Investigation of the abundance and distribution of pilchard eggs and larvae off southern Western Australia (FIRDC Project 91/24).

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# Final FIRDC Report Proposal 91/24

Title

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Page

## Contents

Preface	3
Acknowledgements	4
Executive Summary	5
Introduction	7
Materials and Methods	9
Results	15
Discussion	27
Conclusions	34
Future Research	35
References	36
Tables 1-9	41
Figures 1-21	51
Appendix	73

## Preface

This document is the final report of research undertaken in FIRDC project 91/24. This project, which examined the distribution and abundance of pilchard eggs and larvae along the south coast of Western Australia, forms part of a larger study on the biology and stock assessment of pilchards. This study of pilchards has been in progress since August 1988 the majority of which has been funded by the Western Australian Government.

The knowledge gained in this preliminary study will be used to provide initial assessments of the spawning stock biomass and the degree of stock separation along the south coast of W.A. Such information, together with the data collected during the remainder of the pilchard programme is being used to provide recommendations for the management of this resource which in 1991 had an approximate value of \$5 million.

#### Acknowledgements

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This study would not have been possible without the assistance of the south coast fishermen, particularly Chris Unwin, David Palfrey, Marcus Grey and Robin Pike who provided their boats and experience at reduced costs to assist with this sampling. Thanks must also go to the local Fisheries Department inspectors who helped greatly with the plankton sampling both in Albany and on the west coast. Alan Pearce of the CSIRO Division of Oceanography kindly provided the NOAA sea surface images and helped with their interpretation. Finally the manuscript was aided by comments from staff of the Western Australian Marine Research Labs.

## FIRDC Report Sth Coast Plankton

#### **Executive Summary**

The fishery for pilchards off the south coast of Western Australia has recently expanded to 40 purse seine boats with an annual production approaching 10,000 tonnes. There have been some fears that this rate of exploitation is too high. A lack of knowledge on the distribution of the adult stock during the main spawning period and the number of spawning centres along the south coast has been a major obstacle in the use of a spatial model developed to help determine the stock size for this population.

In this study an intensive plankton sampling programme was undertaken on the continental shelf between Windy Harbour and Bremer Bay on the south coast of W.A. to examine the distribution of pilchard eggs and larvae. From this it was hoped that the parameter estimates for the spatial model could be refined, information could be obtained on the separation of stocks and preliminary estimates of spawning stock size could be calculated.

There were two centres of pilchard spawning, large numbers of day-one eggs were found immediately off the King George Sound area at Albany which was consistent with the low abundance scenario of the spatial model. A minor peak in spawning was also found off-shore of Bremer Bay with the total spawning area (area with day-one pilchard eggs) calculated to be 1500 nm<sup>2</sup>. These peaks in egg abundance coincided with concentrations of pelagic fish, presumed to be pilchards, seen on the echo sounders during sampling.

Later pilchard stages (day-two eggs, yolk-sac and post larvae) were found progressively to the east, with post-larvae mostly located off Bremer Bay. This

suggests that although spawning was inside of the main influence of the Leeuwin current, the easterly flow on the shelf was 0.5-0.9kts. Consequently, there is little possibility that the spawning populations at Albany and Bremer Bay could be genetically separated.

Concurrent adult sampling found that the mean size of mature pilchards was 163 mm L.C.F. with an average weight of 37.9 g. The batch fecundity of this size female was 11,800 and the spawning frequency was 0.1, indicating that females spawn at approximately 10 day intervals. Utilising these estimates and those calculated above for egg production, it was possible to calculate an estimate of the spawning biomass of this region using the egg production area method developed for anchovies and sardines of California. This technique suggested that the spawning biomass was 15,800 tonnes which is also consistent with both the low abundance scenario for the spatial model and also with the calculated rates of total mortality for the Albany population.

It appears that the pilchard stock off Albany is not large, supporting our recent management initiatives to reduce boat numbers and restrict catches using ITQ's. Nonetheless, there are a number of areas which need to be addressed. First, the distribution of eggs and larvae between Bremer Bay and Esperance needs to be determined to help understand the relationships amongst all these south coast areas before the fishery develops in Esperance. Second, sampling needs to be repeated in December when there is a second spawning period, a time when catches are traditionally poor. Third, these tows should be repeated over a number of years to investigate both the variable influence of the Leeuwin current on the pattern of egg abundance and also to allow for the variation in biomass estimates to be determined.

#### FIRDC Report Sth Coast Plankton

#### Introduction

The fishery for pilchards off the south coast of Western Australia has been expanding rapidly during the past 10 years and now forms the largest volume finfish fishery in the state. Prior to 1980, catches of pilchards along the south coast of W.A. were less than 1,000 tonnes per year. Subsequently, the catch has increased to levels approaching 10,000 tonnes with up to 40 purse seine boats licensed to fish in 6 locations. Such an increase in effort has been accompanied by fears that the rate of exploitation, especially in the Albany region, may be too high. Information on the number and size of pilchard stocks along this area is, therefore, required to determine if these fears are warranted and whether further expansion in other areas, such as the Esperance region, are justified.

As part of a major study on the pilchard fishery of Albany, a spatial model has been developed to simulate the behaviour of the fishery to provide estimates of the abundance of the stock (Fletcher, 1992). The lack of specific information on this stock has, however, precluded a definitive conclusion being made on the size of the stock. One important requirement is knowledge of the distribution of pilchards during winter when their catch rates have historically been high (Fletcher, 1991). One scenario compatible with historical data is that the increased winter catch rates are due to an intense aggregation of a relatively small stock off the Albany region. The alternative (also compatible with historical data) is that there is only slight aggregation and the stock is more extensive.

An additional consideration is the number of pilchard stocks along the south coast. The present structure of the model assumes a relatively isolated stock for Albany, situated between Windy Harbour and Dillon Bay (Fig. 1). It is obviously important to establish if this assumption is correct or if the Bremer Bay fishery and also the developing fishery in Esperance are harvesting the same stock as that caught off Albany.

In this study, it was proposed to conduct an intensive plankton sampling programme between Windy Hbr (Pt D' Entrecasteux) and Bremer Bay along with samples taken at other locations to :

(1) Examine the distribution of eggs and larvae of pilchards along the south coast of Western Australia.

(2) Use the results of the survey to refine and further validate the estimates of stock size derived from the spatial model.

(3) Obtain information to aid in the decision of whether to treat the pilchard stock off Albany as local and small or part of more extensive south coast population.
(4) Calculate preliminary estimates of spawning stock size utilising the egg production techniques developed for anchovy and sardine populations off California.

