by standard mass spectrophotometric techniques, with values reported in standard delta notation relative to the PDB-1 standard (Epstein *et al.*, 1953).

2.2.3 Statistical analyses

Analysis of covariance (ANCOVA) was used to test the hypotheses of no difference in $?^{18}O$ or $?^{13}C$ between pilchard fishing regions in WA. Following the ANCOVA design used

previously for adult pilchards (Edmonds and Fletcher 1997), and which has been adopted elsewhere (Newman *et al.* 2000), region and date (i.e. year) were treated as fixed effects, with mean otolith weight used as a covariate. Significant effects were then further compared using Tukey's Honestly Significant Difference (HSD) test for unequal sample sizes. Because the south coast region was the main interest of this project, the sites from this region were also analysed in isolation from the west coast sites, but using the same statistical technique. In order to make the statistical tests more balanced and thus more robust, these tests for the south coast were restricted to the seven years from 1988 to 1994, which are those for which at least two samples of otoliths were available for each region in each year.

2.2.4. Short term variability in isotopic ratios

Samples of 0+ fish were collected from the Geographe Bay in November 1998 (n = 118) and December 1998 (n = 232) and from Esperance on the 2nd (n = 102) and 5th (n = 150) April 1999. The samples were sorted into 2 mm length (LCF) categories, ranging from 46 to 68 mm for Geographe Bay and from 66 to 86 mm for Esperance. The otoliths were removed from a sufficient number of fish within each 2 mm length interval so as to obtain a least 6 mg of otolith material for isotope analysis.

In addition to the analyses for particular length intervals, before the juvenile pilchards were sorted into the size classes, a random sample of fish was taken from one of the Geographe Bay and Esperance samples. The otoliths from these fish were included as samples from the year at age 1 analysis and were the sole data for Geographe Bay in 1998 and Esperance in 1999.

2.3 Chemical marking of otoliths

More traditional methods of physically tagging fish could not be applied to pre-recruit pilchards due to the extremely high mortality and the difficulty associated with handling the fish and the immense numbers of fish that would need to be tagged. As a consequence, a method for bulk tagging juvenile pilchards by using a chemical dye such as oxytetracycline or calcein to deposit a fluorescent mark on otoliths and other calcium based structures such as fin rays or vertebrae was investigated. Both calcein and oxytetracycline have been used extensively for reliably marking otoliths in age validation studies for fish. Calcein was used in this study rather than oxytetracycline as it is less harmful to fish at high concentrations and does not degrade in ultraviolet light, unlike the latter.

Due to the difficulties in handling live pilchards, calcein could not be administered to juvenile pilchards via injection, which has traditionally been the most common method used in age and growth studies (e.g. Tanaka, 1990; Babaluk and Craig 1990; Francis *et al.*, 1992; Sadovy *et al.*, 1992; Speare, 1992; Monaghan, 1993; Rien and Breamesderfer, 1994; Francis *et al.*, 1999; Gauldie, 2000). Immersion in a solution of trace elements such as strontium, calcein or other fluorescing dyes, is another method that has been proven to be effective for marking otoliths and other calcified structures (Beckman *et al.*, 1990; Ennevor, 1994; Brooks *et al.*, 1994; Nagiec *et al.*, 1995; Mohler, 1997; Pollard *et al.*, 1999). However, immersion is better suited for aquaculture where small fish such as larvae can be treated in situ, in a relatively small bath (i.e. using smaller quantities of expensive chemicals) and kept alive in that closed system for a number of hours. Immersion has been used on wild fish, with success on a species of damselfish (*Pomacentrus amboinensis*) by immersing the

developing embryos with tetracycline, thus leaving a mark on the otoliths of juvenile fish (Jones *et al.*, 1999). In that study, female damselfish were encouraged to spawn onto PVC tiles placed adjacent to breeding sites by the researchers. These plates were then placed into plastic bags temporarily, while being immersed in a 400 µg L^{-1} solution of tetracycline in situ for one hour.

It is unlikely that the immersion method could be applied to a pilchard bulk tagging program as the fish would first need to be captured in a purse seine, then brailed into 1000 L bins on the deck of the boat before immersion in calcein could begin. The act of pursing the fish up close enough for them to be brailed out causes extreme stress on the fish. It increases the likelihood of collisions with the net and each other, leading to scale loss, bruising and cuts, all of which may facilitate infection and later death (Mitchell *et al.*, Submitted). Similarly, costs of filling a 1000 L bin with a 250 mg L^{-1} solution of calcein (as suggested by Mohler, 1997) are extremely high given that we believe less than 1000 juvenile pilchards could be maintained for the duration of the immersion procedure in the bin at one time.

Due to the practical and economic limitations of the injection and immersion techniques for 'bulk tagging' pre-recruit pilchards, it was decided that the method most applicable to tagging pilchards in the field would be to introduce calcein via ingestion (i.e. food laced with calcein). This method would require the use of a purse seine vessel to locate and entrap a school of pre-recruit pilchards. The pilchards would then be fed whilst inside the net, where the amount of food added (pollard or crushed trout pellets with a known concentration of calcein) would be based on an estimation of the size of the school in the net. The fish would then be released by dropping the purse rings and opening the net, allowing the fish to swim out. This method has the advantage over other methods in that it does not require fish to be bunted up alongside the vessel and they do not need to be handled in any way.

If this method for bulk-tagging pre-recruit pilchards can be developed, knowledge of the growth rates of pilchards (Fletcher, 1995) will subsequently permit a particular size class of pilchard to be targeted during the search for 'tag returns'. However, as with the tagging, searching for returns will also require processing large numbers of fish so methods to undertake this will also be assessed. Bulk quantities of otoliths from appropriately sized pilchards from each zone will be removed by blending heads in a kitchen blender, a technique successfully used to remove otoliths from large numbers of sandy sprat. Otoliths will be mounted whole on microscope slides and examined under UV light. Since the identity of individual pilchards is not required, mounting as many otoliths as possible on each microscope slide will decrease the processing time. Tests will be made of the rate of successfully detecting marked fish by mixing marked fish with larger numbers of non-marked fish and then following the procedure(s) from extraction to viewing under the microscope, with the ratio of marked fish not known to the reader.

Note that even if the tagging trial was successful, no effort specifically aimed at searching for returns was planned during this 15 month pilot study. Rather, the successful techniques developed during the pilot study were subsequently to be used in a program aimed at tagging very large numbers of small pilchards.

2.3.1 Esperance chemical marking trial 1

The first chemical marking trial was conducted at Esperance in October 1999 with the primary aim being to determine whether pilchards could be transported live into a holding

cage and kept alive for the duration of a calcein feeding experiment. If this was successful, we would then introduce a specially formulated diet laced with calcein. The floating sea cage (2x3x3 m deep) was initially placed inside an enclosed boat shed near the northern end of the Esperance Port Authority wharf. However, this location was partially exposed to swell and surge in extremely rough conditions and the fish placed into the cage at this location died overnight due to bad weather. The sea cage was subsequently placed at the southern end of the Esperance Port Authority wharf in a significantly more sheltered location. Live pilchards were captured on the 17th October at approximately 20:50. Fish were brailed into a 1000 L fish bin using a brail net with a waterproof canvas liner so that the pilchards would be supported in water whilst being transferred, thus reducing damage to the fish. The pilchards were kept alive during transportation by bubbling pure oxygen through the bin and by continuously running the deck hose into the bin. Back in port, the bin with the fish was loaded onto a truck and transported about 5 km to the wharf at the Esperance Port Authority and transferred into the floating sea cage.

Fish were fed approximately 2 cups of food laced with calcein twice per day for a period of 12 days, starting from the first day after capture. However, it was three days before the pilchards were observed actively feeding. A small number of fish were sacrificed daily during the period of the trial and taken back to the laboratory so that the otoliths could be examined for calcein marks.

The calcein laced diet consisted of the following ingredients:

- 600 g Prime fish meal (Gibson's)
- 200 g plain wheat flour
- 100 g wheat gluten
- 90 g fish oil
- 3 g vitamin premix
- 50 mg calcein
- 250 mL tap water

The ingredients were mixed, extruded through a pasta maker, chopped into short lengths and dried in an oven for 2.5 hours at 80 °C.

Unfortunately this trial was found to be unsuccessful following both visual and chemical analyses of the collected otoliths. This prompted further investigation, revealing that despite the obvious advantages of feeding calcein to pilchards as opposed to more traditional methods of administration, there could be a few potential problems associated with the technique. Firstly, the calcein could bind to the food or be degraded by elements within the food making it inactive. Secondly, the calcein may be destroyed in the fish's stomach before it can be absorbed into the blood stream. And thirdly, the calcein (which is particularly soluble in water) may be dissolved in the water before the food could be eaten. To overcome these potential problems we entrapped the calcein within semi-permeable biocompatible microcapsules in a technique used by Polk *et al.* (1994) to protect vaccines used in aquaculture during oral administration. In theory, the microcapsule should protect the calcein from the food and from degradation within the stomach allowing it to pass in to the intestines of the fish where it could be absorbed.

2.3.2 Preparation of calcein microcapsules

The microcapsules used in this study consisted of a calcium-alginate and chitosan shell, which is formed around a core of a 5 gL^{-1} solution of calcein. The microcapsules were formed by blowing fine droplets of alginate chitosan and calcein into a gently stirring solution of $CaCl_2$ (Fig. 6). When the fine droplets of alginate mixture hit the $CaCl_2$, the calcium bound to the alginate and formed an insoluble membrane around the calcein solution. The microcapsules were then filtered and repeatedly rinsed with seawater to ensure that the microcapsules were not ruptured by the osmotic pressure of freshwater. The microcapsules were then dried overnight in a desiccator and stored in a refrigerator at 5 ^{o}C until they were required for use.

2.3.3. Esperance chemical marking trial 2

A school of approximately 300 kg of adult pilchards were captured by the fishing vessel 'Firebird' at 20:00 on the 2nd February. Fish were brailed into a 1000 L fish bin, transported back to the Esperance Port Authority and transferred into the floating sea cage, as previously described. Research staff monitored the fish for the following week, slowly introducing them to a diet of crushed trout pellets (up to one cup per day), while any dead fish were removed from the cage on a daily basis.

The pilchards in the sea cage at Esperance had their first dose of calcein 31 days after they were captured. The fish were fed the calcein microcapsules mixed with trout pellets every afternoon for 5 days from the 4th of March through to the 8th of March 2000. The feed recipe consisted of:

- 1 cup of trout pellets
- 30 small spatulas (15 g) of 5gL^{-1} calcein microcapsules
- 20 mL of fish oil (used to encourage feeding)
- 50 mL of water

These ingredients were mixed together at least one hour before feeding and allowed to dry. The feed was then crumbled and fed to the pilchards in the cage in very small amounts in an effort to ensure that all of the food was being eaten.

At the end of the fifth day a sample of six pilchards was removed in order to see if the calcein had marked the otoliths and to check the stomach contents of the fish. The remainder of the fish in the cage were kept alive and fed for a further 29 days before they were removed. This was done to ensure the calcein would have sufficient time to mark the otoliths or any other calcium structures, including fin rays or vertebrae.

2.3.3.1 Visual analysis:

A random selection of 20 otoliths were extracted from the pilchards and visually examined for calcein marks. These otoliths were embedded into epoxy resin and a transverse section (about 350 μm) was cut and mounted on a microscope slide. The sections were viewed at 100x magnification under ultraviolet (UV) light.

2.3.3.2 Chemical analysis:

A further selection of otoliths, fin rays and vertebrae were chemically degraded in order to extract any calcein that may have been deposited in the otoliths. A total of 144 otoliths with

a combined weight of 158.53 mg from 87 pilchards were crushed in an agate mortar and pestle and immersed in ethanol and sonically stirred at 50 °C for 3 hours. The supernatant was removed and refreshed 5 times throughout the procedure to give a total volume of 50 mL. It was then filtered and evaporated using a nitrogen blowdown apparatus, giving a final volume of 1 mL. The supernatant was analysed using UV spectroscopy for calcein, which has a lambda max of 499 nm. However an absorbance range of 350-600 nm was also tested in case of the presence of a calcium-bound form of calcein.

2.4 Relative growth rates

Relative growth rates will also be compared within and between regions by regressing fork length against otolith weight. The benefit of this method is that it does not actually involve an estimation of age, with both variables able to be precisely measured. This method assumes that for pilchards of a given length, one with larger otoliths will be older and thus will have had a slower growth rate (Fletcher 1991, 1995). Differences in growth rates would indicate that growth conditions were different, possibly resulting from fish having lived in different environments. Due to insufficient samples from each of the south coast regions this method has yet to be applied.

3.0 Results and discussion

3.1 Capture of juvenile pilchards

Given that the horizontal gill net used to capture juvenile pilchards relied on the current to hold the net away from the boat, wind direction and swell played a major role in determining the position that the net hung from the boat in relation to Cull Island. Therefore, it was not possible to sample the exact same area off the north east corner of Cull Island despite anchoring in approximately the same position for most sampling sessions. Figure 7 shows the three most common net directions, areas and depths fished from a common boat position. The percentage values accompanying the arrows on this figure are based on a total of 24 sampling sessions spanning 10 months from December 1999.

