A COLLABORATIVE INVESTIGATION OF THE USAGE AND STOCK ASSESSMENT OF BAITFISH IN SOUTHERN AND EASTERN AUSTRALIAN WATERS, WITH SPECIAL REFERENCE TO PILCHARDS *(SARDINOPS SAGAX).*

FRDC Project 94/029

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94/029. A Collaborative Investigation of the Usage and Stock Assessment of Baitfish in Southern and Eastern Australian Waters, with Special Reference to Pilchards (*Sardinops sagax*).

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Objectives

1. To carry out a literature search on pilchards and other small pelagic fish species, specifically investigating:
   a) methodologies for stock assessment of small pelagic species,
   b) the effect of fisheries for small pelagic fish species on predator species, including fish, seabirds and marine mammals.
2. To describe the main baitfisheries, including catches, areas and by-catch species.
3. To determine the stock structure of the main baitfish species in eastern and southern Australia.
4. To describe the biology of pilchards in Australia, including age and growth, reproductive cycles and fecundity.
5. To evaluate the potential of using egg surveys to estimate the spawning biomass of pilchards in south eastern Australia.
6. To estimate the potential yield(s) of pilchards in south eastern Australia.

RESULTS AND DISCUSSION

This project was initiated in response to a rapid increase in the demand for pilchards and other baitfish species and the subsequent expansion of purse-seine fisheries throughout southeastern Australia. During the course of the project, the need for research on pilchard stocks was further increased by the mass mortality of adult pilchards that occurred between Noosa Heads (Queensland) and Red Bluff (Western Australia) in autumn 1995 and the decrease in the 1997 Total Allowable Catch for the Western Australian pilchard fishery which lead to a shortage of pilchards on Australian markets.

A literature review confirmed the suitability of the daily egg production method (DEPM) for estimating the abundance of clupeoid fishes. It is the chosen method for stock assessment in some of the world's largest fisheries and, if results are used conservatively, is an effective tool for establishing biologically-based Total Allowable Catches. Concurrent use of the DEPM, age-dependent estimation procedures and hydroacoustic surveys can provide synergistic advantages. For example, age-dependent estimation procedures can be used to convert estimates of spawning biomass into estimates of absolute abundance. Similarly, information on stock size can be used to refine and calibrate hydroacoustic procedures which can in turn enhance knowledge of patterns of distribution and behaviour.

There is a pressing need for research on the effects of purse-seine fisheries on baitfish predators in South Australia, which now supports Australia's largest pilchard fishery, and where there are important populations of southern bluefin tuna, Australian salmon, little penguins, crested terns, Caspian terns, short-tailed shearwaters, New Zealand fur seals and Australian sea lions. Similarly, Victorian populations of seabirds and seals must be included in assessments of the potential impacts of expansion of the fishery into Bass Strait. A wide range of techniques have been developed for modelling complex multi-species fisheries interactions. These provide a framework from which to begin investigations of ecosystem function and will be most readily applied in regions with relatively few pelagic species (e.g. South

Australia). Studies will necessarily include investigations of oceanographic factors, plankton assemblages, predatory species and patterns of energy flow. Population parameters obtained from seabird and seal colonies may act as useful indicators of fish abundance and could provide useful insights into the effects of commercial fisheries on ecosystem function.

There is a large pilchard fishery in South Australia and smaller fisheries for pilchards and anchovies in Victoria. Immature pilchards dominate catches from Spencer Gulf and Port Phillip Bay, but are generally less common than mature fish in catches from Coffin Bay and Lakes Entrance. Both fisheries are driven by local demand and most catches are taken near home ports and markets. The possibility of localised depletion of stocks cannot be discounted, and it is essential that catch monitoring continues in both states. The South Australian pilchard fishery is currently managed using output controls (Total Allowable Catches and Individual Total Quotas) determined from stock assessments presented in this report, but there are no output restrictions on the Victorian fishery. There is a clear need for quantitative data on the stock size and the spawning patterns of pilchards in Victorian waters.

Female pilchards were more common than males in samples from catches taken in both states. In South Australia, 50% of male and female pilchards reached sexual maturity at 14.2 and 14.8 cm LCF respectively whereas in Victoria 50% of male and female pilchards reached sexual maturity at 12.9 and 15.1 cm LCF respectively. In South Australia, spawning occurred during January-April, whereas in Victoria reproductively active fish were collected between September and December. Difficulties in collecting representative samples of spawning fish restricted the precision of estimates of batch fecundity and spawning fraction (South Australia). The estimates of batch fecundity and spawning fraction obtained in 1997 were 13 947 eggs per female and 0.156 females per night respectively.

Application of the DEPM in waters of central and western South Australia suggested that spawning biomass of pilchards was approximately 59 000 t in 1995, 18 000 t in 1996 and 59 000 t in 1997. The low estimate for 1996 may reflect the mass mortality of pilchards that occurred in 1995. Our confidence in the estimates provided is limited by difficulties associated with acquiring reliable estimates of adult reproductive parameters, especially spawning fraction. The effects of this problem were mitigated by the use of conservative values of spawning fraction in calculations of spawning biomass and may recently have been overcome by the development of a fishery-independent sampling method that involves powerful surface and sub-surface lights and a multi-panel gillnet.

Data obtained in this study was used to set the Total Allowable Catch for the SA pilchard fishery in 1998 (11500 t). The pilchard industry in South Australia has agreed to fund DEPM surveys during the period 1998-2001 in order to provide a quantitative basis for establishing future quotas. The current high level of demand for pilchards as fodder for the South Australian tuna mariculture industry suggests there will be considerable pressure to increase the exploitation rate and/or the Total Allowable Catch. The value of industry-funded studies will be maximised if they are conducted in conjunction with integrated studies of factors that control natural fluctuations in the size of pilchard stocks, the development of cheaper and more convenient methods for obtaining indices of pilchard abundance, and the potential effects of the pilchard fishery on other components of the pelagic ecosystem.

ACKNOWLEDGMENTS

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Dr Dan Gaughan (Western Australian Fisheries), Mr David McGlennon (South Australian Research and Development Institute) and Mr Jonathan Staunton Smith (Queensland Department of Primary Industries) reviewed a draft of this report.

CHAPTER 1. GENERAL INTRODUCTION

G.K. Jones, T.M.Ward and M. Kinloch

1.1 Background

In the early 1990s, when most of Australia's commercial fisheries were either stable or contracting, catches of small pelagic fishes increased rapidly. In response, fisheries research and management agencies throughout south-eastern Australia (e.g. South Australian Research and Development Institute, Marine and Freshwater Resources Institute (Victoria), Queensland Department of Primary Industries, East Coast Tuna Management Advisory Committee, Southern Bluefin Tuna Management Advisory Committee) identified the need to assess the stocks and investigate the usage of small pelagic fishes, especially pilchards (*Sardinops sagax*), anchovies (*Engraulis australis*), blue mackerels (*Scomber australasicus*) and yellow-tailed scads (*Trachurus novaezealandiae*) (Glaister and Diplock 1992).

Since the early 1960s, approximately 50 tonnes (t) of pilchards have been taken each year from South Australian waters for use as live bait in the pole and longline fisheries for southern bluefin tuna (Dredge 1969; Jones *et al.* 1995). The recent development of the southern bluefin tuna mariculture industry in Boston Bay (Spencer Gulf), which uses pilchards as fodder, drastically increased demand for this resource. A purse-seine fishery commenced in 1992 and by 1993/4 was the state's largest fishery (by weight) (Jones *et al.* 1995). In 1993, the South Australian Scalefish Management Committee set the Total Allowable Catch at 3500 t for a period of three years (1994-96) while research was undertaken to determine the size and structure of South Australian pilchard stocks.

Beach seine and hoop-net fisheries for pilchards commenced in Victoria's Port Phillip Bay in the 1860s (Blackburn 1950). Purse-lampara and purse-seine nets were used spasmodically in Port Phillip Bay and at Lakes Entrance throughout the 1950s and 1960s. Annual catches for the period 1964-77 were generally less than 500 t (Winstanley 1979). Catches from Port Phillip Bay have increased in recent years and in 1992/93 the total Victorian catch exceeded 3000 t (Neira *et al.* 1997a,b). Concerns grew about possible impacts of the fishery on other species, especially the little penguin (*Eudyptula minor*) which supports a thriving tourist industry. Research was subsequently initiated to investigate potential environmental impacts (see Dann 1992; Hobday 1992) and to assist the establishment of an ecologically-sustainable harvesting regime for the fishery.

The pilchard fishery in New South Wales waters has not undergone the recent expansion that has occurred in other states. Catches have remained stable at approximately 250-500 t per annum 1986 (Dixon *et al.*

1996). Blackburn (1949, 1950) provided considerable biological data on pilchards in New South Wales waters and previous studies carried out on the main fishing grounds in Jervis Bay (Blackburn and Tubb 1950; Joseph 1981) concluded that fishing mortality was low and not adversely affecting stocks. Additional research on the fishery was considered unnecessary and studies in New South Wales concentrated on patterns of baitfish usage (see Dixon *et al.* 1996).

A beach-seine fishery for small pelagic fishes (mainly pilchards) has existed in southern Queensland since the 1950s. Catch statistics prior to 1988 are poor, but annual catches appear to have varied between approximately 50 and 200 t. In early 1995, a Queensland fisher applied for a permit to establish a developmental purse-seine fishery in southern Queensland. Recreational fishers and conservationists strongly opposed this fishery, and the Queensland Fisheries Management Authority initially declined the application. The applicant appealed this decision and on 19 July 1996 a three year permit was issued for a developmental purse-seine fishery for pilchards (*S. sagax*), round herrings (*Etrumeus teres*), anchovies (*Engraulis australis*) and sandy sprats (*Hyperlophus vittatus*). The permit was for one vessel with a maximum annual catch of 600 t. Queensland researchers subsequently proposed to investigate the biology of pilchards and the capacity of stocks to support a purse-seine fishery.

In November 1993, a meeting of agencies considering pilchard research programs was convened at the Sydney Fish Markets. This meeting led to the development of a joint proposal for funding from the Fisheries Research and Development Corporation. In 1994, the Fisheries Research and Development Corporation funded a collaborative study (94/029) of the stocks and usage of small pelagic fishes, especially pilchards, in southern and eastern Australia. Research was expanded to include southern Queensland in late 1995 (95/043). The rationale and methods of these projects are similar to those of an investigation (92/025) of the use of egg surveys to estimate the spawning biomass of pilchards in Western Australia (Fletcher *et al.* 1996a, b).

1.2 Need

The need for this research was identified in response to (i) the history of collapse of many of the world's fisheries for anchovies, sardines, pilchards and herrings (e.g. Murphy 1977; Hempel 1978; Kondo 1980; Lasker and McCall 1983; Lasker 1985); (ii) increased demand for small pelagic fishes as bait for recreational and commercial fisheries, fodder for the southern bluefin tuna mariculture industry and food for human consumption (Fletcher 1992; Anon. 1994); (iii) subsequent increases in the catches of small pelagic fishes throughout Australia (Kailola *et al.* 1993); (iv) the relatively small size of Australian fish stocks (Kailola *et al.* 1993); and (v) the potential effects of fisheries for

planktivorous fishes on predatory species, and the structure and function of marine ecosystems (Hilborn and Walters 1992).

Prior to the current study, most of the data on the biology of pilchards from southern and eastern Australia had been obtained from New South Wales (Blackburn 1941, 1949; Blackburn and Tubb 1950; Joseph 1981), although Blackburn (1950) also included some data from Victoria and South Australia. The paucity of information on patterns of distribution and abundance, age, growth and reproduction, and the genetic structure of stocks prevented the establishment of scientifically-based strategies for managing pilchard resources.

During the course of the current project, two events occurred that further increased the need for estimates of stock size and the establishment of biologically-acceptable Total Allowable Catches. Firstly, in 1995, millions of pilchards died in a mass mortality event that occurred between Noosa Heads in southern Queensland and Red Bluff in Western Australia (Griffin *et al.* 1996; Fletcher *et al.* 1997). Secondly, in 1997, the Total Allowable Catch for the Western Australian pilchard fishery was reduced (D. Gaughan, Western Australian Marine Research Laboratories, personal communication) and the quantity of pilchards available for sale on Australian markets fell, thus further increasing pressure on other states to expand their fisheries.

1.3 Objectives

1. To carry out a literature search on pilchards and other small pelagic fish species, specifically investigating:
   a) methodologies for stock assessment of small pelagic species,
   b) the effect of fisheries for small pelagic fish species on predator species, including fishes, seabirds and marine mammals.
2. To describe the main baitfisheries, including catches, areas and by-catch species.
3. To determine the stock structure of the main baitfish species in eastern and southern Australia.
4. To describe the biology of pilchards in Australia, including age and growth, reproductive cycles and fecundity.
5. To evaluate the potential of using egg surveys to estimate the spawning biomass of pilchards in south eastern Australia.
6. To estimate the potential yield(s) of pilchards in south eastern Australia.

1.4 Rationale and Approach

This project investigated the biology of pilchards in southern and eastern Australia, but the main aim was to develop estimates of abundance required to identify sustainable yields and establish biologically-based strategies for the management of stocks. A literature review was conducted to assess the relative merits of three commonly used stock assessment techniques for estimating the biomass of Australian clupeoids: classical fisheries models; hydroacoustic techniques; and the Daily Egg Production Method. Direct investigation of the effects of baitfisheries on predatory fishes, seabirds and marine mammals was beyond the scope of the project, but a literature review was conducted in order to identify suitable directions for future research. Scientists in South Australia and Victoria described the fishing methods, markets and management arrangements for baitfisheries in their waters and collated catch statistics by season, area and year. Monthly pilchard samples were obtained from commercial catches in South Australia and Victoria. Fish were measured, weighed and sexed to provide basic biological and fisheries data. Otoliths were sent to the Central Ageing Facility at Marine and Freshwater Resources Institute (Victoria) for age-determination. Samples from South Australia, Victoria, New South Wales and Queensland were supplied to the University of New South Wales for stock discrimination studies. Gonads were staged and weighed to elucidate the reproductive cycle, and histological studies were conducted at the University of Adelaide in order estimate fecundity (spawning fraction and batch fecundity). Egg surveys and adult sampling programs were undertaken in South Australia waters during all three years of the project in order to estimate spawning biomass using the Daily Egg Production Method. Information from South Australia have been used to identify sustainable yields and present options for the management of pilchards. Research conducted in southern Queensland waters will be reported separately.

CHAPTER 2. REVIEW OF METHODS FOR ASSESSING THE STOCKS OF AUSTRALIA'S SMALL PELAGIC FISHES.