## FIRDC Report Sth Coast Plankton

Methods

#### Plankton Sampling Locations

The main plankton sampling locations along the south coast of Western Australia extended along the region between Bremer Bay (34.26<sup>o</sup> S 119.36<sup>o</sup> E) and Windy Harbour (34.43<sup>o</sup> S 115.51<sup>o</sup> E) which was divided into 11 regions of approximately 40 km length of coast (Fig. 1). Nine of these sampling areas (B - J) corresponded approximately to the nine spatial zones defined in the modelling exercise done on this population (Fletcher, 1992). In all of the 11 zones, there were two sampling sites at each of 4 and 6 nm from the coast, which were defined as being in the inner shelf (IS) region. There were also two locations in each zone that were 12nm from the coast, classed as being mid-shelf (SH), in six of the blocks there were two locations which were only 1-2 nm from the coast (OB), generally off-shore of embayments, whilst off Bremer Bay there were also two sites that were 16nm from the coast, near the edge of the shelf (OS) (Fig. 2).

Additional sampling locations were also planned for sites at Fremantle, Dunsborough, Augusta and Esperance (Fig 1), where samples were to be taken at 2, 4 and 6 miles from the coast.

#### Tow Methods

A number of boats (local pilchard or tuna fishing boats plus some smaller Departmental inspection and research boats) were used independently or in unison to complete these tows in the shortest possible time. For the main south coast sampling area, up to three boats were operating per day, one east of Albany, one west of Albany and a third operated in the area immediately off Albany repeatedly sampling the same locations. The majority of samples in this south coast region were done between the 25th and 29th of July 1991 with some preliminary tows done in the Albany region during early July. Plankton tows in Fremantle were done on the 5th of July, in Dunsborough on the 18th of July and on the 25th of August in Esperance. Adverse weather conditions resulted in the cancellation of the tows planned at Augusta.

The plankton tows were completed between 0500 and 2010 h during the day, with the majority done between 0800 and 1400 h. The sampling gear used two nets of 500*um* nylon mesh each with a mouth diameter of 60 cm which were combined to form a standard bongo arrangement (Smith & Richardson, 1977). These were hauled in a double oblique fashion; from the surface to a depth of at least 50m and back to the surface at an overall speed of the net of approximately 2 knots, which took an average of 7 minutes. Each net filtered on average 100 m<sup>3</sup> of water with the contents of the two nets combined for analyses.

A flow meter (Rigosha Company) was suspended in the mouth of one of the nets with readings taken at the end of each tow. A hard cod-end of 90mm PVC tube with two circular ports covered by 500 *um* mesh was used to collect the samples which were immediately preserved in 5% formalin solution buffered by borax. These were subsequently transferred to a 3% formalin solution within 2 weeks of collection. At each sampling location the surface water temperature was measured and a sample of water was collected for the later determination of salinity.

#### Acoustic Survey

During the plankton sampling cruises, notes were taken on the abundance and size of schools of fish that appeared on the echo sounders of the boats. The species identity of the schools, and their approximate size in tonnes were assessed by the skippers of the boats who were all experienced pilchard fishermen.

#### Adult Sampling

Concurrent with the south coast plankton sampling, samples of adult pilchards were obtained from the local purse seine fleet in Albany and Two Peoples Bay. A total of 7 samples each of 25 fish were obtained, which had been captured either in the evening or early in the morning. The fish were immediately measured and weighed with the gonads of the first 10 fish (both male and female) in each sample fixed in either 10% formalin or in Bouin's solution. These gonads were subsequently transferred to 70% alcohol and prepared for histological analysis. Additional female gonads, which were visually staged as ripe (stage 4 or late stage 3; see Table 1), were collected and kept in 10% formalin for the determination of their batch fecundity.

#### Plankton Sorting

The settled volume of the collected material being determined using a 1" measuring cylinder. Subsequently, all the eggs and larvae of pilchards plus all the other fish larvae were removed from the sample and stored separately. Samples where the numbers of pilchard eggs was estimated to be very large (> 1000), only a subsample of the material was sorted with the total numbers being scaled

upwards to represent the entire collected volume. The index of abundance for the samples was defined as being the amount found in  $200 \text{ m}^3$  total (100 m<sup>3</sup> filtered by each net).

The abundance of non-pilchard fish eggs and the numbers of all other species/groups of invertebrates were determined for a total of 12 samples (at least one from each block). At least 10ml of material (25 - 80% of total) from each of the randomly chosen sample from each block was sorted, this was sufficient to estimate the total numbers present in a sample (see Appendix).

## Pilchard Eggs

The stage of development of each pilchard egg removed was determined using the criteria developed by Baker (1972) and included the additional opaque stages (representing dead eggs) as identified by Fletcher & Tregonning (1992). Using the average speed of development for pilchard eggs at 18<sup>o</sup> C (from King, 1977), we assumed that all eggs less than stage 7 were spawned the previous night. Similarly all eggs greater than stage 9 were assumed to have been spawned two nights previously, those between 7 and 8 were classified as day one or day two eggs depending upon the time of capture. Consequently, separate calculations were made of the abundance of all pilchard eggs, pilchard eggs spawned one night previously and for those spawned two nights previously.

#### Larvae

The length of each pilchard larva collected in the samples was measured with the aid of a video camera attached to a binocular microscope. The image of each

#### Fletcher et al 1992 FIRDC Report Sth Coast Plankton

larva (which were mostly curled or bent in shape and therefore difficult to measure using an eyepiece micrometer) was measured on a television screen using a length of flexible plastic which was bent to fit the shape of the larva. The larval length to the nearest 0.5 mm was then determined using a conversion scale.

The larvae were separated into three classes based on their length using the results of studies by Baker (1972) and King (1977). Yolk-sac larvae were defined as being less than 6mm; early post-larvae were 6 - 10mm; and late stage post-larvae were defined as those larger than 10mm. Indices of abundance were then calculated for each of these 3 larval groups.

#### Histology

Small pieces of each of the female gonads collected for histological analysis were embedded in wax blocks, from which thin transverse sections were made. These sections were mounted on slides and stained using standard Hematoxylin and Eosin solutions. Each of the slides was examined for the presence of postovulatory follicles as shown for *Sardinops sagax* by Hunter & Macewicz (1985).

#### Fecundity

The batch fecundity of female fish collected was only determined for those with gonad stages of 4 or late stage 3 (see Table 1 for details). A standard gravimetric method of calculating fecundity was used (Le Clus, 1977; Hunter et al., 1985) whereby the gonads of each fish were weighed and two sub-sections from each ovary were removed and weighed. These four sub-sections, each of approximately 0.01 - 0.02 g, were teased apart on separate slides and the number

13

of mature oocytes (there are three easily identifiable stages of oocytes in ripe female pilchard gonads; Clark, 1932) were counted enabling the mean number of mature oocytes/g to be determined. The batch fecundity for each individual was calculated by the product of this mean and the total gonad weight.