FWA Research staff were successful in capturing juvenile pilchards using the light fishing method (Table 3, Fig. 8). The first juvenile pilchard captured at Cull island using the light fishing method was in April, 12 days after a juvenile pilchard was caught in a purse seine net by a commercial fishermen. May was the most successful month for capturing juvenile pilchards where a total of 18 fish were captured from 3 sampling sessions. These data are reflected by the catch rates of juvenile pilchards, which exhibited a peak in May (Fig. 8). Four more juvenile pilchards were captured in late May/early June, one of which was caught with the dip net at the back of the boat.

The relatively large standard error associated with the catch and effort data (Fig. 8) suggests that even during months that juvenile pilchards were present in Esperance Bay, their occurrence near Cull Island is highly variable. Thus, although the light-gill net technique does catch pilchards, a static fishing method applied at a set location may provide quantitative information (i.e. catch rates instead of simply obtaining some fish) that is relevant only to that specific location. While it would be expected that changes in the abundance of pre-recruit pilchards in the Esperance Bay would be reflected by their catch rates near Cull Island, the high variability of the data suggest that the changes in abundance would have to be large in order for an accompanying signal measured at Cull Island to be reliable. A longer time series of sampling may prove or disprove this assumption but the level of effort required is not warranted by the size of the industry. Indeed, large influxes of pre-recruits or recruit pilchards are normally observed by the purse seine industry and reported, even if only in an ad hoc manner, to FWA research staff.

Regarding collection of pilchard otoliths for isotope analyses, the low numbers caught using the light-gill net technique were insufficient to represent a reasonable sample size. Given the size of the purse seine industry in Western Australia and the concomitant level of resources for this type of research requires, specifically a small vessel with a generator, collecting samples of pre-recruit pilchards at night time at some distance from port requires the direct assistance of industry at sea, as well as the invaluable onshore assistance. Thus, if the Bremer Bay and Albany purse seine fisheries had been operating during this study, attempts to collect of samples of pre-recruit pilchards for isotope comparisons would probably have best been met by obtaining the irregular samples of pre-recruits from the commercial catches and through periodic targeting of pre-recruits at those particular times they were observed near the fishing grounds.

3.2 Oxygen and carbon isotope analysis

The isotopic data ($\delta^{18}O$ and $\delta^{13}C$ values) from both the degraded and 1 year old otoliths, were plotted for all locations as a scattergram in order to ascertain if any clear patterns exist in the data (Fig. 9). In order to clarify interpretation of these results, the oxygen and carbon isotopic data were averaged for each location (Fig. 10). This figure shows that there is a clear difference between the west coast and south coast regions of WA for both oxygen and carbon isotopes. The patterns of mean isotope values for each of the five WA locations from 1984 to present supports previous information suggesting that the west coast locations of Fremantle and Dunsborough constitute a separate stock from the south coast locations (Fletcher *et al.*, 1996; Edmonds and Fletcher, 1997). Similarly, the samples from Victoria and South Australia have quite different $\delta^{13}C$ values to those from all of the Western Australian samples, and also have different $\delta^{18}O$ values to the west coast samples. Individual plots of $\delta^{18}O$ and $\delta^{13}C$ values (Figs. 11 and 12) clearly show this progression of isotopic differences from the west coast, to the south coast, to South Australia and to Victoria.

In order to ascertain whether the isotopic ratios of juvenile pilchards (represented in this study) differ to those of adult fish from the same regions, the mean oxygen and carbon isotopic values from this study were compared to those data collected on adult pilchards by Edmonds and Fletcher (1997). The data from the present study and the Edmonds and Fletcher data show an almost identical trend for both the oxygen (Fig. 13) and the carbon (Fig. 14) isotopes. The values for both isotopes from the current study are slightly lower than the Edmonds and Fletcher (1997) data for the south coast locations, and are markedly lower for the west coast locations. These data indicate that juvenile pilchards from both the south and west coasts either live in warmer water than the adults, or oxygen isotopes are not deposited in equilibrium to ambient seawater at all times. These two alternatives were similarly concluded by Edmonds and Fletcher (1997) for adult *Sardinops*.

This trend is further illustrated in Figure 15 which shows the extent of the separation between juvenile pilchards from Fremantle and Dunsborough in the current study from all other locations in both studies. However, for the south coast regions the $\delta^{18}O/\delta^{13}C$ plots reveal that juveniles show the same trends as the adults but with lower values and more variability, as mentioned above. Indeed, the mean south coast $\delta^{18}O$ values for juveniles were spread as widely as those for the adults. However, there was consistently more variation in the otolith isotope data (Figs. 16 and 17) for the juvenile pilchards in the current study than for the older pilchards examined by Edmonds and Fletcher (1997). While a certain level of correlation is expected because the samples used in this study encompass those of Edmonds and Fletcher (1997), in the case of $\delta^{18}O$ data the less variation for the adults is indicative of a "narrower" thermal history. This supports the concept that adult pilchards do not move a great deal once recruited into a particular region. By contrast, juveniles in any one region appear to have experienced a broader range of temperatures. This is not to say that individual fish have made extensive migrations between areas of differing temperatures, but that the recruits coming into an area may have come from different areas.

3.2.1 Trends for year at age 1

As the age of the pilchard otoliths taken from the archives is known, and otoliths were degraded down to the size of a 1 year old fish, the $\delta^{18}O$ and $\delta^{13}C$ values correspond to the year of capture. For example, a five year old pilchard captured in Albany in 1994 and a seven year old fish captured in Albany in 1996 were both 1 year old in 1990. That is, these

fish were likely to have recruited into the Albany fishery in the same year. This technique enables us to gain an insight into any annual patterns that may exist within and between locations.

The statistical analyses considered only the WA samples from the period 1988 to 1994.

The ANCOVAs showed that $\delta^{18}O$ (Table 4) and $\delta^{13}C$ (Table 5) from pilchards in southwest WA differed between sites and between years. As expected from examination of Figures 10, 11 and 12, both $\delta^{18}O$ and $\delta^{13}C$ differed significantly between the west and south coasts of WA, with Tukey's HSD test indicating that for $\delta^{18}O$:

Fremantle = Dunsborough < Bremer = Albany = Esperance, where the order for 'equal' sites is from lowest to highest.

Similarly, Tukey's HSD test indicated that for $\delta^{13}C$:

Fremantle = Dunsborough < Esperance = Albany = Bremer.

There was no significant difference in $\delta^{18}O$ nor in $\delta^{13}C$ between Albany, Bremer Bay and Esperance on the south coast (Tables 6 and 7). However, there was a significant difference between years for $\delta^{13}C$ ($p<0.05$). While this was not the case for $\delta^{18}O$, as with the $\delta^{13}C$ data the year effect did account for most of the explained variance.

As highlighted earlier, a feature of the whole data set and the reduced set for the south coast was the high level of variability. This was particularly pronounced in the south coast data for each of the two ANCOVAs performed (i.e. for $\delta^{18}O$ and $\delta^{13}C$); the sums of squares (SS) attributable to the error term was close to 75% of the total SS. Thus, a large amount of the variability could not be explained by the effects of region or year; recruitment of pilchards in southern WA is inherently variable. As this variability is a major feature of the south coast data it will be explored in more detail below.

Annual trends in the $\delta^{18}O$ for both the west and south coast are shown in Figure 18. There is a major decline in $\delta^{18}O$ values from 1994/95 to present, which coincides with an increase in SST over this period (Fig. 19a). There is little consistency in the annual differences in $\delta^{18}O$ between the fisheries of Fremantle and Dunsborough, and between Albany, Bremer Bay and Esperance, as indicate by the ANCOVAs. However, although the within-region differences are not significant, they may still help answer some unknown aspects of recruitment processes. The changes in both magnitude and direction of the interannular differences between pairs and triplets of sites highlight, again, the variability in environmental signatures of one year old pilchards. Focussing on the south coast in the years when samples were available for all three regions (Fig. 18c), in some years the mean $\delta^{18}O$ at two regions are similar, and different for the third region (1987, 1988, 1991, 1993, 1994), while in other years all three regions are different (1989, 1990, 1992, 1995). Furthermore, there are also changes in which two regions are similar. Thus, Albany and Bremer Bay were similar in 1987, Albany and Esperance in 1988 and 1991, and Albany and Bremer Bay in 1994. Interannual variations in the relative $\delta^{18}O$ values at each south coast region did not follow any clear pattern. Examination of the variability at this level indicates that for particular years the recruits which enter each south coast pilchard fishery may have resided, in broad terms, in two habitats of different temperatures, and in other years resided in at least three different habitats of different temperatures. Although this broad generality does not provide any information on where these nursery habitats may be, they do preclude the existence of a

single, well defined nursery area. The alternative is therefore that juvenile pilchards utilize a range of thermal habitats.

Further interpretation of each of these scenarios is provided here to bring the results back to a level that is important for management of the WA pilchard resource.

Assuming the oxygen isotope ratio is largely a function of water temperature (e.g. Kalish, 1991; Edmonds and Fletcher, 1997) an attempt was made to determine the temperature at which recruits in each of the five regions had lived for the majority of their first year. A comparison of the $\delta^{18}O$/temperature regression models, for calculating the mean water temperature from the $\delta^{18}O$ data, of Kalish (1991) and Edmonds and Fletcher (1997) showed that the former provided a more realistic range of potential temperatures experienced by southern WA pilchards (Table 8). For example, the $\delta^{18}O$ range for Bremer Bay (Figure 20d) in 1990 is 0.0 to 1.2, which equates to a range in water temperature of 12.15 - 18.28 ^{o}C according to Kalish (1991), and is equivalent to 16.7 - 26.7°C according to Edmonds and Fletcher (1997) (Table 8); 26°C is higher than the temperature range for *Sardinops*.

Overall, juvenile pilchards from various regions apparently experience a temperature range between approximately 10 and 20°C. Within most regions the temperature of the predominant habitats of different juveniles was typically around 4°C (Fig. 19). In some cases, the range of temperatures experienced was ≥6°C (Albany 1990 and 1991, Bremer Bay 1990). Given that the maximum range in annual mean SSTs across southern Australia between Walpole and Melbourne is typically less than 4°C, the within-region variability in $\delta^{18}O$ has two possible explanations. One is that $\delta^{18}O$ is not a precise indicator of temperature and therefore that oxygen isotopes are not always deposited on the otolith carbonate in equilibrium with seawater, as suggested above, for reasons that have yet to be elucidated. The alternative explanation is that southern WA pilchards are derived from a range of nursery habitats that include deeper, (hence cooler) offshore waters. Regardless of whether or not the observed $\delta^{18}O$ values closely reflect water temperature, the results clearly indicate widely varying nursery habitats amongst juvenile pilchards within regions. Rather than using the terms thermal histories or thermal habitats, it is probably more valid to thinking terms of isotopic histories.

As with the inconsistency in magnitude and direction of mean differences between sites, the range of temperatures experienced also varied interannually within regions. As indicated above, this again suggests that in some years the recruits to a particular region have come from a broader range of habitats than others

3.2.2 Short term variability in juvenile fish

Stable isotope analysis of otolith carbonate from multiple length-class intervals within individual samples of 0+ pilchards from Dunsborough (November and December 1998) and Esperance (2nd and 5th April 1999) allowed us to determine (1) if there is any short term variation in the oxygen and carbon isotopes of juvenile pilchards and (2) the level of consistency within a school. Figure 21 and 22 summarises the results for the $\delta^{18}O$ and $\delta^{13}C$ data respectively. The marked difference between the Dunsborough and Esperance samples for both $\delta^{18}O$ and $\delta^{13}C$ are consistent with the results of the main analysis, with the Dunsborough fish having lived in cooler water than those from Esperance. The Dunsborough pilchards were caught in late spring/early summer and had therefore been growing through the late winter/spring period. In contrast, the Esperance pilchards were

caught in early autumn and had therefore just grown through the summer period, when temperatures were higher than those in Geographe Bay during the previous winter.

There was very little difference in either $\delta^{18}O$ or $\delta^{13}C$ between the various length categories within any of the four samples of 0+ pilchards. This consistency indicates that each of the schools sampled were relatively cohesive. Interestingly, the difference between the two Esperance samples that were caught only three days apart also indicates that schools of juvenile pilchards occupying a single region can have different isotopic histories. This confirms that pilchards in a region have typically recruited from a variety of different habitats. Similarly, the $\delta^{18}O$ values for the Dunsborough 0+ pilchards also exhibited little overlap.

3.3 Chemical marking of otoliths

The gut contents of 3 pilchards that were recently fed microencapsulated calcein, were examined by UV spectroscopy in order to ascertain whether the calcein remains active within the microcapsule after being exposed to the stomach. The results from this analysis (Fig. 23) show there to be a slight peak at about 499 nm, suggesting that there is some active calcein within the microcapsules in these gut samples. However, visual analysis of otoliths extracted from the pilchards 29 days after they were fed calcein microcapsules in with their food, revealed no calcein marks on transversely cut sections of the otolith when analyzed under ultra violet light. Furthermore, chemical analysis (via UV spectroscopy) revealed an absence of calcein from the otoliths and vertebrae from both pilchards and yellowtail scad, that were fed microencapsulated calcein in the holding cage (Fig. 24).