T.M. Ward, F.J. Neira, G.K. Jones and M. Kinloch

Objective: To carry out a literature search on the methodologies for stock assessment of small pelagic fishes. Classical fisheries models that rely on fishery-dependent data are inappropriate for new and developing fisheries and their (usual) reliance on catch-per-unit-effort data renders them relatively unsuitable for schooling pelagic fishes such as clupeoids. Use of hydroacoustic methods is impeded by temporal and spatial variation in schooling behaviour and difficulties associated with verifying the species and size composition of schools. The daily egg production method provides rapid estimates of spawning biomass and, if results are used conservatively, is an effective tool for managing clupeoid fisheries. Concurrent use of the three methods has synergistic effects. Data from egg surveys can be used as a fishery-independent index of abundance in classical fisheries models and to calibrate the results of hydroacoustic surveys. Age-dependent estimation procedures can be used to convert estimates of spawning biomass into estimates of total biomass. Hydroacoustic surveys can provide valuable information about the spatial and temporal patters of distribution and abundance of spawning and non-spawning fish.

2.1 Introduction

Factors that hinder accurate and precise estimation of the biomass of clupeoids and other pelagic species include their highly contagious distribution, extreme mobility; high capacity for net avoidance, differential susceptibility to capture of various sex and age classes, and temporal variations in patterns of distribution and behaviour. Methods that have been used to estimate the relative or absolute abundance of stocks of small pelagic fishes in Australia and New Zealand include: visual surveys from boats and aeroplanes (e.g. Blackburn and Tubb 1950); pelagic trawl surveys (Collins and Barron 1981; Stevens *et al.* 1984; Zmiyevskiy in Fletcher 1991a); hydroacoustic surveys using echo-sounders and sonar (Rapson 1953); various mathematical models (e.g. Fletcher 1992); and egg surveys (Fletcher *et al* 1996a, b).

This chapter reviews methods commonly used to assess stocks of small pelagic fishes and examines their applicability to Australian stocks of clupeoids. We define stock assessment in its narrowest sense as estimation of biomass. Components of the broader definition (Hilborn and Walters 1992, p. 3), such as yield optimisation and effects of fishing on biomass, are discussed in Chapter 9. Three types of methods for estimating abundance are discussed herein: classical fisheries models, hydroacoustic

surveys and the daily egg production method (DEPM). Techniques that provide only indices of relative abundance, e.g. aerial surveys, are only discussed in regard to their use in procedures for estimating absolute abundance.

2.2 Classical Fisheries Models

History

A plethora of models that incorporate fishery-dependent data have been used to estimate the biomass of commercial stocks (see Hilborn and Walters 1992), but as such methods are not applicable to new and developing fisheries for small pelagic fishes they are given only cursory attention in this review. Readers with particular interest in these approaches are directed to reviews by De Lury (1947), Ricker (1954), Schaefer (1954), Beverton and Holt (1957), Gulland (1977), Fournier and Archibald (1982), Deriso *et al.* (1985), Megrey (1989), Sparre and Venema (1992) and Hilborn and Walters (1992). Fletcher (1992) used a spatial simulation model to estimate the abundance of pilchards in a Western Australian pilchard fishery.

Models

Most fisheries models can be allocated to one of five major categories: simple production models; (e.g. Schaefer 1954; Pella and Tomlinson 1969; Walter 1973), depletion models (De Lury 1947), age-dependant models (Megrey 1989; Hilborn 1990; Punt 1995), models of the Deriso-type (Deriso 1980; Schnute 1985; Horbowy 1992), and age-dependent estimation procedures such as virtual population analysis (De Lury 1947; Gulland 1965; Murphy 1965; Zang and Sullivan 1988) and catch-at-age analysis (Doubleday 1976; Paloheimo 1980; Fournier and Archibald 1982; Deriso *et al.* 1985; Paloheimo and Chen 1993). Each of these categories of models was derived independently, except for Deriso-type models that were derived from an age-dependent model (Xiaou unpublished manuscript).

Simple production models and depletion models are derived from direct assumptions about the dynamics of a fish population, and relate its present total number or total biomass directly to its previous total numbers or total biomasses. They generally do not consider age-dependent characteristics of fish populations, such as growth and gear selectivity, and are 'perhaps the most abused stock-assessment technique' (Hilborn and Walters 1992).

Age-dependent production models, models of the Deriso-type and age-dependent estimation procedures relate the present total number or biomass of a fish population to its previous numbers or total biomasses through it age structure (i.e. fish numbers and biomasses at age and time). All age-

dependent production models are based on Beverton and Holt's (1957) exponential population dynamics model.

Xiaou (unpublished manuscript) demonstrates that production models, depletion models and models of the Deriso-type can, using three or four assumptions, all be derived from an age-dependent model.

Usage

Simple production models have rarely been used for stock assessments of schooling pelagic fishes (see Schaefer 1954; Caddy and Csirke 1983; Patterson *et al.* 1992). Age-dependent production models have been used to assess stocks of small pelagic fishes in Californian/Mexican waters (Deriso *et al.* 1996), the West Iberian Sea (Borges 1990), and the Bearing Sea (Zheng *et al.* 1996). Age-dependent estimation procedures have been used to estimate the biomass of pilchards, anchovies and mackerels (Parrish and MacCall 1978; MacCall 1979; Butterworth 1983; Pauly and Palomares 1987; Limbong *et al.* 1988; Sparholt, 1990; Butterworth and Bergh 1992; Paterson *et al.* 1992, 1993; Deriso *et al.* 1996; Barange and van der Lingen 1996).

Requirements

Classical fisheries models have mainly utilised commercial catch and effort data, but more recent applications have also incorporated data obtained from sources outside the fishery (e.g. Hilborn and Walters 1992). The dependence of most models on CPUE data restricts their application to stocks of pelagic fishes whose catchability varies over a range of stock sizes, that commonly migrate into or out of the fishing area, and whose populations commonly display large, natural, inter-annual variations in abundance. Age-dependent production models, models of the Deriso-type and age-dependent estimation procedures require a long-term and continuous series of catch-at-age/length data. Such information enables cohorts to be "followed" from the year of first recruitment to the year that they leave the fishery. If length-at-age data are unavailable, these models can be applied to species with distinct modes in length frequency distributions (Pauly *et al.* 1987). Dependence on a relatively long time series of data precludes the use of these models in new and developing fisheries.

Advantages

A major advantage of classical fisheries models is that estimates of biomass can be obtained from catch data and samples collected from fishers. This approach minimises the expenditure of funds, resources and energy required for the application of fishery-independent techniques. These methods were used by fisheries scientists when few other approaches were available.

Disadvantages

The major disadvantages of most classical fisheries models are the assumptions used to estimate parameters. Most applications of these methods have assumed the stock is at equilibrium and that the relationship between CPUE and effort is linear. Application of these methods to declining stocks invariably causes over-estimation of optimal effort (harvest). More seriously though, for tightly schooling clupeoid species such as pilchard and anchovy, the assumption that CPUE is related to abundance is often seriously violated. At low levels of abundance the fish simply become more closely aggregated and, with modern fish-finding devices, remain relatively easy to catch. In addition, the model does not take into account environmental variability causing inter-annual recruitment fluctuations, which are known to occur in most small pelagic fish populations.

2.3 Hydroacoustic Methods

History

Acoustic methods were first used to detect stocks of sub-surface fish around the 1920s and have since been used extensively by the fishing industry in marine and freshwater systems (e.g. Peterson *et al.* 1976; Acker 1977; Traynor and Ehrenberg 1979; Hara 1984; Burczynski and Johnson 1986; Hampton 1987, 1996; MacLennan and Holliday 1996; Porteiro *et al.* 1996; Ransom *et al.* 1996). The first hydroacoustics manual was published by Forbes and Nakken in 1972 and several others have since been produced (e.g. Saville 1977; Burczynski 1979; Amos 1980).

Acoustic systems for estimating fish biomass have undergone massive improvements in the last 20 years, including the development of side-scaning sonar and sophisticated hardware and software for signal processing (Hewitt *et al.* 1976; Coombs 1977; Dickie *et al.* 1983; O'Driscoll and McClatchie 1995; Hedgepeth *et al.* 1996; MacLennan and Holliday 1996). Modern systems can cost as much as US$700,000 and are able to link satellite navigation, water quality sensors and other data inputs and provide the complete, real-time data needed for stock assessment (Hedgepeth *et al.* 1996).

Model

This method employs sonar (**SO**und **NA**vigation and **R**anging) or echosounding equipment (when sound waves are directed downwards) to measure the reflection of a projected sound wave (Hedgepeth *et al.* 1996). A typical acoustic system used in fisheries research comprises four elements: transmitter, transducer, receiver-amplifier, and signal processor (Thorne 1983; Hedgepeth *et al.* 1996). The transmitter delivers electrical energy to the underwater transducer which in turn converts it to an acoustical signal and projects it into the water ("ping"). The projected acoustic energy reflected from fish or other targets (echo) is then returned to the vessel and converted back into electrical energy

(target strength) by the transducer. The receiver-amplifier then intensifies the signal and sends the converted electrical energy to the signal processor which in turn transforms it into an output (eg. paper or video monitor) that can be interpreted by the user (Hedgepeth *et al.* 1996). Although many users prefer real-time data processing equipment, others save outputs in a computer for analysis after the completion of the survey (Thorne 1983; Hedgepeth *et al.* 1996). Acoustic sampling is relatively rapid but a statistically rigorous sampling design is required (Hedgepeth *et al.* 1996).

Usage

Considerable information has been published on the use and application of acoustic techniques in fishery stock assessment (see Thorne 1983; Hedgepeth *et al.* 1996). Acoustic methods have been employed to study the behaviour of fish schools (e.g. Pitcher *et al.* 1996; Soria *et al.* 1996; Fréon *et al.* 1996), and to obtain information on the distribution and abundance of pelagic fishes. For example, Hampton (1996) successfully applied hydroacoustics to estimate the spawning biomass of South African anchovy, and the biomass of anchovy recruits over a period of 10 years. Hara (1985) combined acoustic and aerial surveys to estimate the size and distribution of schools of Japanese pilchard in waters off south-eastern Hokkaido. Garcia *et al.* (1994) employed hydroacoustics to estimate the biomass of anchovy in the Mediterranean Sea.

Hydroacoustics have been used in Australia since the early 1950s to determine the distribution and estimate the abundance of pelagic schooling species such as pilchard, jack mackerel and southern bluefin tuna (e.g. Rapson 1953; Blackburn and Downie 1955; Wolfe, 1976; Stevens *et al.* 1984), and as an aid in midwater trawl fish surveys (e.g. Amos 1976; Gorman and Graham 1977).

Requirements

Before conducting a hydroacoustic survey it is important to design a sampling strategy (see Forbes and Nakken 1972; Hewitt *et al.* 1976; Peterson *et al.* 1976; Saville 1977; Burczynski 1979; Holliday and Larsen 1979; Shotton and Bazigos 1984; Burczynski and Johnson 1986; Jolly and Hampton 1990; Hedgepeth *et al.* 1996), select and calibrate equipment (e.g. single or multibeam systems) and identify methods for data processing. The appropriate system will depend on the target strength of the species, which is the scaling factor required to convert echo intensity to fish density. Other major factors that must be considered are the ability to accurately identify the target species and the collection of representative samples for verification (Hedgepeth *et al.* 1996; MacLennan and Holliday 1996).

The selection of an appropriate survey design is essential when employing hydroacoustic methods (Thorne 1983; Shotton and Bazigos 1984; Jolly and Hampton 1990; Hedgepeth *et al.* 1996). A

stratified random design in which primary sampling units in each stratum are parallel transects that are randomly spaced within certain non-critical limits is recommended (Jolly and Hampton 1990). A transect corresponds to the course followed by the survey vessel perpendicular to the coast whilst the echosounder and sonar are being operated and the raw data are being collected (Hedgepeth *et al.* 1996). The position of each transect in the survey area is selected at random so that each transect can be considered as a sample unit. The total number of transects assigned will depend on the precision desired and the resources available. Zigzag transects are not recommended since they produce poor distribution of sampling effort. A detailed study of the area should be carried out prior to the start of the acoustic survey to obtain variables such as bathometry, oceanographic features and habitat preference of the target species (Jolly and Hampton 1990).

Advantages

Hydroacoustic fishery assessment techniques have a number of advantages over other methods, including independence from fishery catch statistics, relatively low operational costs, low variance and potential for absolute population biomass estimation. In addition, the technique is not affected by environmental parameters and does not reduce the size of the population (Thorne 1983).

Disadvantages

The limitations of hydroacoustic procedures include: the high initial cost; poor species discrimination in multispecies complexes; reduced sampling capability of fish at the surface and at the bottom; high complexity and potential bias associated with target strength and calibration (Thorne 1983; MacLennan and Holliday 1996). Moreover, there are major concerns about the reliability of the estimated fractions associated with length or age groups, behaviour and patchiness (Hedgepeth *et al.* 1996). The correct species identification is still one of the major limitations in acoustic biomass estimations, so ground truthing of echo traces is crucial both to accurately identify the species and to obtain data on the size composition of the population (e.g. Haralabous and Georgakarakos 1996; MacLennan and Holliday 1996; Scalabrin *et al.* 1996).

2.4 Daily Egg Production Method

History

The concept of estimating fish abundance from the total number of eggs produced in a spawning season, the mean fecundity (of females) and the sex ratio was initially proposed by Hensen and Apstein (1887 in Fletcher 1991a). Prior to the 1980s, few attempts were made to use this method to estimate spawning biomass (Buchanan-Wollaston 1923, 1926; Clark 1934); all were relatively unsuccessful. The application of the egg production method was facilitated by the development of

methods for determining batch fecundity (i.e. counting hydrated oocytes) and spawning fraction (i.e. identifying and aging post ovulatory follicles) (Hunter and Goldberg 1980).

Model

The DEPM has been applied to many species of clupeoid fishes (e.g. Parker 1980; Lasker 1985; Armstrong *et al.* 1988; Garcia *et al.* 1991a, 1991b; Alheit 1993; Fletcher *et al.* 1996). The method provides an estimate of the biomass of the adult population of fishes that release batches of pelagic eggs into the water column throughout the spawning season, i.e. serial or batch spawners. It relies on the premise that spawning biomass can be calculated from estimates of the number of eggs produced per day within the spawning area (daily egg production) and the average number of eggs spawned per day per unit mass of the population (daily fecundity).

Spawning biomass (B) was estimated using the equation of Parker (1985):

$$B = \frac{P_1 . A_1 . W}{R . F . S} \qquad (1)$$

where P_1 is the daily egg production (eggs m^{-2} day^{-1}), A_1 is the spawning area (m^2), W is the average weight of mature females (kg), R is the sex ratio (proportion of females by weight), F is the average batch fecundity (eggs day^{-1}) and S is the spawning fraction (proportion of mature females spawning day^{-1}).

Requirements

Criteria which must be met for the application of the DEPM include: fish must be multiple (i.e. batch or serial) spawners; eggs must be pelagic and able to be caught in plankton nets without significant losses; it must be possible to conduct the egg survey throughout the entire spawning area at the time of peak spawning; and spawning and non-spawning adults must be sampled during the egg survey and must be equally catchable (e.g. Parker 1980; Lasker 1985; Alheit 1993; Fletcher *et al.* 1996a,b). Logistical requirements include a research vessel capable of working for extended periods in offshore waters; equipment for sampling eggs, larvae and adults; sufficient personnel to collect and process samples; and histological facilities for processing ovaries. Details of the methods used to estimate parameters employed in this study are provided in Chapter 8.