## Statistical Analyses

Statistical examinations of the abundance of pilchard eggs collected in the plankton tows were made using analyses of variance whereby the factors were the 11 areas along the coast and the three regions within each area (OB, IS and SH). An analysis using only day-one eggs at the IS sites was done with the two eastern and two western IS sites in each area being treated as a nested factor. These analyses were performed using the SAS Proc GLM procedure (SAS Inst., 1987). Cochrans' test for homogeneity of the variances was performed on the variances with log transforms generally being applied to the data prior to analysis. Post analysis of means were accomplished using Duncans' multiple range tests.

Examinations of the relationships between each of the pilchard stages and the other faunal components of the plankton plus the physical properties of the sites were made using both a correlation matrix analysis and stepwise multiple regression techniques. These were accomplished using the SAS Proc Reg procedure (SAS Inst., 1987).

#### Results

#### Plankton Sampling

A total of 119 plankton samples were taken during the project of which 110 were done along the south coast sampling area. During the main period of sampling (25 - 29th July 1991), all stations in the south coast grid (except for those in area A) were sampled at least once, resulting in a total of 77 stations sampled. Most of the additional tows were in the King George Sound area, with stations G1 and G8 sampled on consecutive days from the 25th - 29th plus on some additional days prior to this period.

## Pilchard Eggs

#### Distribution

<u>All eggs</u>: Pilchard eggs were found in 48 of the 77 samples with abundance values for a number of samples being in excess of 1000 eggs with one sample exceeding 8000 (Fig. 3). The overall mean abundance of pilchard eggs was 319. The samples with the large number of eggs were mainly clustered around the Albany and Two Peoples Bay region. Consistent but smaller numbers of eggs were found for most samples east of Two Peoples Bay through to Bremer Bay. By contrast, west of Torbay very few pilchard eggs were found, with only one sample off Walpole having a significant number of eggs.

*Egg abundance and Water Temperature*: The abundances of eggs collected at different water temperatures are shown in Fig 4a. This shows that the majority of

eggs, and the majority of samples with eggs, were found at water temperatures between 17.5 and  $18.5^{\circ}$  C. The mean temperature for the location of the eggs, which was used in the calculation of development rates, was  $18.05^{\circ}$  C.

*Egg Staging*: The distribution of egg stages found in the samples formed a bimodal distribution (Fig. 4b), with a peak at egg stage 6 and a further peak at egg stages 11 & 12. This distribution supported the assumed cut-off points between day one and day two eggs using the development rate at 18.0<sup>o</sup> C.

Normal (not opaque) day-two eggs made up a much larger proportion of the eggs that were collected (57%) than did day-one eggs (24%). Opaque (dead) eggs made up a significant part of the samples comprising almost 20% of the total eggs captured. In contrast to the normal eggs, there were far more opaque eggs with stages less than 6 than those with older stages (Fig. 4b).

*Day-One Pilchard Eggs*: A total of 38 of the 77 samples contained day-one pilchard eggs, with an overall mean abundance of 138 (co-efficient of variation = 3.21). For this stage there appear to be two regions of higher abundance, one in the inner shelf region from Torbay to Two Peoples Bay, and a second peak covering a more widespread area off Dillon Bay and Bremer Bay (Fig. 5). The mean concentration for day-one eggs in each area is plotted in Fig. 6a, this also shows three separate zones, the area west of Torbay (area F) having basically no day-one eggs except for one site off Walpole (area D), there were, however, two peaks in abundance, one off area G (Albany) and another minor peak off area J (Dillon Bay).

Fletcher et al 1992 FIRDC Repo

A number of analyses of variance were done on the numbers of day one eggs. To homogenise variances, a logarithmic transform  $(\log_{10} + 1)$  were applied. A planned nested analysis of IS (inner shelf) sites indicated that there was no significant nesting effect (Table 2), therefore these sites were pooled for subsequent analyses. A two factor un-balanced analysis of variance showed that there were significant differences both among the areas along the coast and between regions within an area (Table 3). A post-hoc analysis of the means showed that there were three groups of areas, (J, K, I); (G, H, F, D) and (E, B, C). There was also a difference in the off-shore extent of pilchard spawning with the two inner sampling regions IS and OB (Outer Bay) having significantly greater abundances of eggs than did the off-shore sampling areas of the mid shelf (SH) and the two samples taken on the outer shelf region (OS) off-shore of Bremer Bay (Fig. 5).

*Day-Two Pilchard eggs*: There were 40 samples with day-two eggs with an overall mean abundance of 184 (coefficient of variation of 3.00). The distribution of day-two eggs also showed clumping, but this time the largest numbers of eggs were found off the Two Peoples Bay and Cheynes Beach areas (Fig. 7). Unlike day-one eggs, the mean concentration of this group had only a single peak in its distribution, off area H (Fig. 6b). An analysis of variance for this group also indicated significant differences among samples but there was a significant interaction between area and region (Table 4), so therefore no definitive statement can be made concerning the main effects.

17

#### Consistency of Sampling

A number of tests of the repeatability and reliability of this plankton sampling technique to measure the distribution and abundance of pilchard eggs were possible during this project. The sampling which was done at two locations over a number of days provided one measure of daily variations in egg production at one area over a large number of days (Table 5). The mean number of all pilchard eggs found outside of King George Sound (Sites G1 & G8) varied between 30 and 619 during the 5 day period of sampling and from 5 to 1000 over all the samples taken during July. When only day-one eggs are used, the variation was reduced markedly to between 6 and 70 during the main sampling period (Table 5a). Variation in the mean number of eggs at all sites sampled on the three days when all three boats were operating showed even less variation. The overall mean abundance of pilchard eggs varied between 570 and 675, whereas for just day-one eggs, the variation was between 86 and 252 (Table 5a).

A further test was the examination of the variation at the 8 sites which were sampled twice (Table 5b). For both all eggs and day-one eggs, the values at the stations were similar with no significant difference found between days ( $t_{pair} = 1.13$ , P > 0.05, n = 8). The only large differences were between G7 for day-one eggs and H2 for all eggs. Thus, there was a reasonable level of consistency in the abundance of eggs at a site on different sampling days.

## Spawning Area and Rate of Egg Production

The total spawning area of pilchards, defined as the area in which day-one pilchard eggs were found, was calculated by integrating the areas between

## Fletcher et al 1992 FIRDC Report Sth Coast Plankton

adjacent tows containing eggs. Thus, the area within which just day-one eggs were found was 1,500 nm<sup>2</sup>. The daily production of eggs per  $0.05m^2$  of seasurface was calculated using day-one eggs with no allowance made for egg mortalities. This was done by assuming that the nets only sampled the region between 50 m and the surface in a uniform manner. Given that the abundance index was the number of eggs per 200 m<sup>3</sup> this relates to a sea surface area sampled of approximately 4 m<sup>2</sup>. Therefore, to obtain the rate of production per 0.05 m<sup>2</sup>, which is used in egg production calculations (Lasker, 1985; Wolf & Smith, 1985), the overall abundance index for day-one eggs at positive stations (270) was divided by 80. This resulted in an estimated rate of egg production of 3.4 egg/0.05m<sup>2</sup>/day.