In conclusion, it is unlikely that feeding an enclosed school of pilchards microencapsulated calcein would be successful. For this technique to work it would be necessary to investigate the processes involved in the absorption of calcein into the bloodstream and further refining the microcapsules ensuring that they efficiently deliver calcein to the intestine.

4.0 Benefits

Increased understanding of recruitment variability of pilchards in WA has the following benefits. This variability in the level of connectedness (i.e. similar nursery conditions in some years but not others) between pre-recruits indicates southern WA pilchards are not reliant on a single nursery area nor on a single type of environmental condition. Through benefiting our general understanding of recruitment processes, this knowledge will permit more appropriate development of hypotheses relating to variability in the magnitude of recruitment, which is the prime factor affecting stock sizes.

Determining that marking of otoliths with calcein is not a useful approach for tagging pilchards means that assessment of mixing rates between stocks may be better addressed using advanced techniques for DNA analysis.

5.0 Further development

Relative growth rates of juvenile pilchards could not be compared between regions in this study because none were available from Albany and Bremer Bay. However, juveniles were caught at these sites in December 2000 and January 2001, respectively, so one set of comparisons can be made. These same samples will also permit further comparisons of isotopic rations of otolith carbonate to add to the series obtained thus far. These data will also allow further assessment of the within school variability.

The oxygen isotope data will be further analysed with respect to sea surface temperatures

The new aspects on relationships between juvenile pilchards will be used directly to assist with the interpretation of the time series of recruitment strength for each of the WA pilchard fisheries.

6.0 Conclusion

There is an urgent need to determine the relationship between pre-recruit stages from the different stocks of adult pilchards amongst regions of southern WA. In particular, whether pre-recruits which originate in each region largely remain separate or mix together needs to be determined. Following this, knowledge on the rates of mixing of pre-recruits should be investigated so that the relative contribution from any one region to any other region can be estimated.

The Key Issues relating to recruitment processes of pilchards in WA were identified to be as follows

1. Do specific pilchard nursery areas exist?
2. Do recruits to each region come from a common pool of pre-recruits (e.g. one year olds)?
3. Is there significant eastward and or westward movements of pre-recruits between zones?
4. Does each zone of the fishery contribute similar numbers of recruits or is one zone (or two) more important than the others.

These issues were used to develop the formal Objectives (below) of this project; the conclusion of each of are presented here.

1. Ascertain if pre-recruit pilchards can be caught on a regular (or even semi-regular) basis at each of the south coast regions or if a major nursery area exists for the entire stock.
5. Assess whether there is a potential to develop a fishery independent index of recruitment.

Due to the often unfavourable weather and sea conditions along the southern coast of WA, fishing for juvenile pilchards was significantly more difficult for Fisheries WA staff than anticipated, particularly due to the constraints of fishing at night from an open vessel and being restricted each month to fishing only during the dark phase of the moon. Because fishing for pre-recruit pilchards could be undertaken on a regular basis by research staff at

only Esperance, the concomitant lack of opportunity to search for juvenile pilchards in different areas did not allow the existence, or otherwise, of specific nursery areas to be ascertained (Key Issue 1). With respect to Objective 5, considering both the value of WA's commercial pilchard fishery and the high level of resources required for researchers to catch juvenile pilchards, sampling of the commercial catches by Fisheries WA staff continues to provide the best means of assessing recruitment levels each year.

2. Undertake an analysis of the oxygen and carbon stable isotope ratios for otoliths of pre-recruit and young post-recruit pilchards from each fishing zone to determine if separate groups of pre-recruits can be identified.

3. Undertake an analysis of the oxygen and carbon stable isotope ratios for the central region of otoliths from fully recruited pilchards at each region caught over the past 8 years to determine if separate groups of pre-recruits can be identified consistently over several years.

Conclusions relating to these two objectives are presented together.

The within-region variability in oxygen isotope ratios showed that separate groups of pre-recruits typically contributed to the influx of juveniles to most regions in most years. Examination of the average recruitment, in terms of the isotope ratios, indicates that in some years juveniles that move into each of the three regions had lived in similar habitats prior to recruitment but in other years had lived in quite different habitats. The similarity of habitats occupied by juveniles in some years supports a hypothesis of a large, single pool of recruits that supplies each adult assemblage (Key Issue 2). Furthermore, the similar habitat-history implies that the pool is either widespread with a fast spatial turnover, or is possibly delivers recruits to each region (to even out isotope signatures) in quick succession (to minimise differences in isotopic signatures). However, the differences in other years suggests that there can be up to three distinct groups of recruits which have utilized different nursery areas. Although the question of a specific or several nursery areas (Key Issue 1) could not be directly addressed because sampling effort was concentrated at only one site, the analyses of otolith carbonate allows the conclusion that there is not a specific nursery area in the environmental sense within or between years, which probably equates to a lack of a geographically consistent nursery area. Thus, the whole south coast region may act as a nursery to some extent, including regions east of Esperance and into the Great Australian Bight. A broad spread of pre-recruits may act to reduce the impacts on the south coast breeding stock of localised negative influences, which may be particularly important for pelagic filter feeders in oligotrophic waters.

4. Attempt to tag large numbers of pre-recruit pilchards using tetracycline, calcein and possibly other 'dyes' to mark otoliths and other calcium based structures such as fin rays to determine if this is a viable research tool for pilchards on the south coast of WA.

Pilchards caught in Esperance on a commercial vessel and held in a cage for several weeks were given a chemical dye mixed into a high-energy food. Although the food was readily eaten by the pilchards, analyses in the laboratory could not detect the chemical either on the otoliths or other bones. This study was able to show that chemical marking with fluorescent dyes is not a useful tagging method for pilchards in WA, so there is currently not a method to assess the extent to which juvenile pilchards may move between zones (Key Issue 3). If an alternative tagging method cannot be developed, examination DNA may offer a technique for determining the origins of juveniles within each zone and thus whether some regions are produce more recruits than others zones (Key Issue 4).

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8.0 Tables

Table 1. Otolith weight categories used to determine the age of Sardinops sagax samples extracted from the archives.

Age	Otolith Weight (x10-2 mg)
1	0 - 80
2	90 -104
3	119 - 133
4	142 - 157
5	167 - 181
6	188 - 202
7	208 - 222
8	> 233

Table 2. Number of Sardinops sagax samples extracted from the otolith archives, backdated to the year at age 1, and used in the stable isotope analysis.

Year at	Location						
Age One	Frem.	Dunsb.	Albany	Bremer.	Esp.	Sth. Aust.	Vict.
1984	-	-	-	1	-	-	-
1985	-	-	2	3	-	-	-
1986	3	-	2	2	-	-	-
1987	1	-	4	4	1	-	-
1988	2	1	5	5	3	-	-
1989	4	1	5	4	4	-	-
1990	5	2	6	5	5	-	-
1991	4	2	6	6	7	-	-
1992	3	1	5	4	5	-	-
1993	4	1	2	3	4	-	-
1994	3	3	2	3	4	-	-
1995	4	2	1	1	2	-	-
1996	3	1	3	-	1	-	-
1997	3	-	2	-	1	1	1
1998	3	1	1	1	-	1	1
1999	-	-	-	-	2	-	1
2000	-	-	-	-	1	-	-

Table 3. Summary of juvenile Sardinops sagax captured during light fishing sessions off the coast of Esperance WA.

Date	Location	Mesh Size (mm)	Net Type	LCF (mm)	Weight (g)	Otolith Weight (mg)
08/04/2000	Cull Island	13	Horizontal gill	58	1.78	0.34
03/05/2000	Sandy hook	19	Horizontal gill	103	9.82	0.66
03/05/2000	Sandy hook	19	Horizontal gill	87	6.8	0.38
05/05/2000	Limpet Rock	13	Vertical gill	65	2.54	0.22
05/05/2000	Limpet Rock	13	Vertical gill	91	7.15	0.51
07/05/2000	Cull Island	13	Vertical gill	67	2.35	0.25
07/05/2000	Cull Island	13	Vertical gill	68	2.46	0.22
07/05/2000	Cull Island	13	Vertical gill	71	2.88	0.21
07/05/2000	Cull Island	13	Vertical gill	68	2.62	0.24
07/05/2000	Cull Island	13	Vertical gill	69	2.85	0.21
07/05/2000	Cull Island	13	Vertical gill	64	2.12	0.33
07/05/2000	Cull Island	13	Vertical gill	63	2.16	0.30
07/05/2000	Cull Island	13	Horizontal gill	68	2.61	0.25
07/05/2000	Cull Island	13	Horizontal gill	71	3.14	0.25
07/05/2000	Cull Island	13	Horizontal gill	69	2.54	0.21
07/05/2000	Cull Island	13	Horizontal gill	66	2.44	0.21
07/05/2000	Cull Island	13	Horizontal gill	66	2.33	0.21
07/05/2000	Cull Island	13	Horizontal gill	70	2.68	0.27
07/05/2000	Cull Island	13	Horizontal gill	39	1.58	0.23
31/05/2000	Cull Island	5	Dab net	83	3.98	0.35
02/06/2000	Cull Island	19	Horizontal gill	95	7.48	0.47
02/06/2000	Cull Island	19	Horizontal gill	98	8.58	0.47
02/06/2000	Cull Island	19	Horizontal gill	98	8.44	0.56

Table 4. Results of ANCOVA of $\delta^{18}O$ for Sardinops sagax from five zones (Fremantle, Dunsborough, Albany, Bremer Bay and Esperance) in southern Western Australia.

Effect	df	SS	MS	F-value	Level of significance
Mean otolith weight	1	2.113	2.113	22.686	<0.001
Region	4	8.256	2.064	21.945	<0.001
Year	16	4.874	0.305	3.240	<0.001
Region x year	41	3.075	0.075	0.797	0.800
Error	162	15.237	0.094		
Total	224	47.573	0.212		

Table 5. Results of ANCOVA of $\delta^{13}C$ for Sardinops sagax from five zones (Fremantle, Dunsborough, Albany, Bremer Bay and Esperance) in southern Western Australia.

Effect	df	SS	MS	F-value	Level of significance
Mean otolith weight	1	0.166	0.166	2.331	0.129
Region	4	4.219	1.055	14.790	<0.001
Year	16	6.484	0.405	5.682	<0.001
Region x year	41	4.882	0.119	1.670	0.0133
Error	162	11.554	0.071		
Total	224	36.420	0.162		

Table 6. Results of ANCOVA of $\delta^{18}O$ for Sardinops sagax from three zones (Albany, Bremer Bay and Esperance) in southern Western Australia between 1988 and 1994.

Effect	df	SS	MS	F-value	Level of significance
Mean otolith weight	1	0.800	0.800	5.153	0.026
Region	2	0.120	0.060	0.387	0.680
Year	6	1.883	0.313	2.021	0.074
Region x year	12	0.831	0.069	0.446	0.938
Error	70	10.865	0.155		
Total	91	14.332	0.157		

Table 7. Results of ANCOVA of $\delta^{13}C$ for Sardinops sagax from three zones (Albany, Bremer Bay and Esperance) in southern Western Australia between 1988 and 1994.

Effect	df	SS	MS	F-value	Level of significance
Mean otolith weight	1	0.139	0.139	1.768	0.188
Region	2	0.030	0.015	0.195	0.823
Year	6	1.288	0.215	2.738	0.019
Region x year	12	0.507	0.042	0.539	0.882
Error	70	5.488	0.078		
Total	91	7.373	0.081		

Table 8. A comparison of the models suggested by Kalish (1991) and Edmonds and Fletcher (1997) that examine the relationship between $\delta^{18}O$ and water temperature.