Usage

Recent overseas studies have shown that the DEPM is a valuable fishery-independent method for estimating spawning stock size (e.g. Alheit 1993; Lasker 1995). It has been used to estimate biomass in situations as diverse as a small artisinal fishery for tropical anchovy in eastern Indonesia (Milton *et al.* 1997); Australian stocks of blue grenadier, *Macruronus novaezealandiae*, and the major clupeoid stocks of eastern boundary current systems off North and South America and South-west Africa (Lo *et al.* 1996; Baranges and van der Lingen 1996). In California and South Africa, application of this method has reached a high level of sophistication and has been used in combination with hydroacoustic techniques. Results obtained by Fletcher *et al.* (1996a, b) suggest that the DEPM can be applied to pilchards in Australian waters.

Advantages

The advantages of the DEPM are that a biomass estimate can be obtained from a single cruise, estimates of biological parameters are accompanied by estimates of variance, and it can be used in conjunction with other methods, e.g. hydroacoustic surveys. If a suitable research vessel is available, the cost of conducting a DEPM is also relatively low (c.f. cost of hydroacoustic equipment).

Disadvantages

Estimates of parameters required for application of the DEPM are subject to considerable sampling error and these errors are compounded by the multiplicative method used to calculate biomass. Logistical constraints also limit the potential for adhering to the predefined requirements of the method. For example, obtaining a sufficiently large number (i.e. 200-900) of plankton samples from throughout the entire spawning area is expensive and time-consuming. Similarly, collecting samples of adults required for estimation of reproductive parameters (i.e. >30 samples with >50 females per sample) is difficult using sampling methods suitable for most research vessels. Furthermore, the vulnerability to capture of clupeoids in pelagic trawls and purse-seine nets varies according to their sex and reproductive status, and can cause biases in parameter estimates (Hunter and Goldberg 1980; Stauffer and Picquelle 1981; Alheit *et al.* 1984; Alheit 1985

2.5 Discussion

The major constraint to the use of classical fisheries models for stock assessment purposes is their general dependence on data obtained from the fishery. The absence of extensive time series data for several of Australia's pilchard fisheries precludes the immediate use of these models or spatial models of the type used by Fletcher (1992). If, however, while data from the fishery are accumulating, fishery-independent estimates of abundance are obtained and knowledge of pilchard ecology is

expanded, the outcomes obtained by applying these models to Australian pilchard stocks will be maximised.

Studies in South Africa have shown that hydroacoustic methods can provide similar estimates of anchovy spawning biomass to those obtained using the DEPM. Hampton (1996) pointed out that DEPM-based estimates were no longer required for estimating anchovy biomass and that efforts should concentrate on improving the accuracy of direct hydroacoustic estimates, particularly in the development of more accurate methods for estimating *in situ* target-strength. It should be noted, however, that South African fisheries scientists possess considerable knowledge of the horizontal and vertical distribution of clupeoid stocks, and have expended considerable energy and resources in developing and adapting hydroacoustic techniques. They have been able to finance this expensive endeavour because of the large size and value of their fishery.

Results obtained by Fletcher (1996a, b) suggested that the DEPM can be applied to stocks of Australian pilchards. Initial problems associated with the collection of representative samples of adult fish have recently been overcome (see Chapter 7). Two of the major practical disadvantages of the method are the costs of conducting cruises and the time required for processing plankton samples. These problems are exacerbated if the spawning grounds are large. Hydroacoustic surveys are, however, also expensive to conduct, and initial costs are considerably higher. One of the most important advantages of the DEPM is that is based on fewer unfulfilled assumptions than the fishery-dependant models that have more commonly been used to estimate stock abundances and sustainable yields.

CHAPTER 3. POTENTIAL EFFECTS OF CLUPEOID FISHERIES ON PREDATORY FISHES, SEABIRDS AND MARINE MAMMALS IN SOUTHERN AND EASTERN AUSTRALIA.

T.M. Ward and G.K.Jones

Objective: To carry out a literature search on the potential effects of clupeoid fisheries on predatory fishes, seabirds and marine mammals in southern and eastern Australia. The potential effects of purse-seine fisheries on pelagic ecosystems vary between states. The need for research on the significance of these effects is particularly urgent in South Australia, which now supports Australia's largest pilchard fishery, and where there are important populations of clupeoid predators, such as southern bluefin tuna, Australian salmon, little penguins, crested terns, Caspian terns, short-tailed shearwaters, New Zealand fur seals and Australian sea lions. In Victoria, populations of seabirds and pinnipeds must be considered in assessments of the potential impacts of expansion of the fishery into Bass Strait. No current management strategy for an Australian fishery incorporates an ecological allocation of biomass, mainly because the data required to estimate the appropriate size of such an allocation are not available. A wide range of techniques have been developed for modelling complex multi-species fisheries interactions. These provide a framework from which to begin investigations of ecosystem function and will be most readily applied in regions with relatively small number of pelagic species (e.g. South Australia). Studies will necessarily include investigations of oceanographic factors, plankton assemblages, predatory species and patterns of energy flow. Population parameters obtained from colonies of seabirds and pinnipeds may act as useful indicators of fish abundance and provide valuable insights into the effects of commercial fisheries on ecosystem function. Studies that use stable isotopes to identify prey types, consumption rates and patterns of energy flow may be provide valuable insights into the trophodynamics of the pelagic ecosystem.

3.1 Introduction

Clupeoids, such as pilchards, anchovies and round herrings, are important components of marine food webs (Pauly *et al.* 1987). They are highly abundant schooling fishes that feed directly on phytoplankton and/or zooplankton (Rapson 1953; Raymont 1980; Southward *et al.* 1988; van der Lingen 1994) and are major contributors to the diet of a wide variety of temperate and sub-tropical fishes, seabirds and marine mammals (Barker and Vestjens 1990; Overholz *et al.* 1991; Jefferson *et al.* 1993; Laugksch and Adams 1993; Blaber *et al.* 1995). Quantitative studies suggest that the biomass of prey fishes required to sustain predator populations can be substantial and that changes in the population sizes of small pelagic fishes can have important implications for the structure and function of marine ecosystems (Furness 1982; Muck and Pauly 1987; Crawford *et al.* 1991, 1992).

Fisheries for small pelagic fishes can have both operational and ecological effects on predator populations (Muck and Fuentes 1987; Croxall 1987; Blaber *et al.* 1996). Operational effects include (i) incidental capture in fishing gear, e.g. dolphins in purse-seine nets, and (ii) opportunistic feeding on catches and by-catches, e.g. seabirds taking fishes encircled and/or escaping from purse-seine nets. Ecological effects, such as reductions in the quantity of food available to predatory species caused by fishery-induced depletions of target species or incidentally captured taxa, may result in changes to the structure and function of ecosystems, (e.g. Cairns 1987, 1992; Montevecchi *et al.* 1988; Montevecchi 1993) and may therefore have significant economic impacts on other fisheries and the tourist industry.

The aim of this review is to assess the potential effects of clupeoid fisheries in South Australia, Victoria, New South Wales and southern Queensland on populations of predatory fishes, cephalopods, seabirds and marine mammals. This aim is addressed by: (i) describing the social and economic importance of species that commonly feed on clupeoids; (ii) assessing the likelihood of operational interactions between baitfisheries and predators, especially the potential for incidental capture in fishing gear; (iii) discussing the nature and significance of the potential ecological effects on each major group (i.e. fishes and cephalopods, seabirds and marine mammals); (iv) identifying the apparent susceptibility of individual species/populations to fishery-induced declines in prey abundance; and (v) identifying priorities for future research. As very little research has been conducted in Australia on the effects of fisheries on food webs, examples from overseas literature are often used to help evaluate the scope and significance of interactions between clupeoid fisheries and predators.

3.2 Fishes and Cephalopods

Social and Economic Importance

Pilchards, round herring and anchovies are commonly eaten by a large number of predatory fishes and cephalopods. Many of these species support important commercial and recreational fisheries. For example, in 1995/96 commercial fisheries in New South Wales, Victoria, South Australia and Western Australia were valued at approximately: $47.5 million (M) for southern bluefin tuna (*Thunnus maccoyii*); $21M for other tunas (e.g. *Thunnus* spp); $2.8M for Australian salmon (*Arripis trutta* and *A. truttacea*); $1.4M for yellowtail kingfish (*Seriola lalandi*) and $1.2M for arrow squid (*Nototodarus gouldii*). At least one species, i.e. southern bluefin tuna, support internationally-significant fisheries and are known to be depleted (Caton *et al.* 1991). Recreational fisheries for billfishes, Australian salmon, tailor (*Pomatomus saltatrix*), mackerels (*Scomberomorus* spp) and tunas have considerable significance for the tourist industry as well as less tangible social values, such as providing leisure and recreation, stimulating outdoor exercise and encouraging environmental awareness.

Significance of Operational Interactions

Relatively few data are available on the operational effects of fisheries for baitfish on species of non-target teleosts. One of the advantages of purse-seine vessels is their capacity to target particular schools. If a school that is encircled by the net is subsequently found not to contain fish of a commercially-valuable species/size, the net can be opened to release the catch. Problems with teleost by-catch can, however, be exacerbated by the use of lights to attract and 'tether' schools. This practice can result in catches that comprise several non-clupeoid taxa, e.g. Australian herring or tommy ruffs (*Arripis georgiana*), blue mackerel (*Scomber australasicus*) and scads (*Trachurus* spp). There is some evidence to suggest that this effect may be most significant at low latitudes (e.g. southern Queensland) where baitfish assemblages include a large number of taxa (Murphy 1977).

Potential Ecological Interactions

Predatory marine fishes tend to feed on a wide variety of prey species. No species of predatory fish found in Australian waters is known to feed exclusively on one taxon. All appear to utilise species that are locally or seasonally most abundant (Kailola *et al.* 1993). No published data are available on the quantity of baitfishes utilised by predatory fishes or the effects of prey depletion on stocks of predatory fish in Australian waters. Overseas studies have shown, however, that annual consumption rates by predators commonly match fisheries catches. For example, tn the Humboldt Current (Peruvian) ecosystem consumption of anchovies by horse mackerel (*Trachurus murphyi*) and bonito (*Sarda chiliensis*) is similar to or exceeds fishery catches (Muck and Sanchez 1987). Similarly, data from the northern Atlantic Ocean suggest that the abundance of cod (*Gadus morhua*) is directly affected by the availability of capelin (*Mallotus villosus*). Low exploitation rates are maintained for capelin in order to ensure the availability of prey for the commercially-important cod (Carscadden 1983; Mehl and Sunnana 1991).

Clupeoids stocks display large, natural inter-annual fluctuations in abundance (e.g. Kawasaki *et al.* 1991). The capacity of piscean predators to utilise a wide range of prey presumably buffers populations from the effects of these variations, but opportunities to switch prey may be limited at high latitudes where food webs are often simple and include relatively few species of small planktivorous fishes (Murphy 1977). It is thus in the low diversity environments of southern and eastern Australia (e.g. South Australia) that the effects of depletion of clupeoid stocks on predatory species may be most apparent. Whilst clupeoid stocks are highly resilient to reductions in their population sizes, longer-lived predatory species that often support valuable commercial and recreational fisheries may require additional time to recover from population declines.

In Australia, many teleost fisheries target large piscivores (Kailola et al. 1993) and the populations of many predatory fishes have been reduced. For example, the populations of southern bluefin tuna, mackerels (Scomberomorus spp) and tailor (P. saltatrix) have been depleted (Caton 1991; Begg 1996). Removal of top predators has been shown to increase the abundance of prey species (Pauly 1979) and in some cases this effect has been incorporated into management strategies (Brown et al. 1990; Sanders 1995). One school of scientific thought (e.g. Christensen 1996) suggests that moderate usage of several trophic levels, i.e. 'harvesting down the food web', may be a better ecological option than exclusive utilisation of upper trophic levels (see Figure 3.1).

Likely Effects on Predatory Species

In eastern waters, juvenile southern bluefin tuna typically feed on euphausids, ocean squid, jack mackerel and clupeoids, whereas in waters of South Australia and Western Australia they feed predominantly on clupeoids (Sheard 1950; Serventy 1956; Young *et al.* 1997). Pilchard stocks may thus be highly significant for juvenile southern bluefin tuna that appear to migrate between the Great Australian Bight and the southern Indian Ocean before finally moving into to their spawning grounds in Indonesia. Research on the closely related northern bluefin tuna (*Thunnus thynnus*) suggest that local abundance of that species may be positively correlated with the local abundance of *Sardinops sagax* (Polovina 1996). It is also possible, however, that fishing-induced reductions in stocks of southern bluefin tuna in the Great Australian Bight may have resulted decreased predation rates on pilchards and perhaps lead to expansion of the population.

There are important fisheries for yellowfin tuna (*Thunnus albacores*) and developing fisheries for species such as long-tailed tuna (*Thunnus tongal*) off the east coast of Australia. Catches of several by-catch species, e.g. broad-billed swordfish (*Xiphias gladius*), are also expanding rapidly and there are some data to suggest that these species may feed on clupeoids (Baker 1966; Kailola *et al.* 1993). Fishing grounds for these species are, however, located offshore and long distances from Queensland's developmental fishery or the small fisheries for clupeoids in New South Wales waters. Several species of tunas are caught by recreational fishers in southern Queensland and New South Wales, but the diversity of food sources available to and apparently used by these taxa suggests that sustainable fisheries for clupeoids may have insignificant effects on these stocks.

Australian salmon and tailor are ecologically similar species (Kailola *et al.* 1993). Eastern Australian salmon (*A. trutta*) occur in waters of Tasmania, eastern Victoria and southern New South Wales and are known to feed predominantly on euphausids (Malcom 1959; Stanely 1980), whereas western Australian salmon (*A. truttacea*) are distributed between Western Australia and eastern Victoria and commonly feed on clupeoids (Hoedt and Dimmlich 1994; Cappo 1987a,b). Preliminary data on *A.*

truttacea in South Australian waters suggest that this species may consume approximately 13 500 t of pilchards annually (Jones *et al.* 1996), i.e. more than the 1998 Total Allowable Catch for the South Australian pilchard fishery (11 500t). Aerial surveys show that areas in which Australian salmon are highly abundant coincide with areas where pilchards are highly abundant and spawn (Cappo 1987b; Chapter 8). It is thus possible that in years in which large numbers of sub-adult Australian salmon are present in South Australian waters (Dimmlich and Jones 1997), stocks could be adversely affected by fishery-induced reductions in pilchard abundance. Few data are available on the composition of the diet, but tailor appear to be opportunistic feeders that utilise diverse array of prey types, including clupeoids.

Recreational fishers in southern Queensland have expressed considerable concern over the potential impacts of the developmental fishery on the abundance and availability of marlins (*Makira* spp) and sailfish (*Istiophorus platypterus*) (J. Pepperell pers. comm). Although these species eat clupeoids, their nutritional reliance on these stocks is poorly understood.