#### Pilchard Larvae

Pilchard larvae were found in most of the plankton samples taken during the study (63 of 77) with an overall mean abundance of 80. The distribution of the larvae was found to be more easterly than the locations where the eggs were found, with large numbers not encountered until sites east of Cheynes Beach (Fig 8).

<u>Yolk-sac larvae</u>: These larvae, which represent the stage approximately 3-4 days after spawning, were only present in 33 samples with a mean of 46 (coefficient of variation = 2.86). They were distributed from Cheynes Beach to Bremer Bay (Fig. 9) with the maximum abundance off area I (Fig. 6c).

*Early post-larvae*: This larval group, which are between 5 - 7 days post-spawning, were found in 55 samples, generally at low numbers with an overall mean abundance of 33 (co-efficient of variation = 2.80; Fig. 10). Samples with large

numbers of these larvae were mostly in the Bremer Bay region with the peak in abundance being area K (Fig. 6d).

Late stage post-larvae (greater than 1 week old) were found in very low numbers (mean = 1) except for one sample in Bremer Bay itself (Fig. 11). There was no consistent pattern or peak in abundance of this group, at least within the area sampled during this study.

## Influence of the Leeuwin Current

A NOAA AVHRR thermal image taken of the south coast on the 28th of July 1991, during the middle of the plankton tow sampling period, clearly shows the well defined path of the Leeuwin current (Fig. 12a). The warmest water (19-20<sup>o</sup> C), and presumably the strongest part of the current, was flowing close to the coast between Cliffy Head and Denmark. East of Denmark, the current moved off-shore to the edge of the continental shelf in the Albany region and it was located off the edge of the shelf past Cape Riche (Fig. 12b). Thus, there was a band of cooler water (17 - 18.5<sup>o</sup> C) along the shelf area from Torbay to past Bremer Bay. This pattern was confirmed from temperature measurements taken during the tows (Fig. 13).

The distribution of pilchard eggs appears to be closely related to this current pattern, especially with the band of cooler water found inside the main influence of the Leeuwin current (Fig. 12a). The plankton samples with large numbers of eggs were located near the boundary between the warm water and the cooler water, particularly in the region between Torbay and Cape Riche. This pattern produced a negative relationship between sea-surface temperature and the abundance of pilchard eggs (r = -0.31, n = 77, P < 0.05). This suggests that spawning was generally inshore of the main influence of the Leeuwin current.

Despite the location of spawning, the longshore variation in the peak abundance of the various stages of pilchard eggs and larvae suggests that there was still some influence of an easterly current in the inner shelf regions (Fig. 6). Thus, the shift in peaks shows that there was movement of the stages of between 30-40km a day, until at least early post-larvae, this corresponds to a current speed of 0.3 - 0.46m/sec.

## Distribution and Influence of other Plankton Species

*Plankton Composition*: Within the samples the majority of organisms were copepods, decapods, chaetognaths, fish eggs and Tunicates (*Salpa* and *Oikopluera*). At smaller densities were amphipods, molluscs, ostracods, polycheates and there were also a small number of rock lobster phyllosoma larvae.

*Plankton Volume*: The settled volume of plankton material collected in the tows did not vary greatly among areas (Fig. 14). For most tows, the amount collected was in the range of 10 - 30 ml, with only one tow from area B having greater than 100 mls (Fig. 14).

*Other Fish Larvae*: The distribution of non-pilchard fish larvae was more even than that for pilchards with reasonable numbers of larvae found in the samples west of Albany (Fig. 15). East of Albany, however, the distribution was similar

#### Fletcher et al 1992 FIRDC Report Sth Coast Plankton

to that of pilchards, with the majority of larvae found in the Bremer Bay region and few found between Torbay and Cape Riche.

22

*Invertebrates*: The abundance of various groups of invertebrates were measured in 12 samples covering each of the 10 blocks sampled. There were marked differences in the abundances of the various groups among samples (Fig. 16a). These differences related to both longitudinal variations, such as for the larvacean *Oikopluera*, which increased with samples taken in the eastern areas, while the decapod crustacean *Lucifer* declined in the east (Fig. 16a). Similarly, the abundance of some species were negatively correlated with temperature (e.g. crab larvae). Other groups showed variations which were not uniformly related to any of the measured physical conditions but may have been influenced by other species. Thus, a number of significant correlations were found amongst the groups (Table 6). The strongest being the positive correlations between copepods, amphipods, crab larvae and *Lucifer* which appear to form a species complex. There was also a positive correlation between salps and cheatognaths but a strong negative correlation between chaetognaths and other non-pilchard fish eggs.

With respect to the various pilchard stages in the samples, stepwise multiple regressions showed that the abundance of day-one eggs were positively correlated with the abundance of copepods and amphipods whilst negatively correlated with chaetognaths (Table 7). Day two pilchard eggs were negatively correlated with Chaetognaths (Table 7), whilst yolk-sac larvae were negatively correlated with salps and copepods (Fig. 16b). Nonetheless, there were simple negative correlations between pilchard yolk-sac larval stages and the abundance of chaetognaths (Table 6). Moreover, we found one chaetognath with a larval

pilchard in its stomach, indicating that this group may potentially have some influence on the numbers of pilchard larvae.

## Acoustic Survey

The relative abundance of pilchards was assessed by summing the estimated tonnages of the acoustic marks, that were assumed to be pilchards by the skippers of the boats, found whilst steaming between plankton sites (Fig. 17). The criteria for the skippers classing the marks as pilchards appeared to be due to the position of the school in the water column, the shape of the school and the intensity of echos. These area the traits that they use during normal fishing operations to distinguish among the species of pelagic fish found on the south coast (other species are blue mackerel and jack mackerel/scad). Assuming their identifications were correct, this indicated that there were three areas where pilchards were abundant with large numbers of soundings seen off Walpole, Albany and Bremer Bay.

#### Plankton Sampling in Other Locations

The results of plankton tows done at the other sampling locations around Western Australia are located in Table 8. Pilchard eggs were only found at Esperance with none found at either Dunsborough or Fremantle. A small number of pilchard larvae were found at Esperance but a reasonable number were found at Dunsborough.