$\delta^{18}O$	Temperature ^{o}C	
	Edmonds and Fletcher (1997)	Kalish (1991)
1.8	11.67	9.09
1.6	13.33	10.11
1.4	15.00	11.13
1.2	16.67	12.15
1.0	18.33	13.17
0.8	20.00	14.19
0.6	21.67	15.21
0.4	23.33	16.23
0.2	25.00	17.26
0.0	26.67	18.28
-0.2	28.33	19.30
-0.4	30.00	20.32
-0.6	31.67	21.34

9.0 Figures

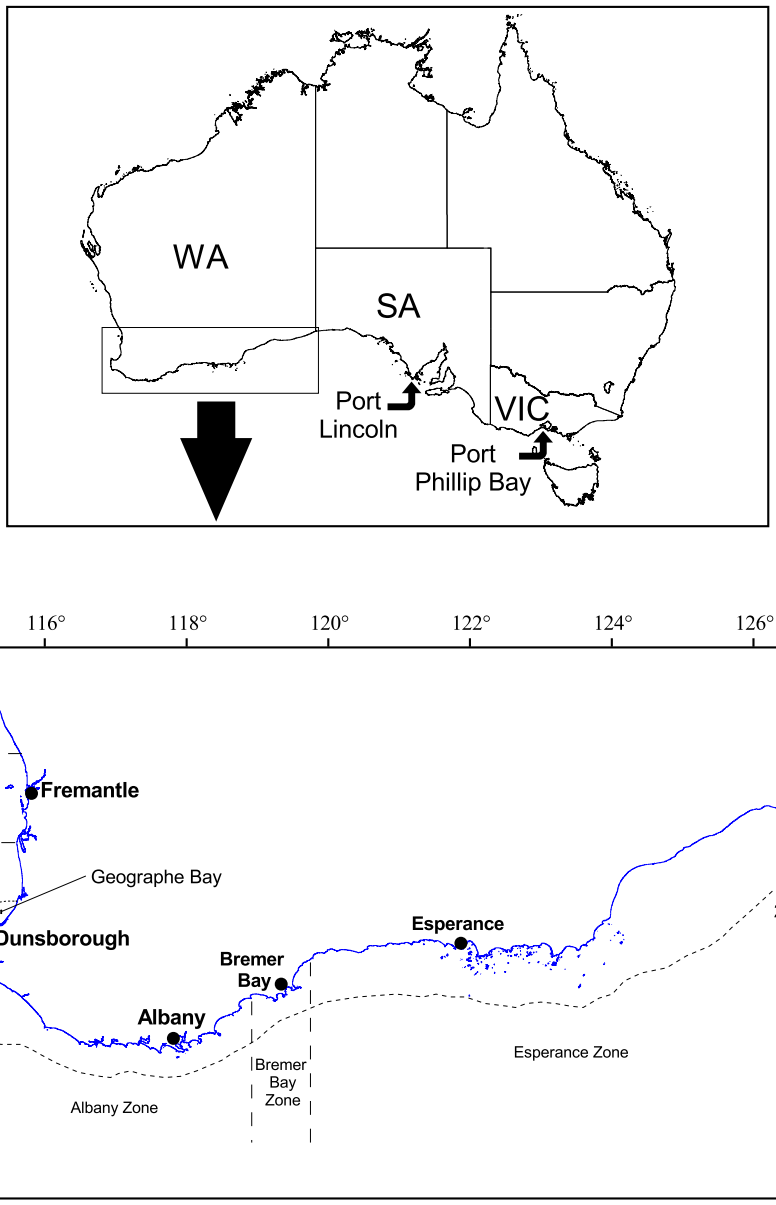


Figure 1. Map of Western Australia of the five zones that make up the fishery for *Sardinops sagax* in southern Western Australia.

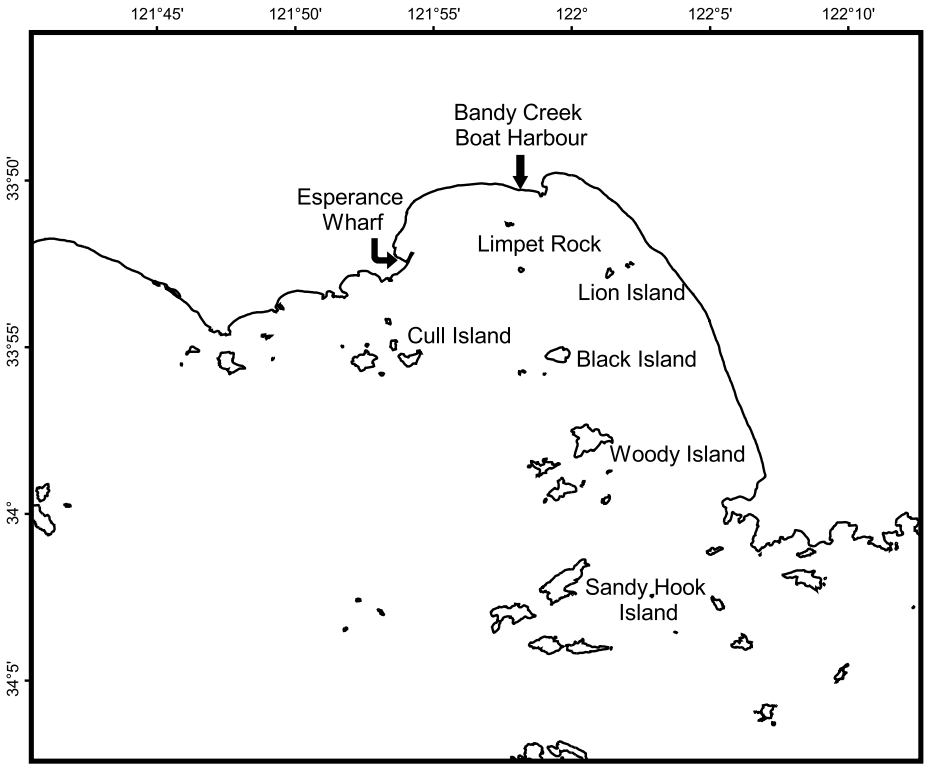


Figure 2. Map of the Esperance bay in southern Western Australia, showing the sites from which the light/gillnet sampling for juvenile *Sardinops sagax* was conducted.

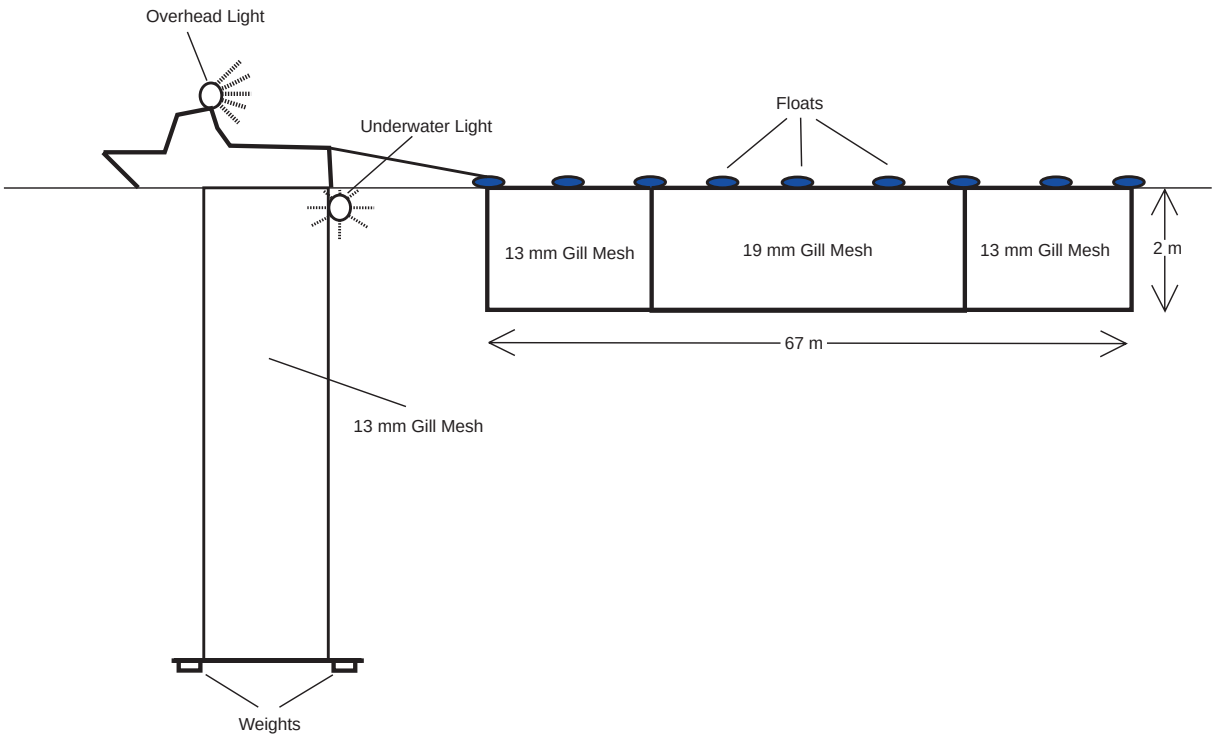


Figure 3. Diagram showing how the two types of gill nets were deployed for the capture juvenile *Sardinops sagax*; a 67 m long floating gill net with a 2 m drop, and a vertical gill net hanging from the gunwale of the vessel to the ocean floor.

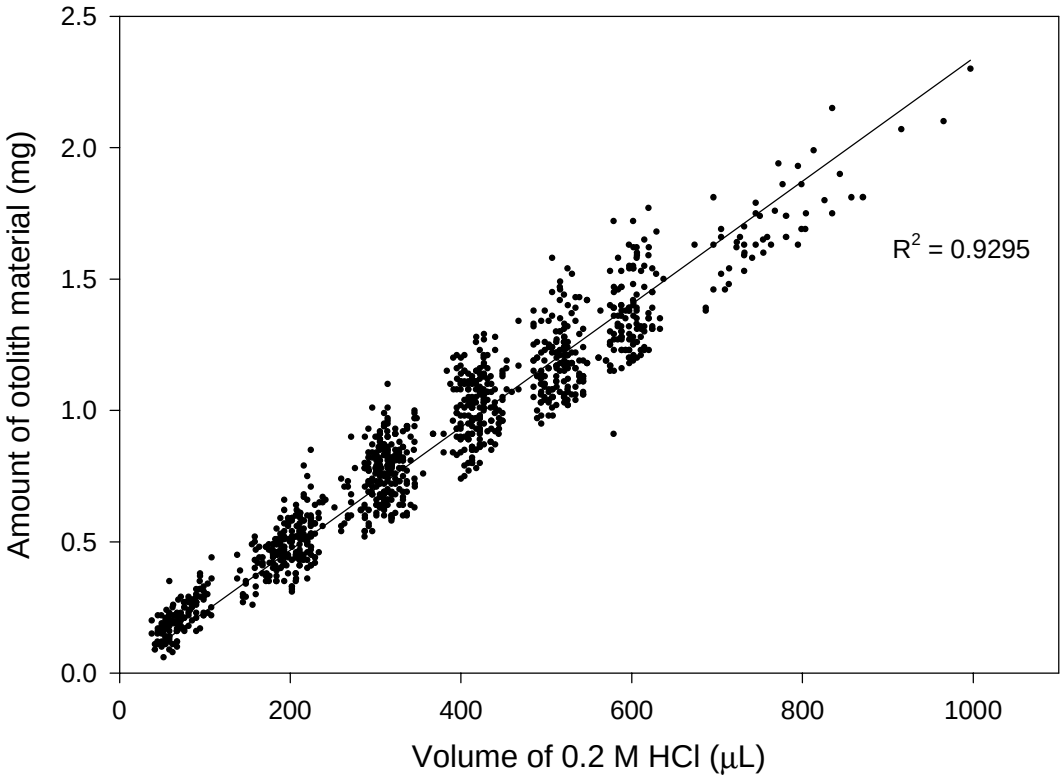


Figure 4. The amount of otolith material degraded in a 20 minute period by an increasing quantity of acid (0.2 M HCl).

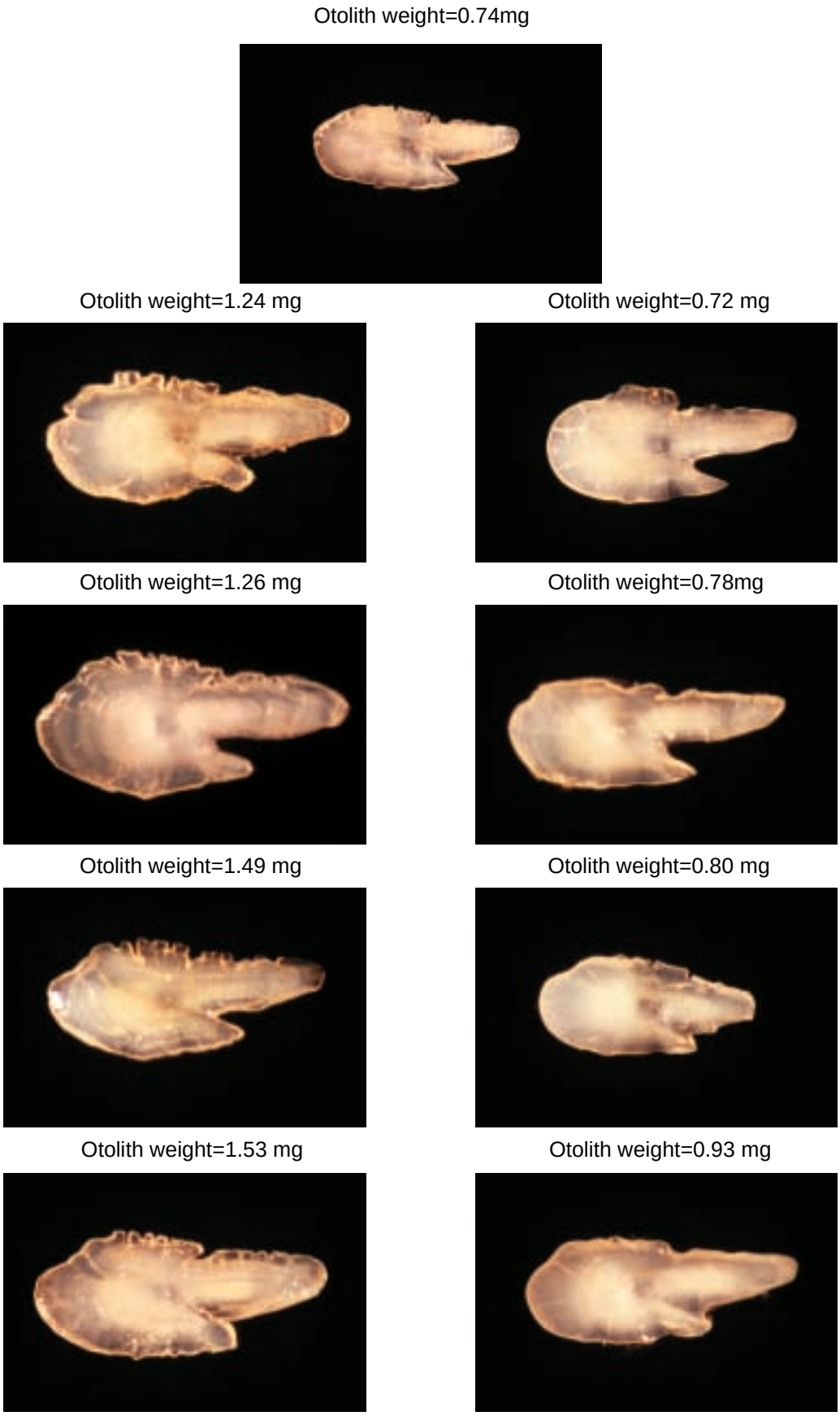


Figure 5. Showing whole *Sardinops sagax* otoliths from both 3 and 4 year old fish from Dunsborough on the left, and the same otolith degraded with HCl on the right. The top photograph in the middle is from a 1 year old pilchard (not degraded).