Mackerels are opportunistic feeders that eat a wide variety of prey, including clupeoids (Begg 1996).

In south-eastern Australian waters barracouta (*Thyrsites atun*) feed predominantly on euphausids whereas in South Australian and Western Australian waters they feed mainly on clupeoids (Blackburn 1957). In South Australian waters, snook (*Sphyraena novahollandiae*) and leather jackets (*Nelusetta ayrandi*) are known to eat pilchards (Grove-Jones and Burnell 1990; Bertoni 1997).

In Bass Strait, clupeoids are an important component of the diet of arrow squid, *Nototodarus gouldii*, and are more commonly eaten during February than in other months (O'Sullivan and Cullen 1983).

Topics for Future Research

Investigations of the interactions between clupeoid and tuna stocks in Great Australian Bight are identified as a globally and locally important priority for future research. Research on Australian salmon, tailor and squid, particularly the potential effects of expanding pilchard fisheries on local stocks, are considered to be a moderately high priority in Western Australia, South Australia, Victoria, New South Wales and southern Queensland. Potential impacts on stocks and catches of billfishes and tunas must be evaluated if fishing effort increases markedly in southern Queensland and/or New South Wales. Research on mackerels, barracouta and snook is currently assessed to be of low priority.

3.3 Seabirds

Social and Economic Importance

Seabirds (i.e. avians that obtain all or most of their food from the ocean) are highly visible components of pelagic ecosystems (Nettleship *et al.* 1984; Cairns 1987, 1992; Nettleship 1991; Montevecchi *et al.* 1988; Montevecchi 1993). Many species are protected by international treaties, such as the Bonn Convention (Blaber 1996), and relatively few species are currently harvested for commercial purposes. Seabirds are, however, important natural assets that help support valuable tourist industries, e.g. little penguins (*Eudyptula minor*) in Phillip Island (Victoria). Expanding interest in eco-tourism suggests that the economic and social value of breeding colonies on offshore islands is likely to grow.

Operational Interactions

Seabirds do not usually become entangled in purse-seine nets. The major operational interaction between seabirds and fisheries for clupeoids is the utilisation of fish encircled by or escaping from purse-seine nets. Seabirds that have been reported taking pilchards and/or anchovies from catches include gulls, several species of terns, boobies, gannets and shearwaters. The ecological effects of this phenomenon are poorly understood but may have implications for the breeding success and learning patterns of juvenile seabirds (see Montevecchi and Myers 1997).

Potential Ecological Interactions

Many of the 120 species of seabirds that occur in waters adjacent to southern and eastern Australia feed on clupeoids, but few data are available on the composition of their diets (Barker and Vestjens 1990; Smith 1993). Data available suggest that relatively few seabirds eat only one species of fish. Field observations suggest that feeding preferences of most seabirds may be more related to prey size than prey type (e.g. Blaber *et al.* 1995). Relatively small birds, especially terns, shearwaters and petrels, commonly feed on cephalopods, crustaceans and small clupeoids such as anchovies, sprats and juvenile pilchards (Ross *et al.* 1996). Larger birds, such as gannets, albatrosses and boobies commonly feed on adult pilchards and round herrings (Wingham 1985; Ross *et al.* 1996).

Many overseas studies have identified the relationship between fluctuations in baitfish stocks and seabird populations (e.g. Furness and Cooper 1982; Crawford *et al.* 1983; Duffy 1983; Muck and Pauly 1987; Tovar *et al.* 1987; Berruti *et al.* 1989). For example, studies off the South African coast suggest that gannets, cormorants and penguins eat approximately 23% of the anchovy biomass (i.e. approximately 30% of the annual commercial catch) and declines in abundance of anchovy and round

herrings result in immediate decreases in the population sizes of these species (Crawford and Shelton 1978). Similar effects have been reported for species of Peruvian guano birds (Tovar *et al.* 1987).

Numerous overseas studies have implicated commercial fisheries as the causal agent for declines in fish stocks and, subsequently, seabird populations. For example, seabird numbers decreased by more than an order of magnitude during development of the Peruvian anchovy fishery (Furness and Cooper, 1982) and, following the collapse of stocks in 1972 (probably through a combination of 'El Nino' and overexploitation), guano bird populations fell to around 20% of their former size (Muck and Pauly 1987). Similarly, Duffy (1983) found a strong inverse relationship between annual fishery landings and the percentage increase in the seabird population; 72% of the variation in the annual percentage of population increase was explained by fluctuations in commercial fishing. The work of Crawford and Shelton (1978) also revealed "tendencies for overfishing to result in decreasing seabird numbers".

Cairns (1987) reviewed the effects of depleted food supplies on seabirds and concluded that only extreme food shortages cause significant adult mortality. Poor to moderate availability of food can reduce adult body weight, clutch size, breeding success, growth rates of chicks, colony attendance and guano production (Cairns 1987, 1992). For example, reductions in the abundance of anchovies off the west coast of North America were correlated with reduced breeding success of brown pelicans (Anderson *et al.* 1980). In addition, many populations of Peruvian guano birds did not breed after the collapse of anchovy stocks (Tovar *et al.* 1987).

Likely Effects on Predatory Species

Little penguins (*Eudyptula minor*) are the most abundant penguin in Australian waters. Islands between Sydney (New South Wales) and Fremantle (Western Australia) support globally-significant breeding colonies. This species has been subjected to considerable research in Bass Strait waters where clupeoids comprise a major component of the diet (Gales and Pemberton 1990; Cullen *et al.* 1992). Gales and Green (1990) estimated that little penguins in Bass Strait consume approximately 25 000 tonnes of clupeoids per annum. Breeding success has been related to annual variations in clupeoid abundance (Cullen *et al.* 1992; Hobday 1992).

Approximately eight species of shearwaters are commonly observed above the seas of southern Australia. Shearwaters mainly feed on planktonic crustaceans and cephalopods, but also eat small pelagic fishes and commonly eat trawl discards and pilfer fish from purse-seine nets (Copley 1996). Species such as the fluttering shearwater (*Puffinus gavia*), Hutton's shearwater (*P. huttoni*), Buller's shearwater (*P. bulleri*) do not breed in Australian waters, whereas the little shearwater (*P. assimilis*)

and wedge-tailed shearwater (*P. pacificus*) mainly breed on islands off Australia's western and north-eastern coasts. Three species of shearwaters have breeding colonies in areas that support purse-seine fisheries: sooty shearwaters (*P. griseus*) breed on islands off Tasmania and New South Wales; all known breeding colonies of short-tailed shearwaters (*P. tenuirostris*) lie between St Franscis Island (South Australia), southern Tasmania and Broughton Island (New South Wales); flesh-footed shearwaters (*P. caneipes*) breed in the south-western and eastern Australia, may be susceptible to the ecological effects of expanding pilchard fisheries in southern and eastern Australia.

Albatrosses are generally wide-ranging birds and several species, including the wandering albatross (*Diomedea exulans*), black browed albatross (*D. melanophrys*) and yellow-nosed albatross (*D. chlororhynchos*), are common throughout southern Australia. Most species feed on a wide variety of fishes, cephalopods and crustaceans (Frith 1977). Several species, e.g. the sooty albatross (*Phoebetria fusca*) and grey-headed albatross (*D. chrysostoma*) are commonly seen in the Great Australian Bight. Although the shy albatross, *D. cauta*, breeds on islands in Bass Strait and off southern Tasmania, relatively few albatrosses breed in Australian waters (most breed on sub-Antarctic islands) and no species seems likely to be significantly effected by expansion of the pilchard fisheries in southern and eastern Australia.

Although several species of petrels, fulmars and prions are sighted in southern Australia (Frith 1977), there are relatively few breeding colonies in the region. Gould's Petrel (*Pterodroma leucoptera*) breeds on Cabbage Tree Island off Port Stephens (New South Wales) and the fairy prion (*Pachyptila tutur*) breeds on islands in Bass Strait. Most species feed on small and juvenile clupeoids, as well as planktonic invertebrates, and the local breeding success of these two species could potentially be affected by the expansion of clupeoid fisheries.

Numerous species of terns occur in coastal waters of southern and eastern Australia. Species such as the crested (*Sterna bergii*) and Caspian tern (*Hydroprogne caspia*) commonly eat clupeoids. Some colonies of these species in South Australia are located close to the pilchard fishery and breeding success could be affected by the fishery's recent expansion (Copley 1996).

Australasian gannets (*Morus serrator*) are common in southern Australia are are often used by fishers to locate schools of pilchards. There are important breeding colonies on islands off Victoria and Tasmania, but the largest are in New Zealand. Feeding regimes vary between locations and seasons, presumably in response to the differences in relative abundance of pelagic fish species. Gannets often feed on clupeoids and carangids that are also targeted by commercial fishers (e.g. Brothers *et al.* 1993;

Norman and Menkhorst 1995). For example, pilchards comprise over 50% of the diet of gannets breeding on islands within Port Phillip Bay. Populations of the closely related South African gannet (*M. capensis*) have been adversely affected by declines in the abundance of clupeoids (Crawford 1991; Crawford *et al.* 1992).

Brown (*Sula leucaster*) and masked (*S. dactylatra*) boobies are commonly sighted above coastal and offshore waters of southern Queensland and northern New South Wales. Both mainly feed on pelagic fish and squid (Blaber *et al.* 1995). Brown boobies are commonly observed feeding on pilchards and in southern Queensland are often used by fishers to locate schools (T. Ward, personal observation). Major breeding colonies are found on offshore tropical islands. The potential impacts of clupeoid fisheries in southern Queensland and northern New South Wales are, therefore, probably low.

Topics for Future Research

Although fluctuations in clupeoid abundance can often be attributed to variations in local productivity (Michelson *et al.* 1992), the potential effects of heavy fishing in 'good years' on the long-term viability of colonies of seabirds requires further study. Such studies are particularly urgent for little penguin colonies in South Australia, where few quantitative data have previously been collected and where there is a relative paucity of alternative prey species (see Chapter 4). Continued monitoring of colonies on Phillip Island (Victoria) and Lion Island (New South Wales) is also recommended. Monitoring programs for colonies of several other species of seabirds in South Australia, Victoria, Tasmania and New South Wales need to be expanded. Species that should be given particularly high priority are Australasian gannets, flesh-footed shearwaters, wedge-tailed shearwaters, short-tailed shearwaters, Gould's petrel, fairy prions, Caspian terns and crested terns.

3.4 Mammals

Social and Economic Importance

The social importance of marine mammals is reflected by the large number of state and federal laws (e.g. *Commonwealth Whale Protection Act 1980*) and international conventions and agreements (e.g. International Whaling Commission's *Southern Ocean Sanctuary*) that ensure their protection. Similarly, the economic value of marine mammals is indicated by the success of eco-tourism ventures based on Australian fur seals (*Arctocephalus pusillus*) on Phillip Island, Australian sea lions (*Neophoca cinerea*) on Kangaroo Island, bottle-nosed dolphins (*Tursiops truncatus*) off Stradbroke Island, Indo-Pacific dolphins (*Sousa chinensis*) in Tin Can Bay, humpback whales (*Megaptera novazealandiae*) in Hervey Bay, southern right whales (*Eubalaena australis*) in the Great Australian Bight and Victor Harbor (South Australia) and Portland/Warnambool (Victoria).

Operational Interactions

Delphinus delphinus and *T. trucatus* have been encircled and drowned in purse-seine fisheries throughout Australia. The number of dolphins killed is generally small, but catch rates per set may be relatively high, and thus the implications for Australia's small coastal populations could be significant. Some anecdotal evidence (D. Gaughan, personal communication) suggests that dolphin catches in Western Australia have decreased over time, perhaps as pods learn to avoid purse-seine nets. International examples, such as the capture of *Stenella attentuata* and *S. longirosteris* in the eastern Pacific purse-seine fishery for yellow-fin tuna (*T. albacares*), clearly show that significant reductions in catch rates can be achieved by advances in gear technology and increases in the skills and knowledge of fishing crews (see McNeely and Holts 1977; Coe *et al*. 1984).

Little is known of the interaction between seals and fisheries in Australian waters, although there have been a few reports of seals becoming entangled in purse-seine nets (Shaughnessy and Davenport 1996). The interaction between Cape fur seals (*A. pusillus*) and the South African purse-seine fishery is well known (Shaughnessy 1984). Hundreds of seals move into the encircling net, eat fish, and become entangled. Efforts to mitigate these effects have been only moderately successful.

Potential Ecological Interactions

Information on the diets of marine mammals are generally sparse. Most data that are available have been obtained from carcasses and may be subjected to biases associated with the cause of death, e.g. old age, stranding, etc. Many species commonly feed on schooling pelagic fishes and potential for ecological interactions with clupeoid fisheries may be relatively high. Most species are, however, relatively opportunistic and may readily switch prey. It seems likely that the effects of expanded fisheries are most likely to be reflected in breeding success.

Likely Effects on Predatory Species

Few quantitative data are available on the diets of the Australian fur seal (*A. pusillus*) in Australian waters. Pilchards made up almost 50% of the diet of *A. pusillus* in South African waters and annually consume similar quantities of pilchards to that taken by the purse-seine fishery (Crawford *et al.* 1987). All of Australia's major breeding colonies of the Australian fur seal are located in Victorian waters, on islands such as the Skerries Group, Norman Island, Kanowna Island and Anderson's Islets in Wilson's Promontory Marine Park (Marsh *et al* 1993; Pemberton and Kirkwood 1994). Expansion of the Bass Strait component of the Victorian pilchard fishery may have implications for these populations.

Few quantitative data are available on the diets of the New Zealand fur seal (*A. forsteri*) in Australian seas. There are globally significant breeding sites of this species on Neptune and Kangaroo Islands (South Australia) (Shaughnessy *et al.* 1995). Anecdotal evidence suggest that high pup mortalities may have resulted from the mass mortality of pilchards that occurred in 1995 (Shaughnessy *et al.* 1996). The expansion of the South Australia pilchard fishery into offshore waters could therefore potentially effect breeding colonies of this species.

The Australian sea lion (*Neophoca cinerea*) is endemic to South Australia and Western Australia, and is listed as Rare by the International Union for the Conservation of Nature, Rare in South Australia, a Specially Protected Species in Western Australia and as Lower Risk, Near Threatened in the Action Plan for Australian Seals (Dennis and Shaughnessy 1996). Its geographic range, which previously included Bass Strait, and population size have declined since European colonisation (Warneke 1982). Approximately 70% of the population now lives in South Australian waters, but monitoring programs are only in place for Kangaroo Island, the Pages Islands and Dangerous Reef (Gales *et al.* 1994. There is some evidence to suggest that South Australian populations of this species may be affected by declines in the abundance of pilchard stocks caused by expansion of the purse-seine fishery. For example, high mortalities of pups on Dangerous Reef and the Pages Islands were temporally coincident with the 1995 pilchard mortality event (Shaughnessy *et al.* 1996).

Few data are available on the diet of the common dolphin (*D. delphinus*) in Australian seas, but gut contents of stranded and entangled specimens from South Australia suggest cephalopods may be more commonly eaten than pilchards (Kemper and Gibbs 1997). In South Africa, however, *D. delphinus* often feeds on pilchards and anchovies (Crawford *et al.* 1992).