## Adult Pilchard Sampling

A total of 7 samples each of approximately 25 fish (184 in total) were obtained during the five day period 24 - 29th of July. Five were obtained from boats which captured the schools in the morning (approximately 0900hr), the other 2 catches were made in the evening at about 1800hr. The sizes of fish collected were unimodal (Fig. 18a) with the mean size of each of the samples only varying between 160 - 164 mm. The overall mean length of the fish was 162mm which is close to the mean value for the entire 1991 year (> 4000 fish) of 163mm. The somatic indices of the fish (somatic weight/length<sup>3</sup>) were relatively low with a mean of 8.35 compared to the maximum values obtained during late summer of > 10.0 which explains the relatively low mean individual weight of 37.7g as compared to the yearly average of 43.9g.

The female gonad stages, as assessed visually, were distributed between stage 3 and 4 gonads, indicating developing and ripe gonads, and stages 6 & 9 which represent those which appear to be partially spent or fully spent/recovering (Fig. 18b; based on the stages developed for *S*. *sagax ocellatus* by King, 1956). Males were either classes as being in a developing/ripe stage or were in the partially spent stage. The sex ratio of the samples collected during the study was 0.62 females, which compares to the overall sex ratio of 0.59 for all of 1991 (> 4000 fish).

#### Batch Fecundity

The batch fecundity of 25 female pilchards over a size range between 148 and 175mm L.C.F. (the total range available with late stage 3 or stage 4 gonads) was

determined for the late July period corresponding to the times of the plankton sampling (Fig. 19). The minimum estimate of fecundity was 6,300 oocytes and the maximum during this period was 15,100. Thus, the batch fecundity for the average individual of 162 mm during late July was 11,360 which is lower than was found earlier in the month of July, at 12,860, and even lower than the 16,010 for pilchards caught during June (Fig. 19).

#### Histology

A total of 50 female gonads were prepared for histological examination. Of these, only 5 showed signs of having post-ovulatory follicles (Fig. 20). This makes a spawning frequency of 0.1 suggesting that each female individual spawns, on average, every 10 days during this period.

## Spawning Biomass

Given all the data collected in this study it is possible to construct an estimate of the spawning biomass of pilchards using a variation of the egg production method (Parker, 1980; Lasker, 1985), the related egg production area method (EPAM; Wolf & Smith, 1985). This latter technique estimates the spawning area over which a specified spawning biomass would be assumed to occur. It has advantages for our purposes because it allows the use of parameter estimates already determined for the Californian sardine to be used as a baseline. This is useful because Wolf & Smith (1985) have showed that oblique tows, such as that used in this study, have a tendency to over-estimate the amount of surface area sampled resulting in lower estimates of daily egg-production. Furthermore, given

## Fletcher et al 1992 FIRDC Report Sth Coast Plankton

that we have no estimate of the rate of egg mortality our estimates of egg production and, therefore, the spawning biomass will be a minimum value.

The best parameter estimates for the Californian sardine and those from the study off the south coast of W.A. along with the EPAM equation are located in Table 9. The major differences between the parameters between the two regions were the average weight of individuals (W) and the batch fecundity (F) but these two tend to cancel one another out. Using these variations plus the slight variations in egg production ( $P_o$ ), spawning frequency (S) and the measured spawning area of 1500 nm<sup>2</sup>, the spawning biomass for the south coast of W.A. was calculated to be 17,400 short tons, which equates to 15,900 tonnes.

#### Discussion

The results of this sampling programme have been successful in helping to answer the main objectives of the study concerning the longshore distribution of pilchards along the Albany region, the degree of overlap of the populations along the south and west coasts and providing an estimate of the spawning stock size of pilchards along the south coast. These were the criteria listed in the proposal for judging the success of the project.

Numerous pilchard eggs were found along the south coast of W.A. during this study, further confirming July as a time of major spawning activity of pilchards in this region (see also Fletcher & Tregonning, 1992). The distribution of these eggs, however, was not uniform over the entire sampled area with large differences found in their abundance among the regions. Thus, there appeared to be two centres of spawning along the main south coast sampling area; one was located off-shore of Albany, and there was also a smaller peak in the region off Bremer Bay. This is consistent with the pattern found for acoustic marks during the study which also found concentrations off Albany and Bremer Bay, and a third concentration off Walpole (which was the only place on this area of the coast which had any pilchard eggs). This, therefore, supports the assumption that the density of eggs is related to the density of adult pilchards. The distribution of pilchards in the south coast region was closest to the model scenario in which there is a relatively small population which clumps together off Albany during July (cf Fig. 21 with Fig. 6).

The two centres of pilchard spawning found during this study are, significantly, the two areas where the major pilchard fisheries are located in this region (Fletcher, 1991). These two areas (and the third area off Walpole) are also notable in that major estuaries flow into the ocean at these locations. Chesney & Alonso-Noval (1989) found that the eggs of *Sardina pilchardus* off N.W. Spain were most abundant in regions were higher nutrients tended to occur. Similarly, Palomera (1992) found that anchovy spawning was also centred around two major rivers flowing into the Mediterranean. She postulated that these provided an enhanced environment for the larvae due to the nutrient enrichment they provided. The presence of such outflows on the south coast of W.A. could also be a possible source of nutrients to this region which are generally lacking due to the influence of the Leeuwin current (Pearce, 1991). But in this instance it may be more related to providing food for the adults rather than the larvae.

The positioning of the Leeuwin current appeared to have a direct affect on the location of pilchard spawning. Thus, in the region where its influence was strongest and it was situated close to the coast, virtually no pilchard eggs were found. Instead, the majority of pilchard eggs were found in the cooler band of coastal water in the region near Albany where the strongest part of the current had moved off-shore to the edge of the continental shelf. Thus, most of the eggs were found in water that was 18° C, whereas the Leeuwin current water was 19.5° C and above. Interestingly, King (1977) found that 18° C was the optimum temperature for the development of pilchard eggs, nonetheless, there were large numbers of dead eggs found in the tows. This has been a consistent phenomenon in tows done during July off Albany, whereas in December the percentage of dead eggs is lower (Fletcher & Tregonning, 1992). It has not been determined conclusively whether the eggs are dead before or after capture (Moser et al., 1985) from which conclusions concerning overall egg survival could be made.

#### Fletcher et al 1992 FIRDC Report Sth Coast Plankton

Despite the evidence that the main current was located near the edge of the shelf at Albany, and beyond the shelf at Bremer Bay, pilchard eggs were still mostly located in the inner shelf region (up to 6 nm from the coast). Few eggs were found in the mid-shelf regions, particularly near Albany and whilst there were some in the mid-shelf region past Cape Riche, there were no pilchard eggs at the edge of shelf near Bremer Bay. The pattern of pilchard eggs being found predominantly close to the coast is identical to that found in the previous study of pilchard spawning off Albany (Fletcher & Tregonning, 1992). It is also consistent with the distribution of pilchard eggs for other populations of *Sardinops*, particularly when they are in small biomass phases (Lluch-Belda et al., 1989; Kuroda, 1991). It is possible that the off-shore extent of spawning could be used as a rough index of abundance, with the larger this extension, the bigger the biomass.