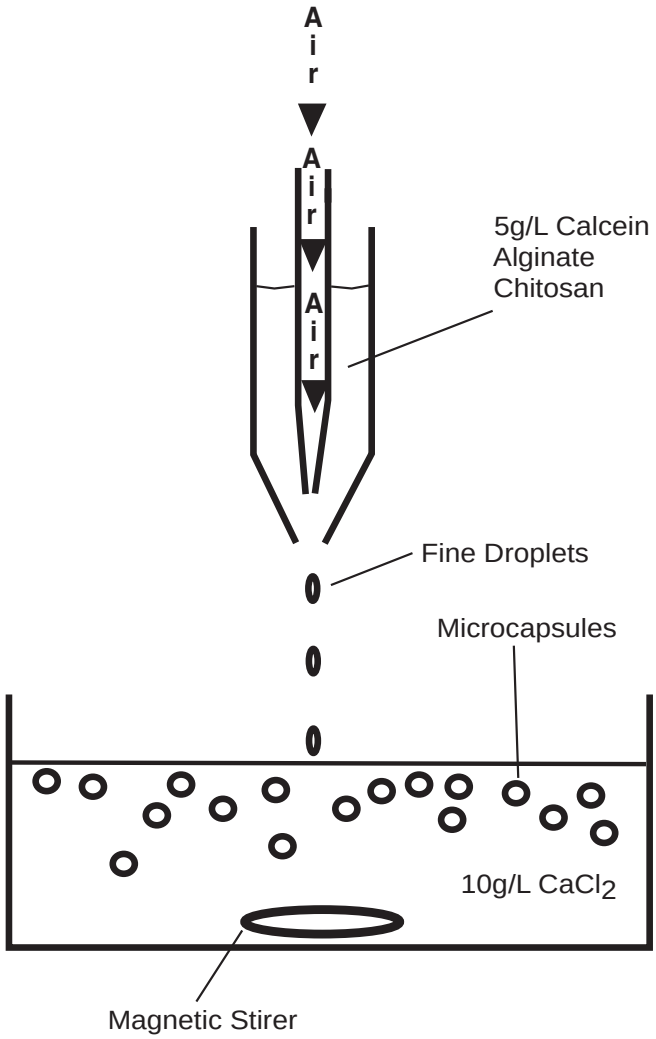


Figure 6. Shows the procedure and apparatus used in the preparation of calcein microcapsules that were mixed with food and fed to a number of *Sardinops sagax* held in a sea cage.

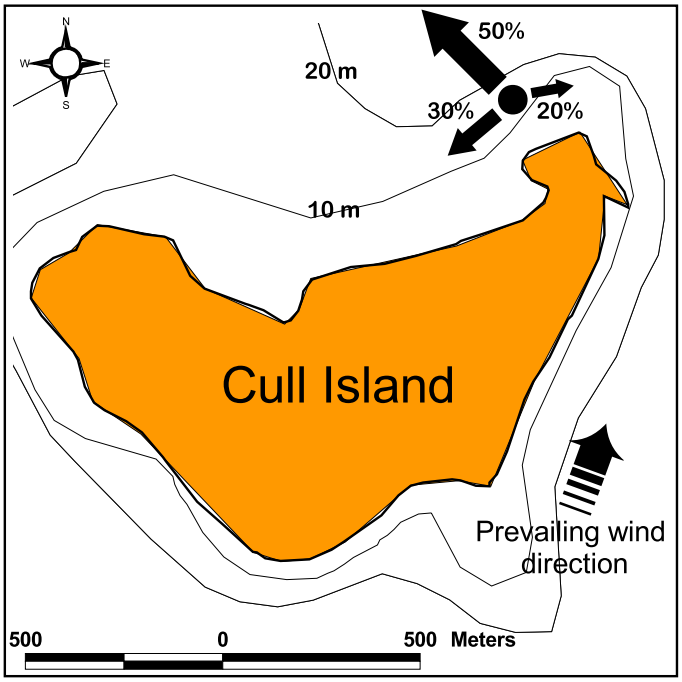


Figure 7. Cull Island, Esperance, showing the location of the anchoring position, the prevailing wind direction, the frequency of the three most common directions that the net hung from the anchored vessel and the bathymetry adjacent to the island.

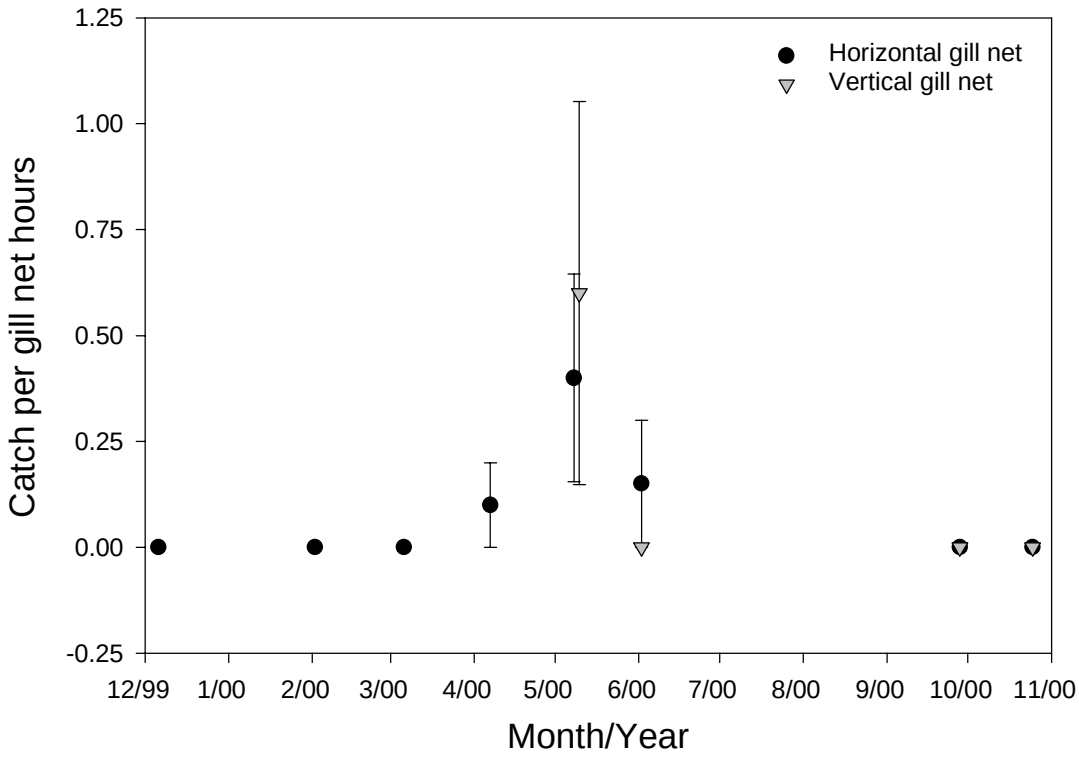


Figure 8. Shows the catch and effort (± se) for juvenile *Sardinops sagax* using both the horizontal and vertical gill nets during light fishing sessions at Cull Island, Esperance.

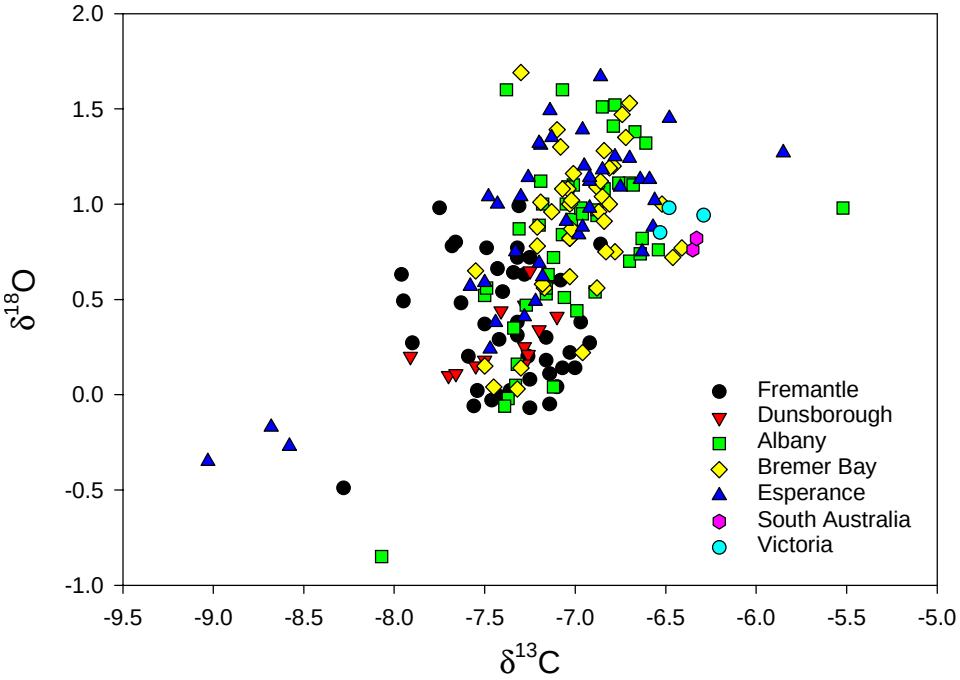


Figure 9. Isotopic ratios of oxygen and carbon for otolith samples of *Sardinops sagax* from Fremantle, Dunsborough, Albany, Bremer bay, Esperance, South Australia and Victoria.

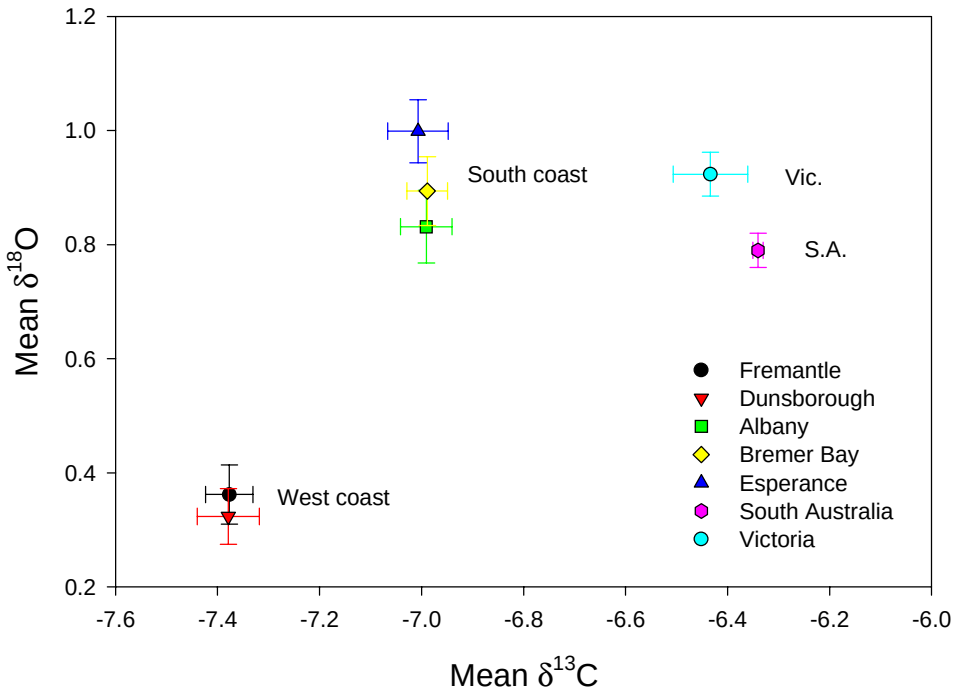


Figure 10. Mean isotopic ratios of oxygen and carbon for all locations (± SE).