Tursiops truncatus is often seen feeding on pilchards schools and data available from gut analyses suggest pilchards are an important component of the diet of this species (Kemper and Gibbs 1997). The importance of pilchards to populations of this species is, however, difficult to assess as this species is an opportunistic feeder that readily switches to alternative prey.

Several species of baleen whales, e.g. southern humpbacks and southern right whales, have been observed feeding around schools of baitfish but is not known whether these whales were actually feeding on baitfish or plankton (P. Corkeron pers. comm). There is, however, clear evidence of humpback whales in the Northern Hemisphere feeding on herring and capelin (Baker *et al.* 1992).

Topics for Future Research

Breeding success of colonies of Australian fur seals, New Zealand fur seals and Australian sea lions should be monitored as part of ongoing assessment of the ecological effects of expanding pilchard fisheries in Victoria and South Australia. Colonies in the Great Australian Bight may provide an important genetic link between eastern and western populations (Dennis and Shaughnessy 1996). The need for research on Australian sea lions and their interactions with commercial fisheries was identified as a priority in the Great Australian Bight Marine Park Management Plan (DENR 1997).

Research is urgently required into methods for reducing the entanglement of marine mammals, especially dolphins in purse-seine nets. Additional information on the importance of pelagic fishes in the diets of dolphins, humpback whales, southern right whales and other baleen whales, are needed although it may be prohibitively expensive to acquire using normal means. Methods that utilise stable isotopes (e.g. Gales and Green 1990) are particularly suitable for investigating this issue.

3.5 Discussion

Regional Issues

The potential effects of purse-seine fisheries on pelagic ecosystems vary between regions. A summary of the key predators of clupeoids in southern and eastern Australia is shown in Figure 2. In the four mainland states of south-eastern Australia different issues and priorities can be identified for researchers and managers responsible for assessing and mitigating possible adverse effects of expanding clupeoid fisheries.

In southern Queensland, the potential effects on valuable recreational and commercial fishes such as marlins, sailfish, tunas, mackerels and tailor require further investigation before a fully licensed (and potentially expanded) fishery is established. Seabird research is not a priority in southern Queensland as there are no significant breeding in that region. There is, however, a clear need for research on procedures for mitigating operational effects on *T. truncatus*. In addition, increased information are required on the diets and behaviour of migrating humpback whales.

The pilchard fishery in New South Wales has remained relatively stable in recent years (Dixon *et al.* 1996) and unless effort increases rapidly research on its ecological effects will not be considered a high priority for that state. Potential effects on fishes and marine mammals in New South Wales are generally similar to those in southern Queensland but New South Wales waters support important breeding colonies of several seabirds, e.g. shearwaters and little penguins, that may be vulnerable to a variety of anthropogenic effects.

In Victorian waters there are important and valuable populations of prions, shearwaters, petrels, gannets, penguins, pinnipeds that seem likely to provide the state with increasing revenue via eco-tourism and must be included in assessment of the potential impacts of expansion of the clupeoid fishery into Bass Strait. Investigations of ecological effects on populations of predatory fishes (e.g. southern bluefin tuna, Australian salmon) may be most useful if they are conducted in conjunction with similar studies in South Australian waters.

In South Australia, considerable attention must be given to potential impacts of the purse-seine fishery on severely depleted stocks of economically-important southern bluefin tuna, socially-significant stocks Australian salmon, locally-significant populations of little penguins, crested terns and Caspian terns, and globally-important breeding colonies of the short-tailed shearwaters, New Zealand fur seals and Australian sea lions.

Management Options

To date, management arrangements for Australian fisheries have largely focused on a single target species, although some consideration has been given to species with particular conservation or other social value that are incidentally captured by fishing gear, such as turtles in prawn trawls (e.g. Poiner *et al* 1990; Robins 1995), dugongs in seine and gill-nets (H. Marsh, personal communication) and marlins and seabirds on commercial long-lines. No current management strategy for any Australian fishery incorporates an ecological allocation of biomass, mainly because the data required to estimate the appropriate size of such an allocation are simply not available. The need to provide ecological allocations is most pressing for commercially-important species, such as southern bluefin tuna, and species with particular conservation needs, such as seabirds and pinnipeds, whose populations have been adversely affected by other human impacts.

Research Options

A wide range of techniques have been developed for modelling complex multi-species fisheries interactions (see Overholz *et al.* 1991). For example, Pope (1979) developed Schaefer's surplus-production model to account for predator-prey and competitive relationships between species. Multi-species virtual population analyses have been used to analyse the interactions between predator and prey populations in the Northern Hemisphere (e.g. Walters *et al.* 1986) and have been described as the 'flagship of multi-species research' (Shelton 1992). Age-structured simulation models have also provided important insights into the mortality rates of fish prior to their recruitment into fisheries (Overholz *et al.* 1991). Recently developed ecosystem modelling packages, such as ECOPATH and ECOSIM, have become widely available and although still crude appear to provide a simple and

useful framework from which to begin investigations of ecosystem function (Polovina 1984; Walters *et al.* 1997).

Acquisition of the detailed knowledge of ecosystem function that is required for the development of integrated multi-species fishing strategies necessitates the expansion of classical (single species) fisheries studies to include investigations of issues as diverse as oceanographic factors, plankton assemblages, predatory species and patterns of energy flow. Concurrent studies of the biological requirements and breeding success of predators, such as seals and seabirds, will be particularly important in some areas. The success of these investigations will largely depend upon the development of good interactions with non-fisheries organisations, e.g. universities and wildlife agencies, and may require the acquisition of funds from organisations that typically support conservation orientated research (e.g. National Heritage Trust and World Wide Fund for Nature).

Population parameters obtained from colonies of seabirds and seals may act as useful indicators of fish abundance and thus provide valuable insights into the effects of commercial fisheries on ecosystem function (e.g. Cairns 1987, 1992). Studies that use stable isotopes to identify prey types and consumption/metabolic rates are cost-effective and may provide particularly valuable insights into ecosystem trophodynamics (Gales and Green 1990). The disturbance effects associated with dedicated studies mean that these parameters can most appropriately be used to identify temporal changes in food availability at particular locations. Seabirds and seals, like commercial fishing fleets, concentrate on local prey aggregations and use selective fishing methods, however, certain parameters may prove suitable for developing abundance indices (see Hoedt and Dimmlich 1995). These may supplement tools typically used by fisheries biologists to monitor spatial and temporal variations in fish stocks. For example, species with preferences for small prey may provide useful information on recruitment success and improve estimates of natural mortality.

Conclusions and Recommendations

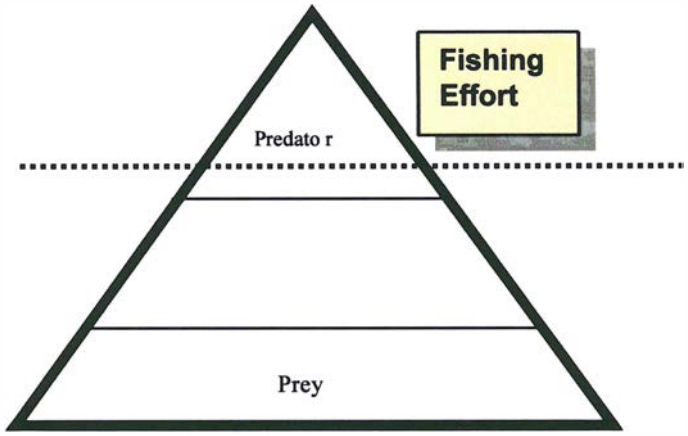
The need for ecological research is most pressing in South Australian waters, that now support Australia's largest pilchard fishery as well globally-significant populations of Australian sea lions, little penguins and southern bluefin tuna.

Studies of natural fluctuations in the abundance of clupeoids are urgently required and will provide most valuable insights if they include detailed investigations of oceanographic parameters and plankton assemblages, and their effects on clupeoid recruitment and abundance.

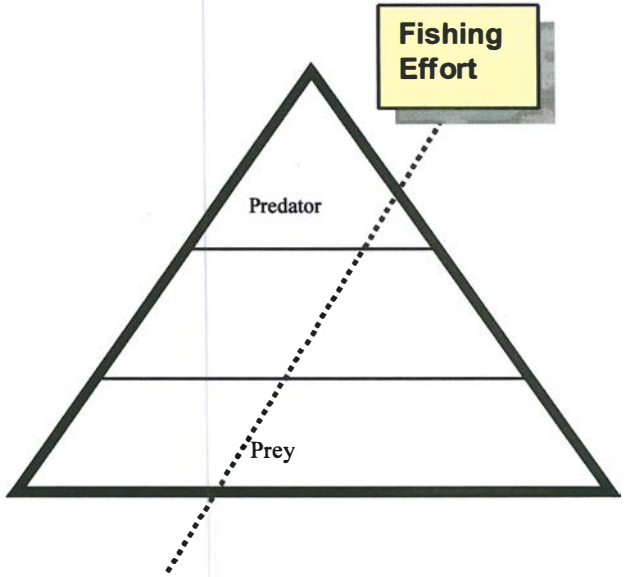
Investigations of trophic relationships, e.g. nutritional sources and consumption rates, will be enhanced by use of procedures that involve the use of stable isotopes.

Acquiring the data required to apply numerical approaches to investigation of predator-prey relationships, e.g. multi-species virtual population analyses, may be easiest in regions with a relatively small number of species (e.g. South Australia).

Monitoring populations of species with reproductive strategies that include a terrestrial phase, e.g. pinnipeds and seabirds, may provide valuable insights into effects of fisheries on ecosystem function.



Exclusive fishing of upper trophic levels



Fishing down the food web

Figure 3.1 Exclusive fishing of upper trophic levels versus 'fishing down the food web'.

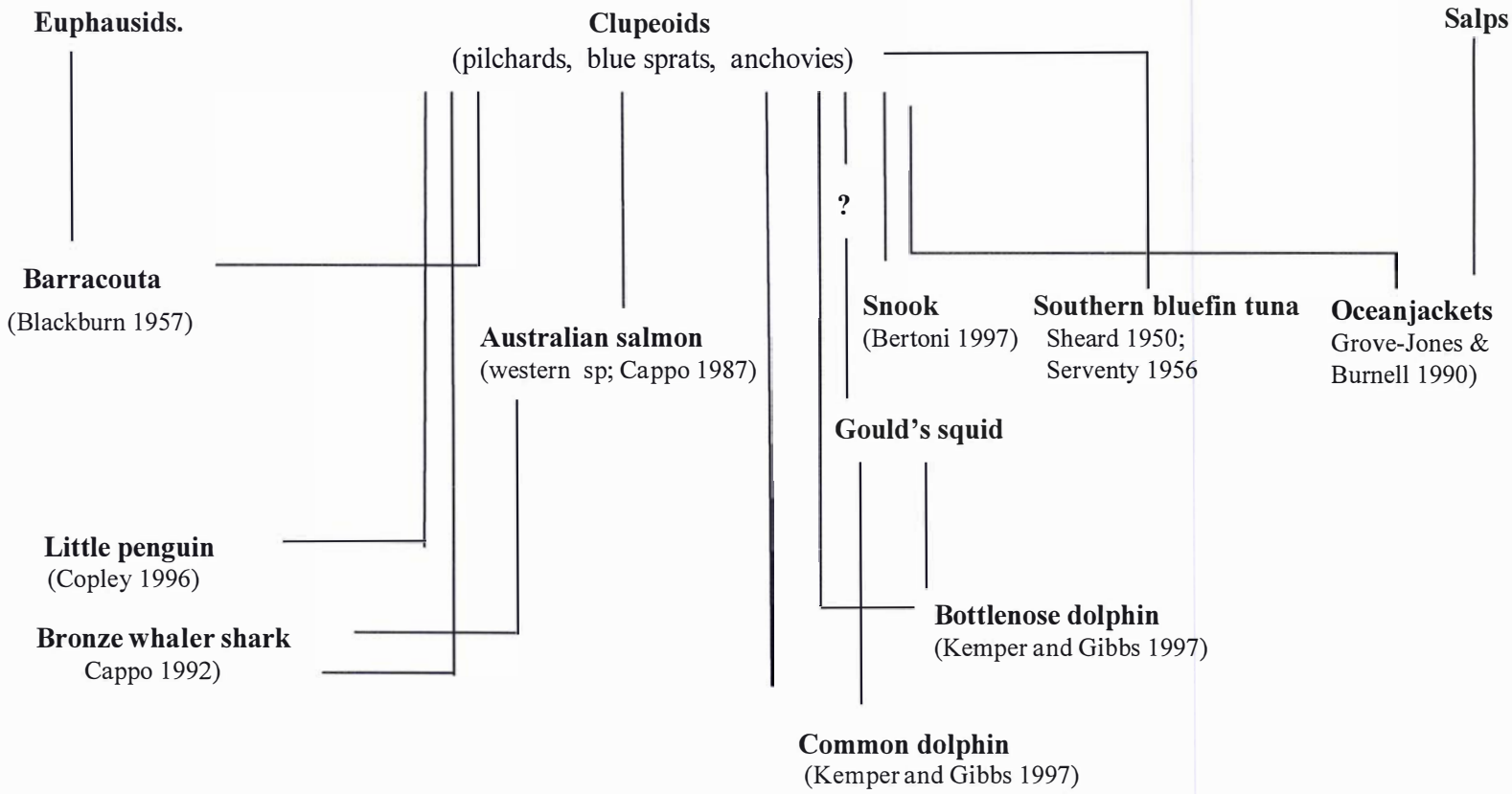


Figure 3.2A Marine food web in South Australian waters, with special reference to clupeoids.