The abundance of the pilchard eggs also appeared to be related to the presence of other components of the plankton. Thus, the abundance of day-one eggs was positively correlated with the abundance of copepods and amphipods. It is possible that the abundance of these small crustaceans, which are major prey items of adult pilchards (King & MacCloud, 1977) could influence the adults and, therefore, the location of spawning. The other major relationships were negative correlations for day-two eggs and larval pilchards with the abundance of cheatognaths and salps. The possible influence of salps, which are filter feeders of phytoplankton and the smaller stages of copepods (Zyl van, 1959), remains unknown but is likely to be minimal. The predatory nature of chaetognaths, combined with the evidence that a larval pilchard had been eaten by a chaetognath suggests, however, that they may a major influence on the local abundance and survival of larval pilchards. In fact, Butler (1991) suggested that the zooplankton

29

## Fletcher et al 1992FIRDC Report Sth Coast Plankton30

component of ichthyoplankton tows (excluding the gelatinous components) should be assumed to measure the level of potential predators not larval food. To measure this, 75 *u*m mesh nets must be used.

The reliability of the patterns of abundance of pilchard eggs found in this study was enhanced by the use of several boats. This meant that the time taken to complete sampling was greatly reduced providing a "snap-shot" picture of egg abundance. Moreover, using multiple boats enabled sites which were quite distant to each other to be sampled on the same day, thereby significantly reducing the risk that any longshore patterns of abundance were merely due to daily differences in spawning. Finally, a number of sites were sampled more than once and two of the sites were sampled on 5 consecutive days, therefore providing data on daily variability. The analysis of these data indicated that although there was some variation between days in the numbers of eggs found at a site, in general, the numbers of eggs collected at a site were reasonably stable, at least during the main week of sampling.

One possible complication to the reliability in the pattern of egg abundance was that the numbers of day-one eggs were less abundant than the numbers of day-two eggs, which is the opposite to that expected. This could indicate that our ageing of the eggs was incorrect and that they may not hatch for three days. This, however, is unlikely given the consistency of egg development times among different studies (Tibby, 1937; Baker, 1972; King, 1977) which all suggest development of pilchard eggs at 18<sup>o</sup> C takes just 2 days. There were also only 2 peaks found in the egg stages and the majority (80%) of day-two eggs were stages 11 or 12 which are only a few hours apart. Another possibility is that our towing methods were not sampling the day-one eggs properly, with perhaps day-one eggs

being deeper than 50m. Again this is unlikely given that most studies of the vertical distribution of pilchard eggs have found that they tend to be above 50m and are generally close to the surface (Ahlstrom, 1959) but is obviously something which should be investigated. An alternative explanation is that the spatial distribution of the day-one eggs was more contiguous than later stages due to spawning occurring in discrete patches. There is some evidence for this in that the co-efficient of variation for this stage was the highest and it decreased with age down to post larvae, this pattern has been found in other similar plankton studies (Smith & Hewitt, 1985).

The variation in the longshore distribution of the planktonic pilchard stages was consistent with a relatively strong easterly drift of between 0.5-1.0 kt. This speed is consistent with that found by Cresswell (1991) on the shelf near the Albany region during June 1987, and while strong, it is still markedly slower than the 3kt flow he measured in the main region of the Leeuwin current off the edge of the shelf. This easterly movement along the shelf also explains the lack of larvae found in the Albany region during July of both 1989 & 1990 in the previous study done off Albany (Fletcher & Tregonning, 1992). They had postulated that this pattern must be due to either movement of the larvae from the area or that the mortality of larvae at this time of year must be large. The former now appears to be the most likely cause.

The movement of these stages out of the Albany region from which they were spawned across to Bremer Bay indicates that there is no chance that pilchards in these two areas can be reproductively isolated. Nonetheless, given that there were two centres of spawning leaves opens the possibility that there may be 2 functionally separate adult populations in the two regions. There is some additional micro-chemical data (Edmonds, pers comm) and catch-at-age data (Fletcher, unpubl) which also supports this hypothesis. This movement also highlights a potential problem in plankton studies which fail to identify egg stages because the conclusions drawn from this study would be very different if only the distribution of larvae were used.

Finding a few pilchard eggs and larvae in Esperance in August increases the chances that this area may form part of one south coast spawning population. Supporting this was the recent morphological study which found no difference between all three south coast locations (Syahailatua, 1992). There is, however, the microchemical data which indicates separation as adults among the three south coast regions (Edmonds, pers. comm.).

The general lack of spawning along the south coast west of Torbay and up the west coast to Fremantle (despite the fact that pilchards were being caught at this Fremantle during this period) suggests that the pilchards in these two regions are likely to be relatively isolated. The morphological study did find differences between these two regions (Syahailatua, 1992) suggesting reproductive isolation and microchemical and recent oxygen isotope analyses have confirmed that there is at least a difference between the west and south coast pilchards as adults (Edmonds pers comm).

The adult fish collected during the study had similar sizes and sex ratios to those caught in the Albany region over the entire year. The predominance of female fish is similar to that found off California where the sex ratio was also 0.62 (Wolf, in press). The condition of the fish collected were indicative of spawning individuals with the weight per length of each fish (condition factor) being

## Fletcher et al 1992 FIRDC Report Sth Coast Plankton

relatively low and the stages of the female gonads being consistent with fish either preparing to spawn or recovering from spawning.

The average batch fecundity of 11,300 found in this study is low in comparison to other studies done on this species, with both Baker (1972) and Joseph (1980) finding mean values of over 20,000 in their studies. It was not stated in these studies, however, at what stage in the spawning cycle these estimates were made. This appears to be important because the slope of the batch fecundity vs fish length curve was much lower during the period when the plankton tows were done than was found for the previous month when the mean fecundity was nearly 17,000, possibly before spawning had begun. This indicates that individual batch fecundity declines during the spawning season and that most individuals had probably already spawned a number of times by the end of July.

The spawning frequency of females was estimated to be 0.1. This was calculated from the percentage of female fish with post-ovulatory-follicles, and is consistent with other studies of *Sardinops*. Wolf (in press) has found spawning frequencies of 0.03 - 0.18 for the Californian sardine and similar rates have also been found for the northern anchovy (0.12 - 0.17; Hunter & Goldberg, 1979) and for the cape anchovy (0.11 - 0.21; Armstrong et al., 1988).