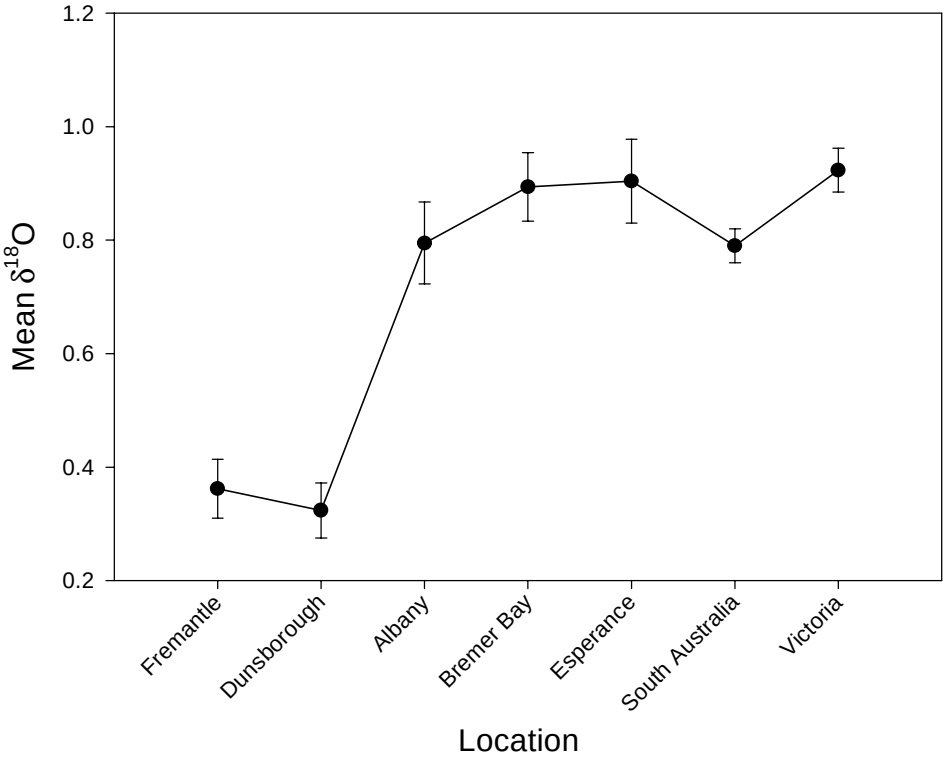


Figure 11. Mean $\delta^{18}O$ of otolith samples from *Sardinops sagax* (± SE) from Fremantle, Dunsborough, Albany, Bremer Bay, Esperance, South Australia and Victoria.

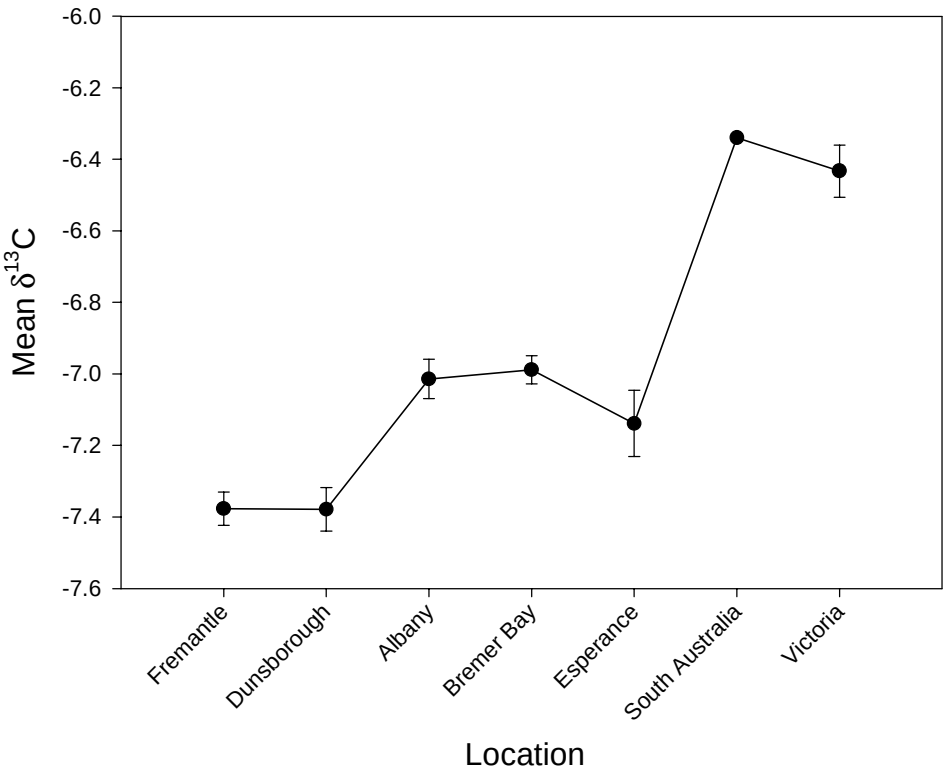


Figure 12. Mean $\delta^{13}C$ of otolith samples from *Sardinops sagax* (± SE), from Fremantle, Dunsborough, Albany, Bremer Bay, Esperance, South Australia and Victoria.

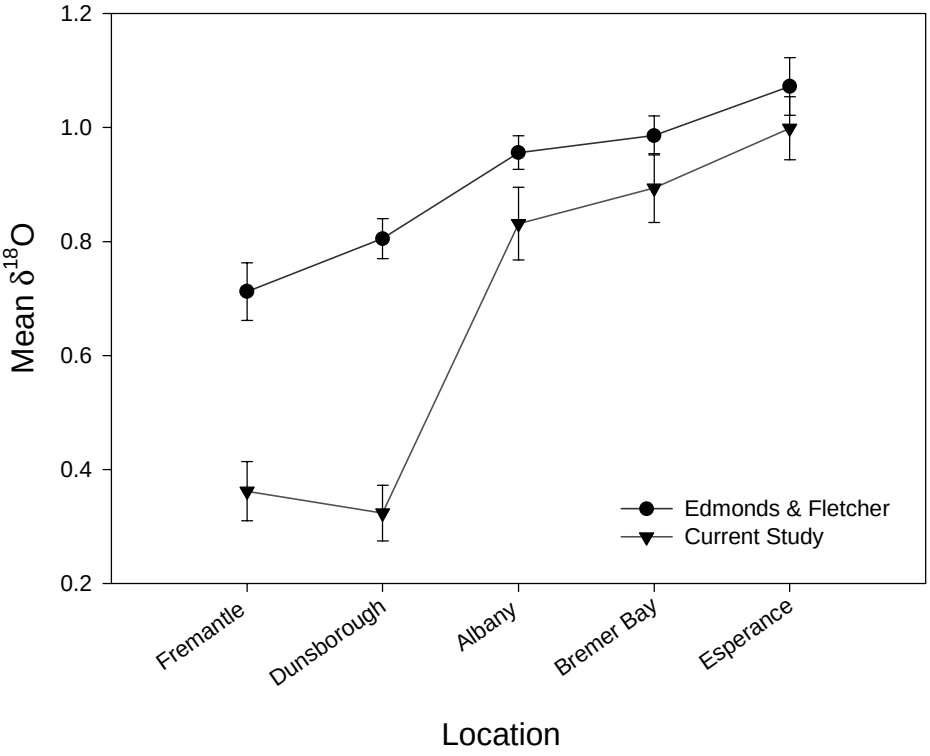


Figure 13. Comparison of the mean $\delta^{18}O$ values (± SE) from the current study (pre-1998) with that of Edmonds and Fletcher (1997).

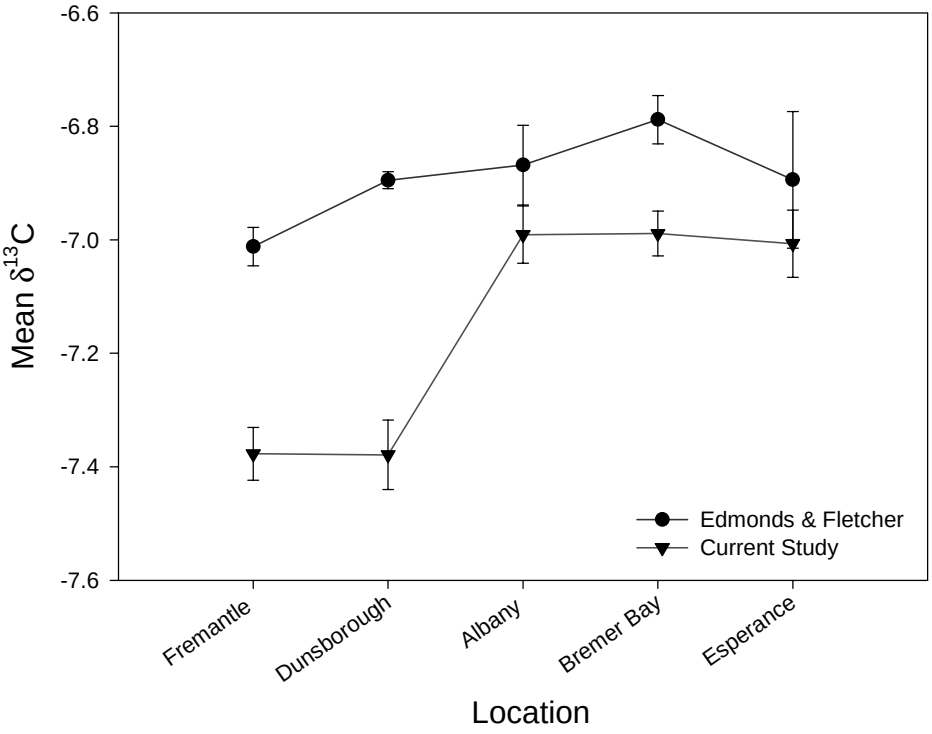


Figure 14. Comparison of the mean $\delta^{13}C$ values (± SE) from the current study (pre- 1998) with that of Edmonds and Fletcher (1997).

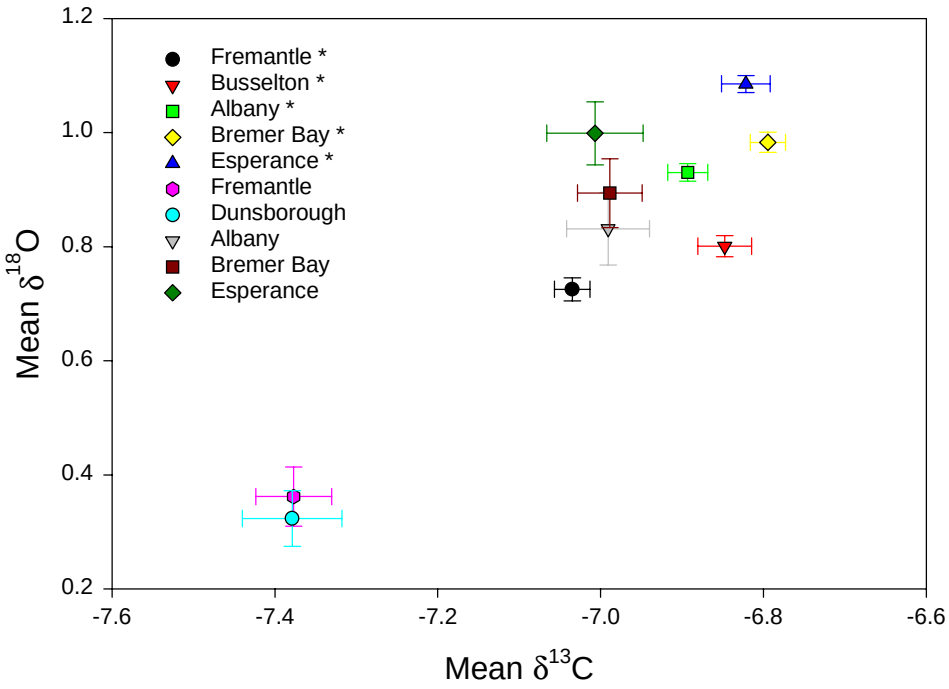


Figure 15. Comparison of the mean oxygen and carbon isotopes (± SE) from the current study with that of Edmonds and Fletcher (1997) (denoted by *). Note that Busselton from the Edmonds and Fletcher (1997) study is equivalent to Dunsborough in the current study.