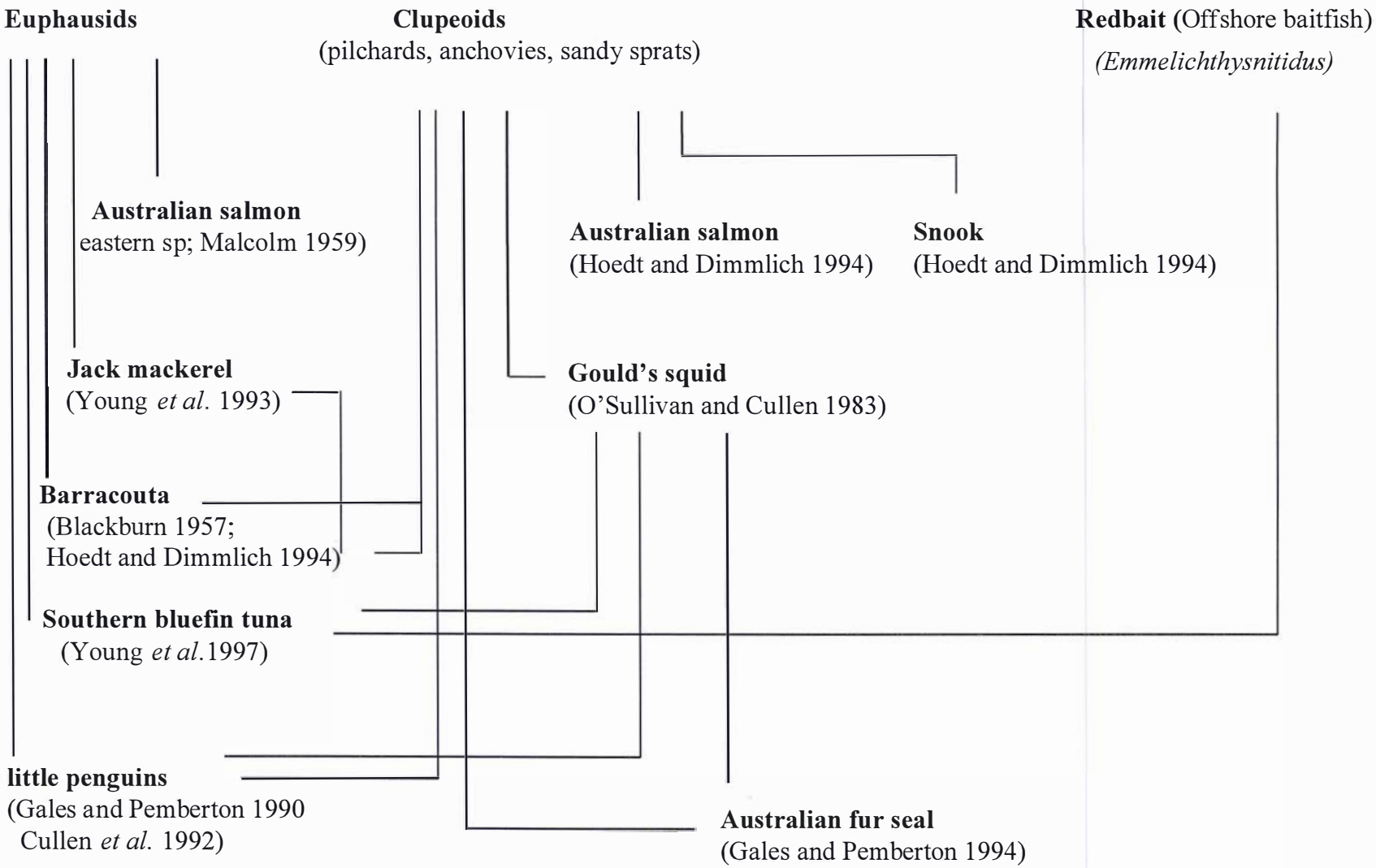


Figure 3.2B Marine food web in Victorian/Tasmanian waters, with special reference to clupeoids.

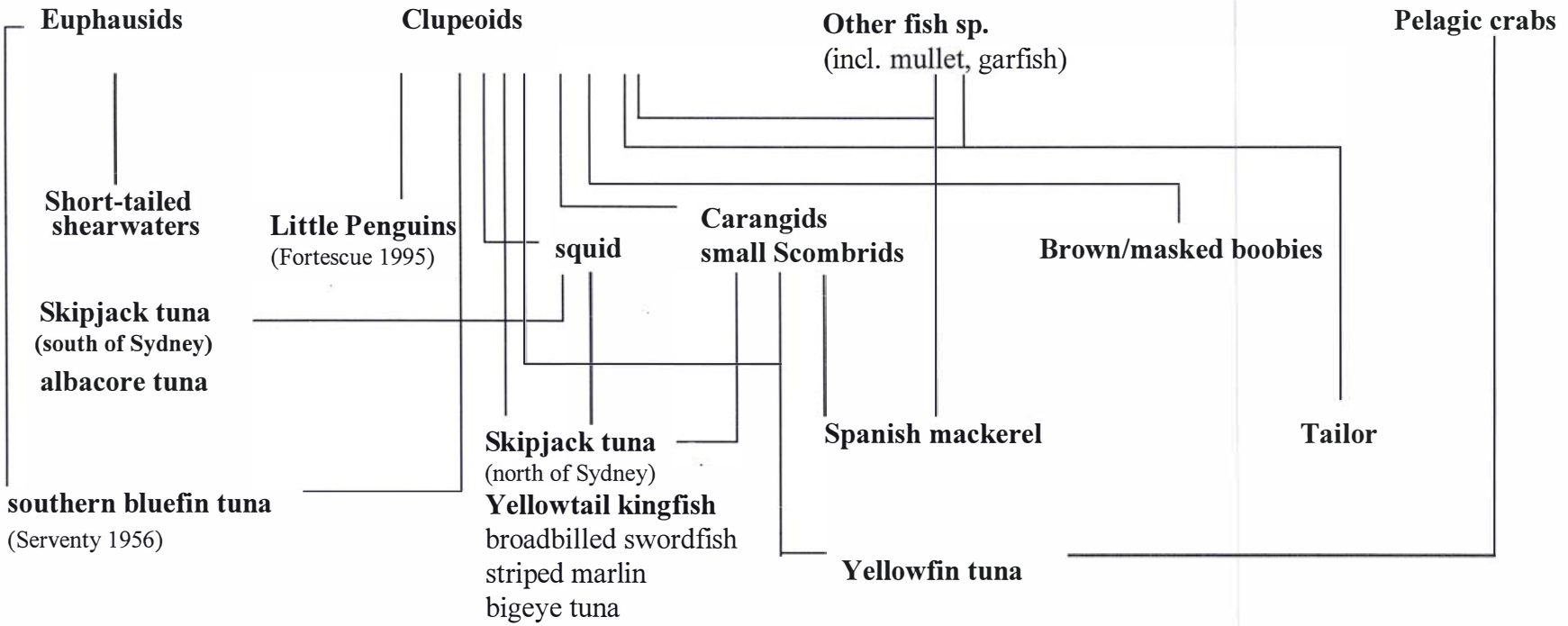


Figure 3.2 C Marine Food web in Eastern Australian (NSW & Qld) waters, with special reference to clupeoids. (Dietary information on fish species mainly based on species summaries in Kailola et al, (1993) unless otherwise noted)

CHAPTER 4. BAIT-FISHERIES OF SOUTHERN AND EASTERN AUSTRALIA

G. Jackson, F.J. Neira, G.K. Jones, T.M. Ward and M. Kinloch

Objective: To describe and compare the historical background, current status, management approach and fishing methods for baitfisheries of South Australia and Victoria, and to analyse catch/effort and size/age composition data for pilchard fisheries in Spencer Gulf, Coffin Bay, Port Phillip Bay and Lakes Entrance. This objective was achieved by collating historical information from both states and by collecting samples of commercial catches taken between December 1994 and April 1997. Age structure of catches was determined from otoliths sent to the Central Aging Facility at Marine and Freshwater Resources Institute (Victria) (see Chapter 5). There is a large pilchard fishery in South Australia and smaller fisheries for pilchards and anchovies in Victoria. Immature pilchards dominate catches from Spencer Gulf and Port Phillip Bay, but are generally less common than mature fish in catches from Coffin Bay and Lakes Entrance. Both fisheries are driven by local demand and most catches are taken near home ports and markets. The possibility of localised depletion of stocks cannot be discounted and it is essential that monitoring of the size/age composition of catches continues in both states. The South Australian pilchard fishery is currently managed using output controls (Total Allowable Catches and Individual Total Quotas) determined from stock assessments presented in this report (Chapters 8 and 9). In contrast, there are no management restrictions on the Victorian pilchard fishery. There is a clear need for quantitative data on the stock size and spawning patterns of pilchards in Victorian waters. Some studies have been conducted on the ecological effects of the fishery in Port Phillip Bay, and such studies are urgently required in South Australia.

4.1 Methods

General

This chapter describes the fisheries for pilchard (*Sardinops sagax* Steindachner), anchovy (*Engraulis australis* White), blue mackerel (*Scomber australasicus* Cuvier), and jack mackerel (*Trachurus declivis* Jenyns) in South Australian and Victorian waters. It discusses the history and markets, management, vessels and gear, and effort, catches and catch-per-unit-effort (CPUE) of the fisheries for each species, and describes the length frequencies and age composition of the pilchard fisheries. Baitfisheries of New South Wales have recently been reported elsewhere (Dixon *et al.* 1996). Information for Queensland will be submitted to the Fisheries Research and Development Corporation funded as part of the final report on a separate project (95/043).

Collection and Analysis of Catch Statistics

South Australian purse-seine fishers are legally required to complete monthly catch and effort forms which record daily catch, the number of boat-days fished in the month, the area fished and the number of crew on board each day. The number of days fished includes days spent searching for schools, even if no fish were caught. Since the commencement of the developmental fishery in November 1991, data for all active licence holders have been summarised annually using the general marine scale-fish catch and effort database *GARFIS*. Monthly and annual summaries of the data were transferred to Excel spreadsheets for analysis and graphical display. Catch and effort data from these forms were compared with the catches recorded from the quota monitoring program, and no discrepancies were found. Catch and effort of live bait in South Australian waters is recorded from southern bluefin tuna fishing log sheets. These data were supplied to South Australian Research and Development Institute by Australian Fisheries Management Authority as an Excel spreadsheet. These data have not been validated and their reliability is unknown.

Commercial catch and effort data for pilchard and anchovy fisheries in Port Phillip Bay and throughout Victorian waters are available from 1935 and 1978 respectively, and were collated from fishing returns completed each month by commercial fishers and archived in the Catch and Effort Unit at Marine and Freshwater Resources Institute (Victoria). These data were used to provide summaries of total monthly and annual catches for purse-seine and lampara nets combined. Catch rates (kg/day) were calculated only for purse-seine vessels since other fishing gear (e.g. beach, estuarine, haul seines) are infrequently used in these fisheries.

Catch Sampling.

Catches of South Australia's pilchard purse-seine fleet were sampled between March 1995 and May 1997 to obtain information on the size/age-structure of the catch and seasonal changes in gonadal development. The length to the caudal fork (LCF) of each fish was measured and otoliths were removed and forwarded to the Central Ageing Facility (Victoria) for age determination. Ages were determined for almost 1300 pilchards ranging from 9.5 to 19.0 cm LCF. Age-length keys were developed and applied to the length frequencies to determine the age-structure of the total catch from Spencer Gulf and Coffin Bay in 1995, 1996 and 1997.

For the Victorian fisheries, random monthly samples of commercially caught pilchards were obtained from the Melbourne Fish Market, fishers and fish processors. Totals of 11 176 fish from Port Phillip Bay and 1 205 fish from Lakes Entrance were measured between December 1994 and September

1996. Otoliths were removed from 1773 pilchards (1168 from Port Phillip Bay and 605 from Lakes Entrance) and sent to the Central Ageing Facility for analysis of age and growth (see Chapter 6).

4.2 Results

Pilchards

History and Markets

In South Australia, pilchards have been caught in the bays of southern and western Eyre Peninsula since the early 1960s when a seasonal, small-mesh lampara net fishery was developed to provide live bait for the offshore southern bluefin tuna pole-and-line fishery. Three main areas were fished: Boston Bay, Coffin Bay and Streaky Bay. Although pilchard was the main species taken, small quantities of juvenile Australian herring (*Arripis georgianus*) and jack mackerel (*Trachurus declivis*) were also caught. The quantity of pilchard used for bait in South Australia, as determined from southern bluefin tuna catch returns, ranged between 12 and 200 t per annum from 1983 to 1997 (T. Skousen, Australian Fisheries Management Authority, personal communication). These figures are believed to substantially underestimate bait usage. Calculations based on the annual landings of southern bluefin tuna at the fishery's peak in the early 1980's, together with estimates of the amount of bait required to catch such quantities, suggest that up to 1700 t of pilchard per annum were harvested (Mackie 1995). Catches for the pole-and-line pilchard fishery have declined in recent years due to the reduction in tuna quotas and the development of the southern bluefin tuna mariculture industry. Since 1991, the South Australian purse-seine fishery has supplied pilchards (as fodder) to the southern bluefin tuna mariculture industry around Port Lincoln.

Commercial pilchard fishing in Victorian waters started in Port Phillip Bay around 1935. Prior to 1949, small catches (<100 t per annum) were taken with hoop nets and small-mesh haul-seine nets, and sold mainly as bait to recreational fishers (Hall and MacDonald 1986). Catches in the bay began to increase in 1949, when lampara and purse-seine nets were introduced (Winstanley 1979). The pilchard fishery is regarded as Victoria's largest inshore fishery in terms of catch by weight. The recent increase in pilchard catches is primarily due to the increasing demand for a low value fish for pet food, although the demand for feed for tuna farms, for commercial and recreational bait and, to a lesser extent, human consumption, has also contributed to this trend. The pet food industry consumes about 80% of the Victorian pilchard catch. The preferred size of pilchard for pet food is between 18 and 12 cm LCF, while fish of around 10 cm LCF are preferred for the bait market.

Management

Since its inception, the South Australian purse-seine fishery has been managed under a policy of output controls involving an annual total allowable catch and individual transferable quotas. The fishery is a limited-entry experimental fishery and, since 1994, has been comprised of 14 licence holders with permits to use purse-seine nets (400 m long, 50 m drop and minimum mesh size of 12 mm) to target clupeoids (Mackie 1995). During the last six years pilchards have comprised almost the entire catch. The total allowable catch was set at 1200 t for the 1991/92 season, but was increased to 3500 t for the period of the experimental fishery (1993-96) (Mackie 1995). At the beginning of each year, each licence holder is granted a 250 t Individual Total Quota, part or all of which can be traded between ministerial permit-holders. There are restrictions in the catches that can be taken from the inner region of Boston Bay and Streaky Bay (500 t per annum). These restrictions were imposed to protect the traditional (lampara) live-bait netting operations in these areas.

There are no current management restrictions on catches in the Victorian fishery. The number of active fishers increased from 14 to 28 between 1992/93 and 1994/95. Currently only one vessel operates from Lakes Entrance, while two additional permits were issued for central and western Victorian coastal waters in 1996/97.

Vessels and Gear

Pilchard purse-seine vessels in South Australia range from 10 to 23 m in length. Fishing generally takes place at dusk, although some fishers continue to work through the hours of darkness depending upon the availability of suitable aggregations of pilchards, which may be influenced by factors such as moon phase (Jennings 1996). Most of the fleet lack on-board freezers and the catch is either boxed on deck or stored below in chilled seawater or ice-slurry. From 1991 to 1996, the bulk of the catch was frozen ashore and stored in Port Lincoln freezers, although more recently there has been a move towards locally caught pilchard being delivered fresh to the tuna farms. In 1996 and 1997, Port Lincoln pilchard fishers received approximately $650/t for pilchards sold to local tuna operators (South Australian Research and Development Institute, unpublished fish processor figures).

The two main pilchard fishing areas are off Port Lincoln in southern Spencer Gulf and near Coffin Bay on the west coast of Eyre Peninsula (Tables 4.1, 4.2; Figures 4.1, 4.2). During the early years of the developmental fishery, some fishers investigated the potential of fishing grounds throughout Spencer Gulf, on the far west coast and in Gulf St Vincent. Moderate quantities of pilchard were found at some of these locations, but distances from the tuna farms, coupled with lack of onboard freezers, have resulted in the recent concentration of fishing effort in areas closer to Port Lincoln.