The EPAM estimate of a spawning biomass of 15,800 tonnes is close to the spawning biomass calculated by the computer model of the Albany fishery using the low abundance scenario which suggested that in July 1991 there would 16,500 tonnes. The higher abundance scenarios indicated that the spawning stocks would be either 23 or 30,000 tonnes. Thus, both the calculated biomass and the distribution of adults (see above) were consistent with the lowest abundance

33

scenario. Further confirmation of these estimates comes from the overall mortality rates calculated for this stock which are in the vicinity of 0.85-0.9 (Fletcher, unpubl). Given a natural mortality rate of about 0.45 (Joseph, 1980; Fletcher, 1992) the fishing mortality would be close to 0.4. This is very similar to the rate calculated using a biomass of 16,000t and an average catch of 6,700t, which yields a rate of 0.41.

#### Conclusions

This study has confirmed our views that the stock of pilchards off Albany is not large and, therefore, supports our management initiatives which saw a reduction in the number of vessels operating in the Albany zone and a TAC of 5,500t being introduced. It has also indicated that the stocks off Bremer Bay and Albany cannot be totally isolated with there at least being an exchange of larvae between the areas. There was still, nonetheless, some evidence that the stocks, as adults, may be somewhat independent. It is apparent that there needs to be an extension to the sampling to cover the region between Bremer Bay and Esperance to determine the relationships along the entire south coast especially before a major fishery develops in the Esperance region. Furthermore, there would be great value in repeating this sampling to determine how the results vary between years, especially if the pattern in the location of the Leeuwin current varies among years. This sampling could, if it proves reliable, eventually be used as a fishery independent method of assessing the status of the stock.

#### **Future Research**

Further plankton sampling of this region needs to be done during December when the is an additional spawning period (Fletcher & Tregonning, 1992) at a time when the catch rate of the fishery is traditionally poor (Fletcher, 1992). This should indicate if the patterns predicted by the models for this time of the year are consistent with the pattern of egg abundances.

Sampling should be extended to include the area between Bremer Bay and Esperance. There was evidence that spawning continued past the last sites at Bremer Bay and this should determine if spawning extends through to Esperance, meaning that the spawning stock is considerably larger in size than is depicted in the present study. Alternatively, there is a possibility that a separate spawning area could be located in the Esperance region. The determination of the connectedness of the populations is of prime concern given that the fishery in Esperance is likely to expand rapidly in the near future.

Finally, these series of tows should be done for a number of years to evaluate the influence of the Leeuwin current on the distribution of the eggs. Furthermore, by repeating the series of tows in different years, this will enable the variation in estimates of spawning biomass to be determined.
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List of gonad stages used to visually assess the reproductive status of pilchards examined during the study.

- 1 Immature
- 2 Inactive
- 3 Developing
- 4 Ripe
- 5 Running Ripe
- 6 Partially Spent
- 7 Fully Spent (recent)
- 8 Fully Spent
- 9 Spent/Recovering

Nested analysis of variance for day-one eggs at the IS sites. The two pairs of IS sites per block was the nested variable, block refers to the 11 spatial blocks along the south coast (Fig 1). A  $\log_{10}$ + 1 transform was used to homogenise the variances. \* indicates significance at P < 0.05; \*\* indicates significance at P < 0.01; ns indicates not significant at P > 0.05.

Source	DF	SS	MS	F
Block	9	29.35	3.26	5.9**
Region(Block)	10	7.95	0.79	1.4 ns
Residual	20	11.04	0.5	

Two factor analysis of variance using a log transformation on the abundance of day-one eggs at the 10 blocks along the coast (B-K) and the three regions within each block (OB, IS, SH). See Table 2 for details.

Source	DF	SS	MS	F
Block	9	29.74	3.3	4.9**
Region	2	5.32	2.6	4.02 <sup>*</sup>
Block x Region	14	12.68	0.9	1.37 ns
Residual	45	29.81	0.66	

Duncans' Test: Means connected with the same underline are not significantly different

Blocks	J	Κ	Ι	G	Η	F	D	E	В	С
							:			
Regions			OB		IS		SH			

A two factor analysis of variance using a log transformation on the abundance of day-two eggs for the 10 blocks along the coast (B-K) and the three regions within each block (OB, IS, SH). See Table 2 for details.

Source	DF	SS	MS	F
Block	9	32.87	3.6	9.6
Region	2 ·	17.75	8.8	23.35
Block x Region	14	20.86	1.49	3.92**
Residual	45	17.11	0.38	

A: Comparison of egg abundances at the same sites taken on different days.

A: Area off Albany (G1 & G8 using PV 20 and the overall mean number of eggs collected for the days when all three boats were operating).

Date	All Pilchard Eggs	Day-One Eggs	Overall Mean All eggs	Overall Mean Day-one Eggs
12/06	10			
14/07	5			
23/07	1000	138		
25/07	42	6	633	86
26/07	30	28		
27/07	224	6	570	252
28/07	139	70	675	135
29/07	619	28		

# FIRDC Report Sth Coast Plankton

46

# Table 5 (cont)

B: Samples taken at the same site on two different occasions by the boats working east and west of Albany.

Site	Date	Abur	ıd.	Site	Date	Abun	d.
		All	Day 1			All	Day 1
Rando Disjo de manascala da di							
G1	25	310	15	G3	25	290	0
G1	27	309	12	G3	27	56	0
G7	25	1100	17	G8	25	1300	1261
G7	27	3460	3130	G8	27	139	139
H2	25	1033	78	H7	25	1322	1143
H2	28	92	62	H7	27	7833	1089
I3	25	2	1	<b>I</b> 4	25	1	1
I3	28	3	0	<b>I</b> 4	28	10	10

FIRDC Report Sth Coast Plankton

47

### Table 6

The table of correlation coefficients for the relationships between components of the plankton and the measured physical variables of the sites. Only those relationships which were significant at the P < 0.1 level are listed.

	Cope	pods	Amphipods	Chaetognaths	Crab Larva	Oikopleura	Lucifer
Longitude							-0.55
Temperature					-0.50	-0.53	
Amphipods	0.808				0.614		0.897
Copepods			0.808		0.509		0.576
Chaetegnaths							
Salps				0.747			
Lucifer	0.580		0.897	0.583			
Shrimps					0.580		
Fish Eggs				-0.648			
		Day-C	One	Day-Two		Yolk-Sac	
		Pilch.	Eggs	Pilch. Eggs		Larvae	
Longitude		0.544		0.757		0.689	
Temperature		-0.502	2	-0.638		-0.57	
Copepods		0.569				-0.40	
Chaetognaths		-0.509	)	-0.650		-0.503	
Salps				-0.685		-0.780	

#### 48

## Table 7

Step-wise regressions for the relationships between pilchard egg and larval stages and other components in the plankton and physical factors of the sampling site.