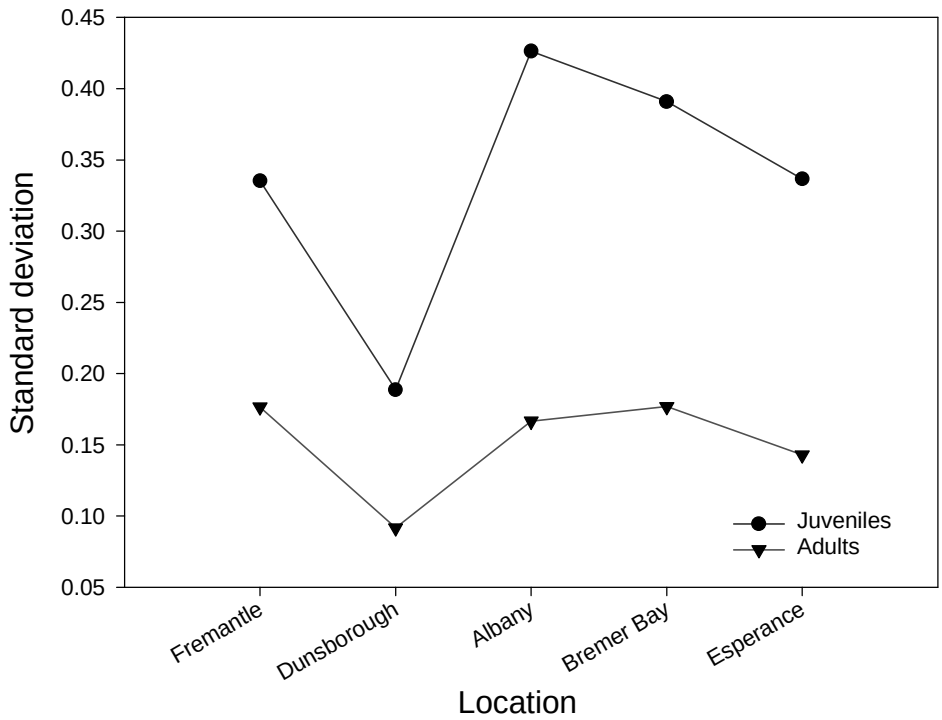


Figure 16. Comparison of the standard deviation of the mean $\delta^{18}O$ data for juvenile (current study) and adult (Edmonds and Fletcher 1997) *Sardinops* in WA.

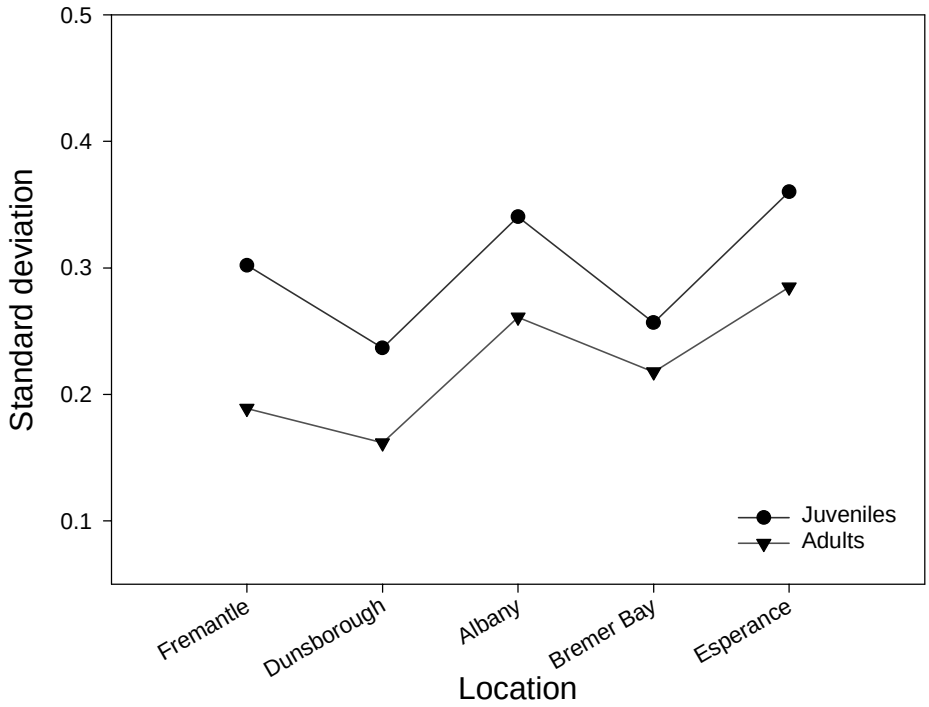
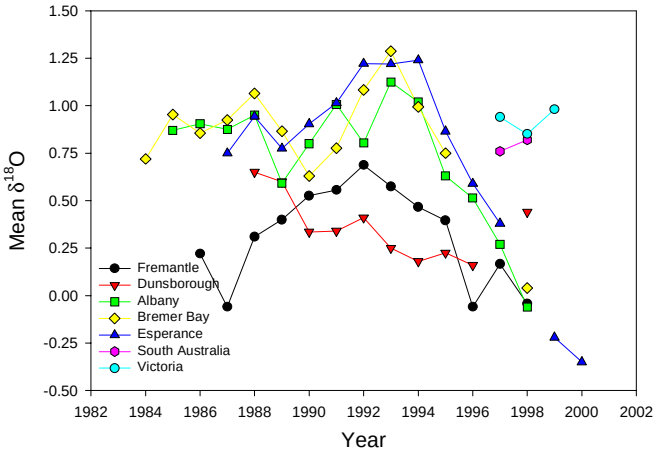
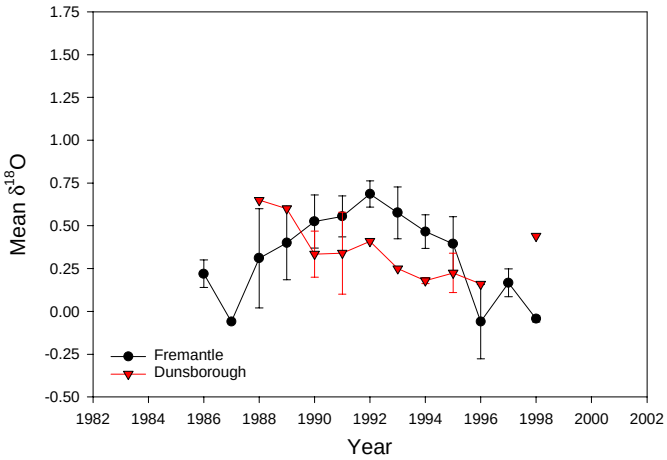


Figure 17. Comparison of the standard deviation of the mean $\delta^{13}C$ data for juvenile (current study) and adult (Edmonds and Fletcher 1997) *Sardinops* in WA.

(a) All locations



(b) West coast (±SE)



(c) South coast (± SE)

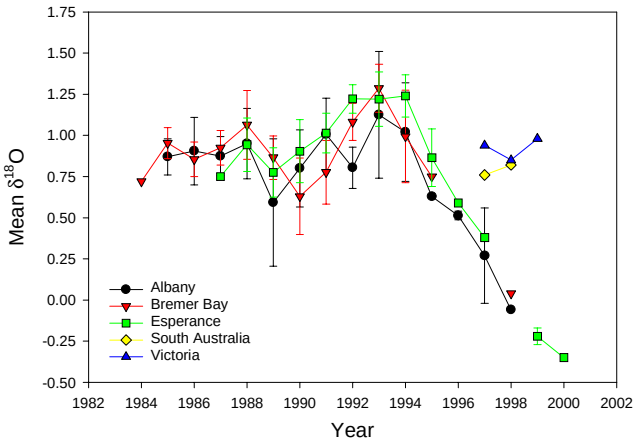


Figure 18. A comparison of the mean $\delta^{18}O$ values backdated to the year at age 1 for all locations (a), the west coast locations (b), and the south coast locations (c).

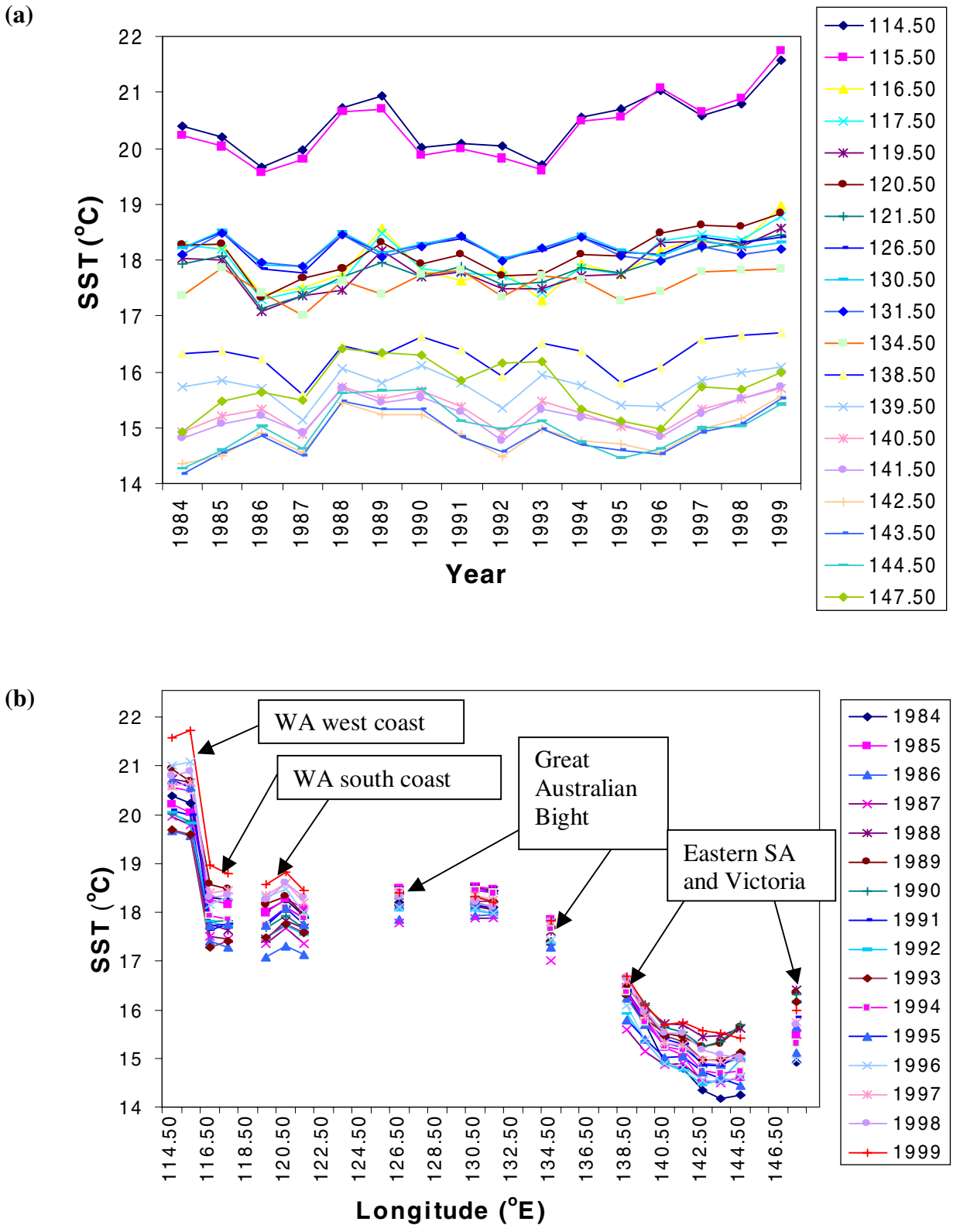
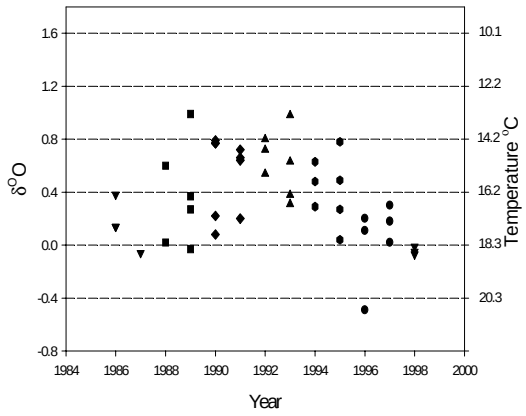
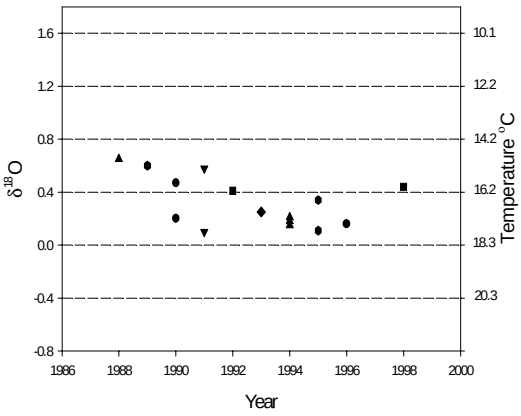


Figure 19. Mean annual sea surface temperatures for ~100 km x ~100 km blocks adjacent to the coast at locations between Lancelin (WA) and Melbourne (Vic.) from 1984 to 1999.