In the last two decades, the commercial pilchard fishery in Port Phillip Bay has changed from a day fishery with small boats that relied on feeding birds to locate fish, into a mainly night fishery with 10-20 m vessels that use sonar (range 200-800 m) to locate fish schools. An advantage of night fishing is that pilchard schools are less likely to disperse. In addition, cooler night temperatures preserve the catch better and landing fish in the morning allows processors to pack fish throughout the day. Most fishers currently utilise purse-seines. According to fishers, the recording of either lampara or purse-seine nets on catch and effort returns is arbitrary and it is thought that no true lampara nets are used in Port Phillip Bay. Purse-seine nets normally vary between 300-600 m in length and 20-65 m in depth, and have 10-12 mm mesh in the bunt (pocket).

Fishing Effort

Between 1991 and 1994, the South Australian purse-seine fishery grew rapidly. Fishing effort peaked at 738 boat-days in 1994 (Table 4.1). During this period most effort was expended in Spencer Gulf. Between 1994 and 1997, the Total Allowable Catch was set at 3 500 t. Fishing effort fell to 368 boat-days in 1996, with most fishing conducted off the West Coast. The decrease in fishing effort was mainly due to improvements in the equipment and skills of fishers.

Monthly trends in fishing effort for the period 1994-96 for the main fishing blocks (27 – West Coast; 30 and 31 – Spencer Gulf) are shown in Figures 4.1, 4.2, 4.3, 4.4. Fishing effort peaked in February-April and was lowest in September-October. There were, however, temporal differences in patterns of effort in the three most important statistical fishing blocks (Figure 4.2). In the area closest to the tuna cages (Block 31) effort was highest during November-April, whereas offshore (Block 30) effort was high in August-September, and in Coffin Bay (Block 27) effort was highest in May-July.

Fishing effort in Port Phillip Bay decreased from 700 boat-days in 1992/93 to approximately 450 days in 1995/96 and 1996/97 (Figure 4.5). Fishing effort at Lakes Entrance was sporadic.

Catches

In most years, the highest annual catches occurred near Port Lincoln but in 1996 significant catches were taken from west coast waters, especially near Coffin Bay (Table 4.2). The rise in catches between 1991 and 1994 reflected the rise in the Total Allowable Catch; 1995 was the only year the Total allowable catch (3 500 t) was not reached. Between December 1991 and June 1997 monthly catches of pilchards fluctuated markedly, but catches were usually highest in the early part of the calendar year, particularly February to March (Figures 4.3 and 4.4). This pattern occurred every year in Spencer Gulf and in 1994, 1996 and 1997 in Coffin Bay. In 1995, catches in April and May

decreased to very low levels presumably due to the massive pilchard kill which occurred throughout South Australian waters in March to April 1995. In every year, catches declined throughout autumn and winter (April - August) and were lowest in September and October, before increasing in November and December (Figure 4.3). The seasonality of catches largely reflects the demand patterns of the tuna farms (S. Clarke, personal communication).

Nearly all of the commercial catch of pilchard in Victorian waters between 1978/79 and 1996/97 was obtained from Port Phillip Bay and coastal waters off Lakes Entrance in eastern Bass Strait, with comparatively insignificant catches from the remaining bays and inlets (Figure 4.5; Table 4.4). Annual pilchard catches in Port Phillip Bay and Bass Strait between 1991/92 and 1996/97 averaged 1495 t (64%) and 855 t (36%) respectively, representing nearly 100% of the Victoria's total catch for that period (Table 4.4; Figures 4.7 and 4.8).

Annual pilchard catches in Port Phillip Bay from 1935 show two distinct fishing periods, the first between 1935 and 1979 with catches below 500 t/yr, and the second from around 1980 with catches over 500 t/yr and rapidly increasing during the late 1980s and early 1990s (Figure 4.7). Pilchard catches in Port Phillip Bay between 1978/79 and 1996/97 increased from 220 t in 1978/79 to 1,443 t in 1988/89, decreased to 836 t in 1990/91 but increased again reaching a maximum of around 2,040 t in 1992/93 and 1993/94 (Figure 4.8 and 4.9). Catches in 1996/97 declined to 596 t, the lowest since 1984/85.

Pilchard catches off Lakes Entrance in Bass Strait declined from 742 t in 1978/79 to less than 0.12 t in 1981/82, and then rose to a peak of 4,841 t in 1983/84. This was attributable to one vessel operating for a South African owned company that processed baitfishes for meal and oil. The factory operated for about 10 years until early 1985. Catches subsequently decreased sharply to 2.5 t in 1987/88 but increased steadily thereafter and remained at around 1,000 t in 1994/95 and 1995/96 (Figure 4.8). Catches in 1996/97 dropped to 177 t, the lowest since 1987/88 (Table 4.4).

The total pilchard catch in Victorian waters in 1996/97 was 773 t, which represented a 64% decline from the total catch in 1995/96 (Table 4.4). Total catches from Port Phillip Bay (596 t) and Lakes Entrance (177 t) in 1996/97 represented a 67% and a 82% decrease from the total 1995/96 catch, respectively (Table 4.4).

Monthly catches in Port Phillip Bay increased from March 1986, when the demand for pilchards for pet food began to increase (Figure 4.9). Mean monthly pilchard catches in Victorian waters between

1990/91 and 1996/97 follow a similar annual pattern, with high catches during late summer/early winter and low catches in spring/early summer (Figure 4.10). Although pilchard are fished throughout the year in Victorian waters, nearly 56% of the mean annual catch between 1990/91 and 1996/97 was obtained between February and May (Figure 4.10).

Catch Rates

Annual pilchard catch rates between 1991 and 1997 (Figure 4.4) indicate that catch rates for Spencer Gulf have increased steadily since the beginning of the fishery. Catch rates in west coast waters increased to over 12 t per day in 1994, decreased to 6 t per day in 1995 and in recent years have been similar to those in Spencer Gulf (19.8 t per day). Prior to 1992 a single vessel operated in Gulf St Vincent, but this licence holder also shifted operations to Spencer Gulf and West Coast waters. Low catch rates in Gulf St Vincent since 1992 reflect exploratory fishing operations by other licence holders in areas other than those fished in 1992. No fishing has been undertaken in Gulf St Vincent since 1995.

Average annual catch rates (t/day) in Port Phillip Bay increased from around 1.6 t/day in 1990/91 to nearly 2.4 t/day in 1995/96, before declining to 1.3 t/day in 1996/97 (Figure 4.5). Monthly catch rates between January 1990 and June 1997 follow a similar seasonal trend to that of the total monthly catches, with high catch rates during late summer and autumn of each year, followed by low catch rates (< 0.2 t/day) during the remaining months (Figure 4.6). Peaks in catch rates between January 1990 and June 1997 fluctuated between 3.5 and 6.0 t/day, with the highest peak occurring in autumn 1995 (Figure 4.6).

Length Frequency

Seasonal variation in the size frequency distributions of pilchards caught in pilchards caught in Coffin Bay and Spencer Gulf (Port Lincoln) are shown Figures 4.11 and 4.12. In Spencer Gulf, fish as small as 7 cm LCF were present in samples and modes were frequently less than 15 cm LCF. Between March 1995 and February 1996 the modal size of samples gradually increased. After February 1996, the modal size of fish fell from 17 to 13.5 cm LCF. This cohort remained in the fishery for the reminder of the sampling period and reached 15 cm LCF in May 1997. In Coffin Bay (Figure 4.12) fish mainly ranged between 15 and 19 cm LCF; no fish were smaller than 12 cm LCF. Distributions were unimodal or bimodal. A cohort of smaller fish (mode 13 cm LCF) entered the fishery in May 1996 and remained in catches until sampling concluded in March 1997.

In 1995, pilchards from Port Phillip Bay catches ranged between 4.5 and 16.0 cm whereas in 1996 the catch was of slightly larger fish (70-210 mm) with a strong mode at 13.0 cm (Figures 4.14 and 4.15). Pilchard samples from Lakes Entrance between February and October 1995 were dominated by fish of 12.0-16.0 cm (Figure 4.16).

Monthly pilchard samples from Port Phillip Bay show a unimodal size distribution between December 1994 and February 1996, and an increasing mean size due to an increasing proportion of fish >14 cm LCF (Figure 4.14 and 4.15). A second mode of smaller fish is evident at around 10 cm from March to May 1995, when fewer fish >14 cm were present. No fish were caught between June and October 1995. In November 1995, the size distribution was again unimodal, with all fish caught being <10 cm. From November 1995 through to the start of July 1996, the distributions remained unimodal except for a small secondary mode at 8.5 cm in January 1996. The mean length of fish caught increased from 6.8 to 14.6 cm over this time, with fish in August and September 1996 showing a continuation of this pattern. While most samples from Port Phillip Bay were from catches made in northern areas of the bay, a sample taken from the southern end of Port Phillip Bay in July 1996 consisted almost entirely of fish >17 cm. Commercial catches in Port Phillip Bay were dominated by 0+ and age 1+ fish, with a small number of 2 to 4 yr-old fish. Most 2 yr-old fish and older were caught in the sample from the southern end of the bay in July 1996 (Figure 4.15).

The length frequency distributions from Port Phillip Bay show a unimodal distribution from December 1994 to February 1995 (1994/95) and an increasing mean size (Figure 4.15) due an increasing proportion of fish over 14 cm fork length. Between March and May 1995, a second mode (1995) of smaller fish is evident at around 10 cm, and fewer fish over 14 cm were present. No fish were caught between June and October 1995 (Figure 4.15). In November 1995, the distribution was again unimodal but all fish caught were less than 10 cm. From November 1995 through to the start of July 1996 (1995/96 mode), the mean length of fish caught increased from 6.8 cm to 14.6 cm. Samples in August and September showed a continuation of this pattern. Pilchards from Lakes Entrance showed similar rapid growth (Figure 4.16), the 1995 cohort reaching a mean length of 12 cm in 12 months. However, the growth of this cohort appeared to be out of phase with 1995/6 cohort in Port Phillip Bay. Most samples in Port Phillip Bay were from catches made in the north end of the bay, but one sample was taken in July towards the southern end of Port Phillip Bay and consisted almost entirely of fish over 17 cm. This sample, and other fish over 18 cm caught in September, were excluded from the plot of mean length against time (Figure 4.15).

Pilchard from Lakes Entrance between February and October 1995 were dominated by a distinct cohort of fish (12-16 cm in February 1995) which gradually became larger (Figure 4.16). The mean length of these fish increased from 13.9 to 16.6 cm over this period. Other size groups which are either larger (e.g. March 1995) or smaller (e.g. May-Aug 1995) than this dominant cohort were evident in samples in some months. Pilchards <12 cm were first evident in samples in May 1995 and were also present as a second mode in the distributions in July, August and October 1995. The mean length of fish <8 cm in May 1995 , the dominant size class at that time, was 5.9 cm, but this group was almost absent from all later samples. Fish between 8 and 12 cm in May 1995 also formed a distinct second mode in July, August and, to a lesser extent, October 1995 . The mean size of this group (estimated to include all fish <14 cm) was 12.5 cm in October 1995 . Pilchards obtained from Lakes Entrance were predominantly 1+ and 2+ fish, with relatively fewer small immature fish (Figure 4.16).

Age Composition

Nearly 75% of the fish aged from South Australia were 2 or 3 years of age, with the oldest fish aged being a single 7 year old. Although more females were collected, very little difference was observed in the age frequency distributions between the male and female samples (Figure 4.17). A slightly greater proportion of males were 2 year olds, while a greater proportion of females were 3, 4, and 5 year olds.

Samples from Coffin Bay showed similar age composition in each of the years sampled (Figure 4.18) and included mainly fish aged between 2 and 3 years old (Figures 4.17 and 4.21). Samples from Spencer Gulf were showed a greater variability in age composition (Figures 4.18 and 4.22), but generally included a greater proportion of younger fish. Samples from Spencer Gulf showed more consistency between samples within each year (Figure 4.18 and 4.27), and 2 year old fish were predominant in most 3 month periods.

Samples from Victoria showed a mode at 1-2 years, but included fish up to 6 years old (Figure 4.19, 4.20 and 4.22). More females than males were collected, but little difference was observed in the age frequency distributions between the male and female samples. Immature fish were either 0, 1 or 2, with the greatest proportion being 1 year old (Figure 4.22).

Significant differences in the age composition between years and areas were apparent. Samples from Lakes Entrance were predominantly of 1 and 2 yr-old fish (Figures 4.20 and 4.24), with no 0+ fish (reflecting the smaller proportion of small immature fish in the samples). The oldest fish were 4 year

olds. Samples from Port Phillip Bay were dominated by 1 yr-old and age 2 yr-old fish (Figure 4.19 and 4.23), showing rapid growth, with a small number of older fish, up to 6 years of age. The majority of the fish aged at 4 years and older were caught in two samples of larger fish caught in July and October, 1996. Samples from Lakes Entrance showed a narrower age range than those from Port Phillip Bay (Figure 4.20, 4.24, with no age 0 fish and no fish older than 4 years.

The age composition for Port Phillip Bay (Figure 4.23) shows a sudden shift from a range of age classes from January-Jun 1995 to a single cohort of immature fish in October-December 1995. No pilchards were caught in Port Phillip Bay between July and September, 1995. This cohort of immature fish dominated the age composition until June 1996, after which a range of older year classes was again represented in the samples. The age composition for Lakes Entrance (Figure 4.23 and 3.28) was more stable and mostly dominated by 2 year olds, but also showed a shift to younger fish in April-June 1995.

Anchovy

History and Markets

There are no records of any historical commercial fishery for this species in South Australia, nor is there currently a dedicated fishery (Jones in Dixon *et al.* 1996), although anchovy are occasionally caught as a very minor by-catch (< 1% by number) of the purse-seine fishery for pilchards particularly when operating in Coffin Bay. There are no records of any recreational catches of anchovy in South Australia.

The commercial fishery for anchovy in Victorian waters commenced in Port Phillip Bay around the 1910s and catches have been recorded since 1944. Initially, most fishers employed hoop nets and catches were sold as commercial and recreational bait (Blackburn 1950a; Hall and MacDonald 1986). By 1946, a Melbourne company started to produce fish-paste flavouring using anchovy and the increased demand for anchovy in Port Phillip Bay resulted in the use of small-mesh haul seines by commercial fishers (Winstanley 1979; Hall and MacDonald 1986). By 1950, the first purse-lampara net (modified purse-seine operated at night with light to attract the fish) was employed to capture anchovy in Port Phillip Bay (Blackburn and Rayner 1951). Today, most anchovy caught in Port Phillip Bay and in open coastal waters off Lakes Entrance are taken with purse-seine nets, although a small percentage are still taken with haul seines and hoop nets.