Day-One	Eggs
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Variable	Partial R <sup>2</sup>	Model R <sup>2</sup>	Prob
Copepoda	0.32	0.32	0.055
Longitude	0.34	0.66	0.014
Chaetognaths	0.17	0.83	0.018

# Day-Two Eggs

Variable	Partial R <sup>2</sup>	Model R <sup>2</sup>	Prob
Longitude	0.57	0.57	0.004
Chaetognaths	0.21	0.79	0.0127

Yolk-Sac Larvae

Partial R <sup>2</sup>	Model R <sup>2</sup>	Prob
0.60	0.60	0.002
0.09	0.80	0.0768
0.06	0.87	0.09
	Partial R <sup>2</sup> 0.60 0.09 0.06	Partial R <sup>2</sup> Model R <sup>2</sup> 0.60       0.60         0.09       0.80         0.06       0.87

Fletcher et al 1992

Table 8

Results of plankton tows done in other locations. Values are expressed as number per 200  $m^3$  water filtered.

Site	Distance from Coast (nm)	Pilchard Eggs	Pilchard Larvae
Esperance 1	1	7	1
Esperance 2	3	2	0
Dunsborough 1	1	1	4
Dunsborough 2	3	0	47
Dunsborough 3	6	0	63
Fremantle 1	2	0	0
Fremantle 2	3	0	0
Fremantle 3	4	0	0

50

#### Table 9

The egg production area method (EPAM) equation, values and parameters used to estimate the spawning area and resulting spawning biomass for both the Californian sardine (Wolf, in press) and for the population off Albany.

EPAM Equation: 
$$A_1 = P k_1 W$$

Where:  $A_1 =$ spawning area of biomass  $B_1$  in nautical miles<sup>2</sup>

 $B_1$  = spawning biomass in short tons

R = sex ratio, fraction of population that is female.

S = fraction of mature females spawning per day

P = daily egg production, in positive stratum

W = average weight of mature females (g).

 $m = \text{conversion from } 0.05 \text{m}^2 \text{ to } \text{nm}^2$ 

 $k_1 =$ conversion from grams to short tons

Site	B <sub>1</sub>	R	F	S	Р	W	А
	Spawning	Sex	Batch	Spawning	Egg	Female	Spaw.
	Biomass	Ratio	Fecundity	Fraction	Prod.	Weight	Area
Calif.	20,000	0.62	62,500	0.115	2.39	172	2,870
Albany	17,320	0.62	11,800	0.10	3.40	38	1,500





### Fletcher et al 1992



Fig. 2 A map of the south coast region of Western Australia indicating the main plankton tow locations.



Fig. 3 A map of the south coast of W.A. indicating the relative abundance of all pilchard eggs collected in the plankton samples.





(b) The development stages (1 - 12) of the pilchard eggs as defined by Baker (1972) and Fletcher & Tregonning (1992). O1 and O2 refer to opaque (dead) eggs between stages 1-6 and stages 7-12 respectively.



Fig. 5 A map of the south coast of W.A. indicating the relative abundance of day-one pilchard eggs collected in the plankton samples.



Fig. 6 The mean abundance of four different planktonic stages of pilchards (a) day-one eggs, (b) daytwo eggs, (c) yolk-sac larvae and (d) post-larvae in each of the 10 blocks along the south coast region.







Fig. 8 A map of the south coast of W.A. indicating the relative abundance of all pilchard larvae collected in the plankton samples.



Fig. 9 A map of the south coast of W.A. indicating the relative abundance of all pilchard yolk-sac larvae collected in the plankton samples.



Fig. 10 A map of the south coast of W.A. indicating the relative abundance of all pilchard early postlarvae collected in the plankton samples.



Fig. 11 A map of the south coast of W.A. indicating the relative abundance of all pilchard late postlarvae collected in the plankton samples.



**Fig. 12** a: A NOAA advanced very high resolution radiometer thermal image of the Leeuwin current flowing along the south coast of Western Australia on the 28th July 1991 (orbit N11/14626). The brightness temperature from band 4 is shown, with the warmest water in dark shades and cooler waters in pale tones; land is white.

b: A diagrammatic version of the satellite thermal picture.

### Fletcher et al 1992



Fig. 13 A map of the south coast of W.A. indicating the surface water temperature measured whilst collecting the plankton samples.



Fig. 14 A map of the south coast of W.A. indicating the settled volume (in a 1" measuring cylinder) of all the collected material from the plankton samples.



**Fig. 15** A map of the south coast of W.A. indicating the relative abundance of all non-pilchard fish larvae collected in the plankton samples.



Fig. 16 a The abundance of the species/groups of invertebrates plotted against the longitude of the sampling location.



Fig. 16 b: The abundance of various pilchard stages plotted against the abundance some of the invertebrates.



Fig. 17 A map of the south coast of W.A. indicating the estimated abundance (tonnes) of acoustic marks which were assumed to be pilchards.



**Fig. 18** A frequency distribution of the (a) length (L.C.F., mm), (b) gonad stages of females and (c) male gonad stages of the pilchards collected during the adult sampling done during this project.



Fig. 19 The batch fecundity of female pilchards collected during the plankton sampling period (late July) and at two earlier periods (early July and June).



**Fig. 20** A photograph of a post-ovulatory follicle found in a female pilchard collected during the plankton sampling period.


**Fig. 21** The relative distribution of pilchards in July 1991 as predicted using the spatial model of the pilchard fishery (Fletcher, 1992) using three different recruitment scenarios from the lowest possible (6) up to the highest possible (9).

# Fletcher et al 1992

FIRDC Report Sth Coast Plankton

72

### Appendix

Examination of the sampling intensity required to adequately sample the invertebrate fraction of plankton samples.

### Methods

For two of the plankton samples, the invertebrate fraction of the collected material was sorted in a number of sub-samples until the entire amount was sorted. The overall abundance for the sample was estimated after each sub-sample and these were then compared to the actual total after the whole sample was sorted.

## <u>Results</u>

The estimates of total abundance of the major groups of invertebrates in the two samples at various stages of sub-sampling are shown in Fig A1. The total abundance estimates of the five most abundant groups were relatively similar for sub-samples of as little as 5ml. For the five rarest species, generally more than 7mls was required to ensure that they were included.

#### Conclusion

These results suggests that at least 10ml of material should be sorted to ensure that a reasonable estimate of the total numbers in the sample will be obtained. This equates to approximately 30% of an average sample with the mean plankton volume obtained during this study being 30ml.

Estimated WHole Number

Estimated WHole Number



Cumulative Sample (ml) Fig. A1 The abundance of various common and rare invertebrate groups found in the plankton for two samples (J4 & G8) as estimated after sorting increasing amounts of the contents up to the entire sample.

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