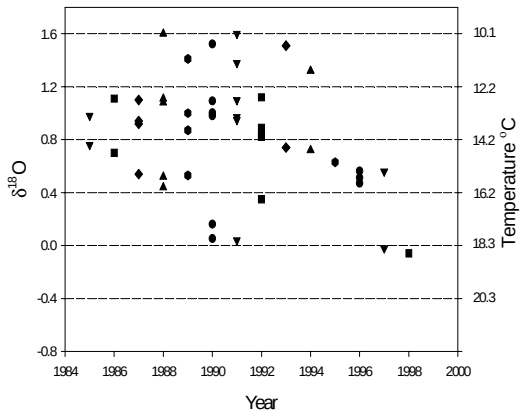
(a) Fremantle



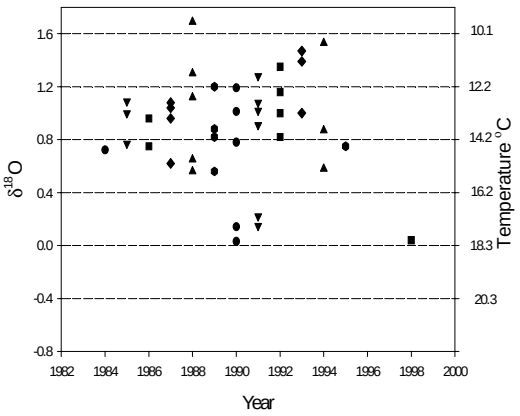
(b) Dunsborough



(c) Albany



(d) Bremer Bay



(e) Esperance

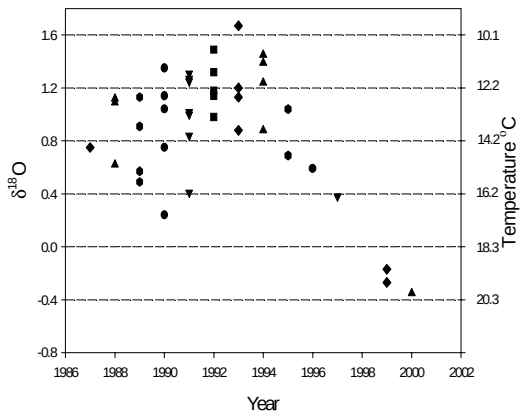


Figure 20. Shows the range of $\delta^{18}O$ values used to calculate the mean $\delta^{18}O$ (described in Figure 18), for Fremantle (a), Dunsborough (b), Albany (c) Bremer Bay (d) and Esperance (e).

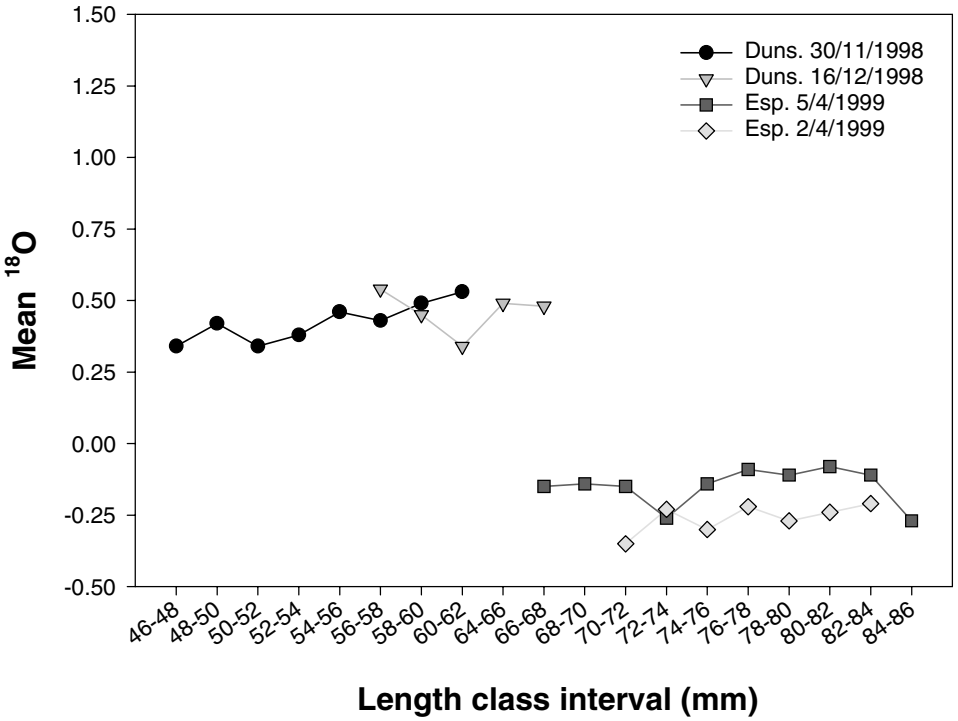


Figure 21. A comparison of two samples of juvenile *Sardinops sagax* from Dunsborough and Esperance showing the variation in ^{18}O for various length categories (LCF mm). Note that the scale for this figure is the same as Figure 18.

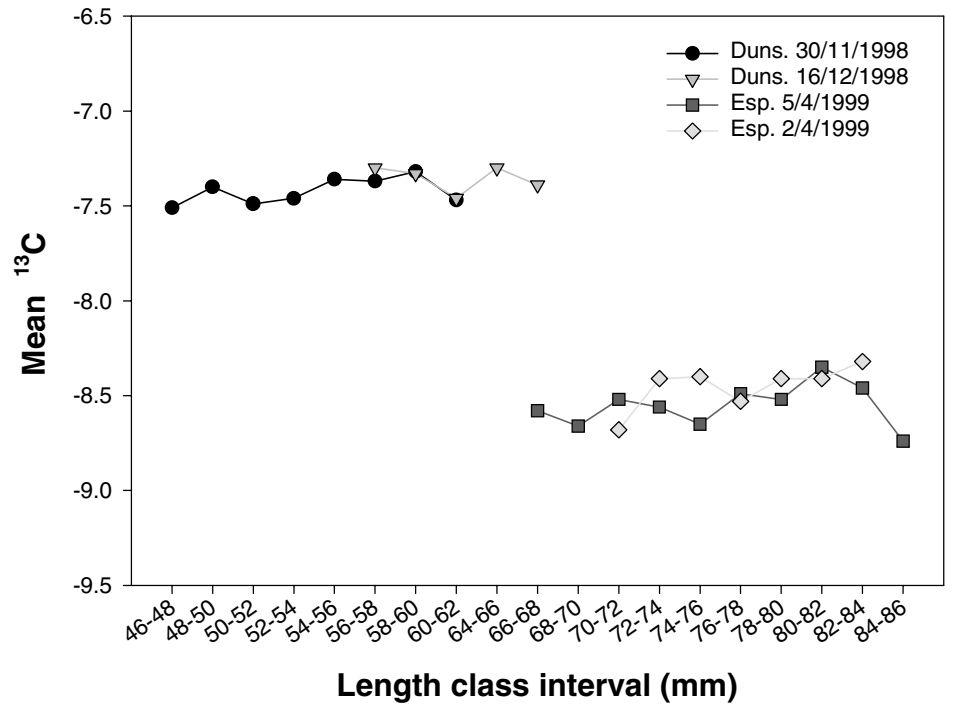


Figure 22. A comparison of two samples of juvenile *Sardinops sagax* from Dunsborough and Esperance showing the variation in ^{13}C for various length categories (LCF mm).

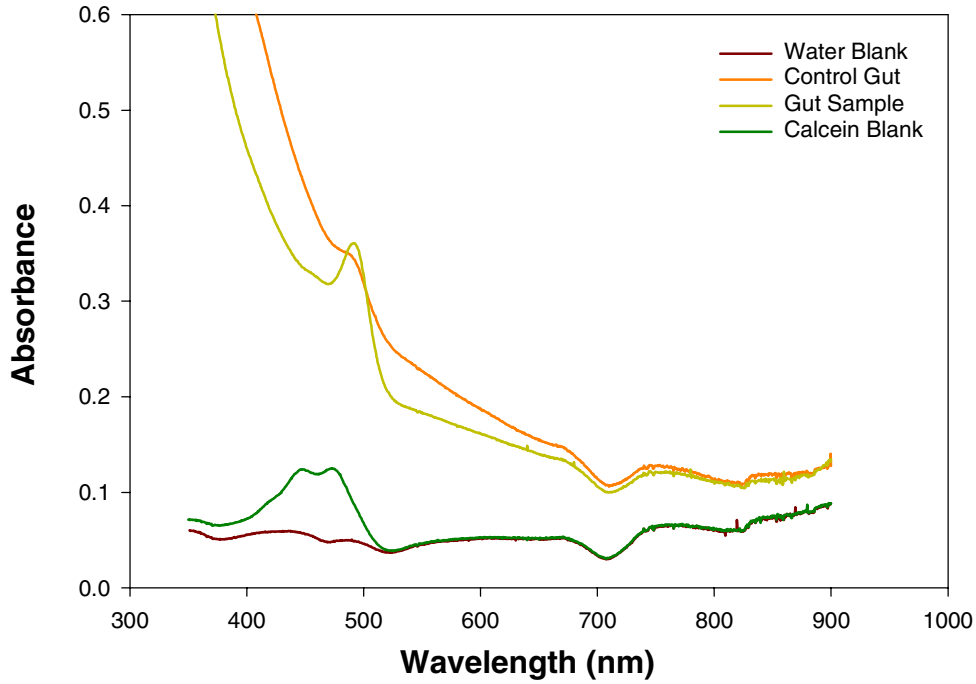


Figure 23. Spectrophotometric analysis of gut samples taken from *Sardinops sagax* to determine if calcein is active in the stomach after they were given calcein microcapsules in their food.

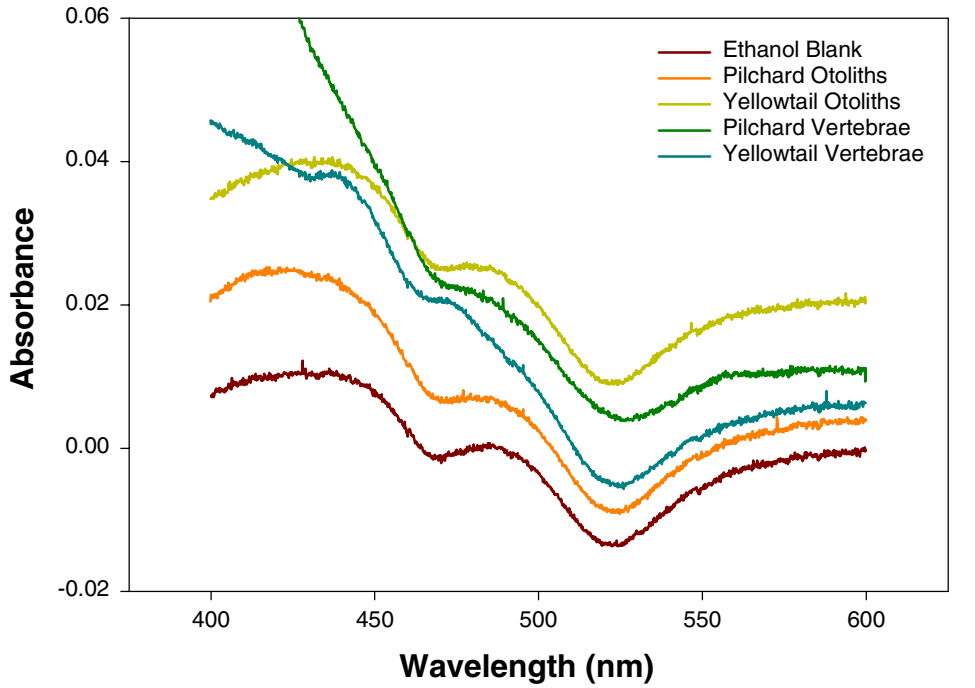


Figure 24. Spectrophotometric analysis of otoliths and vertebrae from *Sardinops sagax* and yellowtail scad that were fed microencapsulated calcein.

10.0 Appendicies

APPENDIX 1: Intellectual property

No saleable items were developed during this project.

APPENDIX 2: Staff

The Department of Fisheries staff who assisted in this project were :

Dr T. Bastow, Mr G. Baudains, Mr John Blaxell, Mr S. Blight, Ms M. Brasseur, Dr D. Gaughan, Mr K. Gittens, Dr L. Glendenning, Mr J. King, Mr T. Leary, Mr E. Loughton, Mr R. Mitchell and Ms S. Seidel.

The Department of Fisheries would also like to thank Marcus and Michelle Gray (and crew) from South East Fisheries in Esperance for their assistance in collecting juvenile pilchard samples, vessel use (Fire Bird and Jumbo II) and gear storage. We also like to thank Aldo Mendolia for the use of his vessel the Amanda Rosa, from which part of the Cull Island sampling was conducted. We thank Dr T. Bastow who was instrumental in suggesting and implementing the technique for the micro-encapsulation of calcein. Finally, the assistance of the Esperance Port Authority was crucial for undertaking the experiments with caged pilchards.