Catch and Effort

Nearly all of the commercial catch of anchovy in Victorian waters between 1978/79 and 1994/95 was obtained from Port Phillip Bay and coastal waters off Lakes Entrance in eastern Bass Strait (Neira *et al.* 1997a). Annual catches in Bass Strait and Port Phillip Bay between 1992/93 and 1996/97 averaged 366 (74.5%) and 187 (24.3%) t respectively, representing almost 99% of Victoria's total catch for that period. Smaller catches were reported from the Gippsland Lakes and Westernport Bay (<1% of total catch; Table 4.5) while none were reported from any of the remaining bays and inlets during that period. Annual catches of anchovy in eastern Bass Strait between 1978/79 and 1996/97 show two distinct peaks at both ends of that period (Figure 4.25). Catches reached 473 t in 1979/80 and declined thereafter, remaining around 13 t between 1980/81 and 1991/92. Catches reached the second peak of 553 t in 1993/94, and declined to 366 t in 1996/97. Annual catches of anchovy in Port Phillip Bay gradually declined from around 200 t in 1982/83 to 16 t in 1989/1990, increased gradually thereafter to 491 t in 1996/97, the highest catch in the history of the anchovy fishery in the bay (Figure 4.26).

Monthly catches of anchovy throughout Victorian waters between 1990/91 and 1996/97 were consistently low between July 1990 and March 1993, averaging 6 t (range 0 - 33 t) during that period. Catches peaked at around 140 t in July and August 1993, 357 t in July 1994, and 152 t in both July and October 1995 (Figure 4.27). Although anchovy are fished throughout the year, around 77% of the mean annual catch between 1990/91 and 1995/96 was obtained between May and August (Figure 4.27). Mean monthly catches of anchovy during that period show a distinct trend, with highest catches in autumn/winter followed by low catches in spring/summer.

Blue Mackerel

History and Markets

In South Australia, during the mid-1980s there was a limited commercial purse-seine fishery for this species in the south-east of the state providing bait for the region's rock lobster fishery. This fishery is no longer active. Since 1993, blue mackerel have increasingly been caught for bait and human consumption by the hooking sector of the South Australian Marine Scalefish fishery, particularly in Gulf St Vincent. However, no information is available regarding the size of fish caught.

In 1996 a Commonwealth licence was granted by the Australian Fisheries Management Authority for a mid-water trawling operation in waters >200m deep in the Great Australian Bight, targeting jack mackerel, yellowtail scad, blue mackerel and redbait. This operation was based at Port Adelaide subsequently moved to Esperance, Western Australia and has since been discontinued. As with

anchovy, blue mackerel are occasionally caught as a by-catch species (< 1% by number) by pilchard vessels operating in Coffin Bay.

There is no commercial fishery for blue mackerel in Victorian waters although significant quantities are caught as a by-catch of the jack mackerel fishery. Reported by-catches averaged 39t between 1978/79 and 1995/96, with the largest catch of 370 t recorded in 1983/84. No catches were reported in 1996/97.

Catch and Effort.

The total annual catch from the purse-seine fishery ranged between 0.06 and 3.6 t, peaking in 1986/87 (Figure 4.32). There is a limited recreational fishery for blue mackerel in South Australia with the total recreational catch recently estimated at 15.5 t (for 1994/95) which represented approximately 75% of the total (recreational + commercial) state catch (McGlennon and Kinloch 1997).

Jack Mackerel

History and Markets

There has never been a fishery for jack mackerel in South Australian waters.

The commercial fishery for jack mackerel in Victorian waters commenced in eastern Bass Strait in the mid-1960s, with the establishment of a fishing company at Lakes Entrance assessing the possibility of processing jack mackerel for fish meal (Maxwell 1979). The company used a large purse-seiner for the fishing operations. Currently most jack mackerel catches derive from eastern Bass Strait and are caught by purse-seine along the coast.

Catch and Effort

The trend in the annual catches in Victorian waters shows highly variable catches. This variability is assumed to be market-driven and as a function of targeting practices, e.g. pilchards are preferred by for the pet food industry. Catches dropped from around 150 t in 1969/70 to below 5 t in the mid-1970's; no data is available for 1975/76 to 1977/78. Catches remained below 150 t until 1988/89 and increased rapidly thereafter, reaching a peak of nearly 450 t in 1992/93. Catches declined sharply thereafter and were 15.7 t in 1995/96.

4.3 Discussion

Overview of Fisheries

The baitfisheries of southern and eastern Australia have one major similarity: they are predominantly driven by local demand, mainly because of the increasing requirement for fresh product and the limited capability of most vessels to freeze fish. These factors, in conjunction with high fuel costs and low prices, encourage fishers to operate only in areas near their home ports and/or nearest market, increase the possibility of localised depletion of stocks.

Baitfisheries in South Australian and Victorian waters display numerous differences. The history of the South Australian pilchard fishery is short and catches have increased dramatically over the last decade in response to the development of the tuna mariculture industry whereas the Victorian pilchard fishery has a long history but catches have contracted in recent years. There is no fishery for anchovies in South Australian waters but the fishery in Port Phillip Bay has operated since 1910 and the highest catch in history was taken in 1996/97. Blue mackerel in South Australian waters have been targeted for use as bait and for human consumption, but have only been taken as by-catch in Victorian waters. There is no fishery for jack mackerel in South Australia, but a small fishery has operated in Victorian waters since the 1960s.

Different management strategies, vessel capacities and patterns of demand in South Australia and Victoria make it difficult to compare catch and effort and age composition information data from the two states. In the South Australian fishery, the seasonally high catch rates during February/March may be due to the relatively high demand of pilchards for tuna fodder, whereas the high catches at similar times of the year, (peaking in April) in Port Phillip Bay are believed to be due mainly to the seasonal immigration of juveniles (0+ fish) into the bay. Declines in catches and CPUE's from June onwards are believed to be due to reduced abundances caused by fishing mortality, emigration from the bay, and (possibly) predation.

Evidence for Localised Depletion of Stocks?

Increases in quotas and catches in the South Australian fishery in the period up to 1993 limit the use of catch rate data in investigations of localised depletion. From 1994-97, however, the Total Allowable Catch was constant at 3500 t and two interesting patterns in CPUE can be observed. The drop of CPUE in 1995 in waters around Coffin Bay, may have be caused by the pilchard kill in March-May, as relatively small catches were taken for the remainder of that year. In Spencer Gulf, CPUE increased in 1996 and remained relatively high in 1997, but fish >2 years of age became less common in the catch. This may have been partly the result of the mass mortality of adult pilchards in

1995, and could also reflect a large stock (strong year class(es)) of immature fish that later matured into produce the high spawning biomass estimate obtained in 1997 (Chapter 8). The possibility of a fishery-induced decline in the age of fish in catches from Spencer Gulf should not, however, be discounted.

In Victoria, analysis of the length and catch data obtained between December 1994 and September 1996 indicated a substantial immigration of juvenile pilchard (40-100 mm) into the Bay in or before March and April 1995, and again in November 1995. A decline in the number of juvenile pilchards in 1995 (May), and also possibly adults (>120 mm), could be attributed to a combination of fishing mortality, emigration of pilchards from the Bay and natural mortality due to predation by the large numbers of barracouta (*Thyrsites atun*) that entered the Bay at that time. The fact that no catches were reported within Port Phillip Bay between June and October 1995, suggests that a large proportion of pilchards could have moved out to sea after May possibly to spawn, as there are strong indications that pilchards spawn in coastal waters and not within the Bay (Jenkins 1986; Hoedt and Dimmlich 1995; Neira and Tait 1996; Neira and Coutin 1998). Alternatively, it is possible that their disappearance could have been associated with the large pilchard mortality that affected southern Australian stocks in March to May 1995. However, no dead pilchards were recorded in open coastal waters between Portland and Lakes Entrance during that period, therefore the real impact of this kill on pilchard stocks in the Bay and in Victoria remains to undetermined. The drop in the CPUE in Port Phillip Bay in 1996/97 may be due to low levels of migration into the Bay. The reason for this low rate of recruitment into the fishery is unknown.

Future Studies

The possibility if the localised depletion of pilchard stocks cannot be discounted in either South Australia or Victoria. Monitoring of fishing patterns and the age composition of catches must continue in both states. There is a growing need to understand the factors causing inter-annual fluctuations in abundance and to investigate the potential effects of the fisheries on populations of predators, especially in South Australia where the fishery has expanded rapidly. In Victoria, additional information are required on the location and timing of spawning and the size of stocks.

Table 4.1 Annual fishing effort (boat-days) expended by the South Australian pilchard purse-seine fleet between 1991 and 1997.

YEAR	West Coast	Spencer Gulf	Gulf St Vincent	Total
1991	0	3	1	4
1992	48	198	3	249
1993	103	342	64	509
1994	31	684	23	738
1995	84	458	0	542
1996	208	160	0	368
1997 (Jan-June)	80	200	0	280

Table 4.2 Annual catches of pilchards (tonnes) in the South Australian pilchard purse-seine fishery between 1991 and 1997.

Year	West Coast	Spencer Gulf	Gulf St Vincent	Total Catch	Annual Total Allowable Catch
1991	0	0.6	6.0	6.6	1,200
1992	4.0	427.7	37.2	468.9	1,200
1993	151.5	1,174.1	132.4	1,457.9	3,500
1994	379.2	3,063.0	68.6	3,510.8	3,500
1995	493.2	2,104.0	0	2,597.2	3,500
1996	2,007.8	1,522.9	0	3,530.8	3,500
1997(Jan – June)	777.3	1,694.5	0	2,471.8	3,500

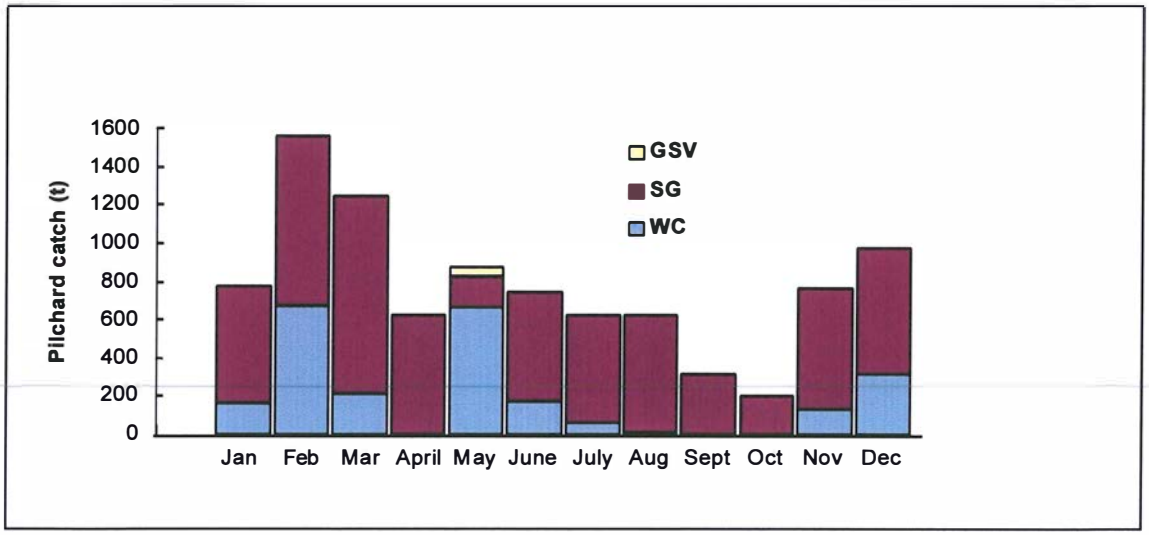


Figure 4.1 Monthly pilchard catches in South Australian waters by fishing area (totals for 1994-96). (GSV – Gulf St Vincent; SG – Spencer Gulf; WC – West Coast).

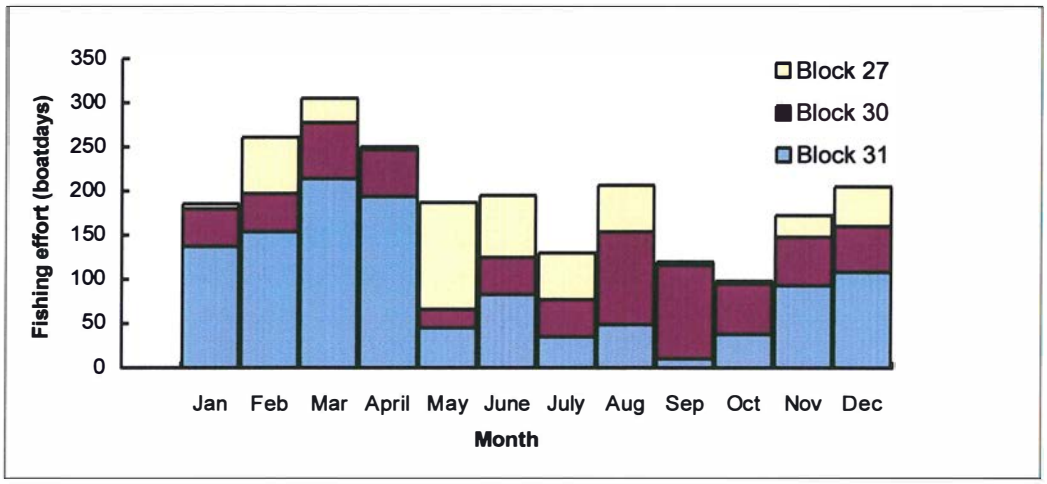


Figure 4.2 Monthly fishing effort for pilchards in South Australian waters per fishing block (totals for 1994-96). (Block 27 – West Coast; Block 30 – Offshore Spencer Gulf; Block 31 – Inshore Spencer Gulf).

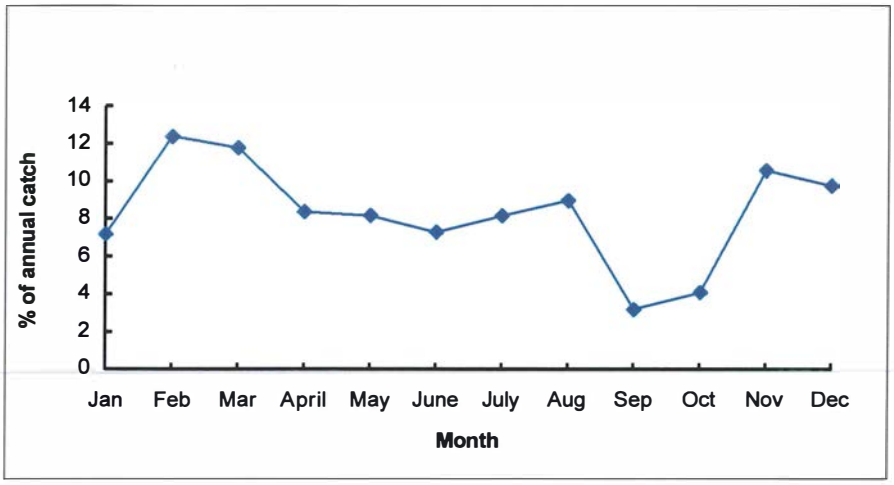


Figure 4.3 Mean monthly catches of pilchards in South Australian waters (expressed as a % of the total annual catch between 1994 and 1996).

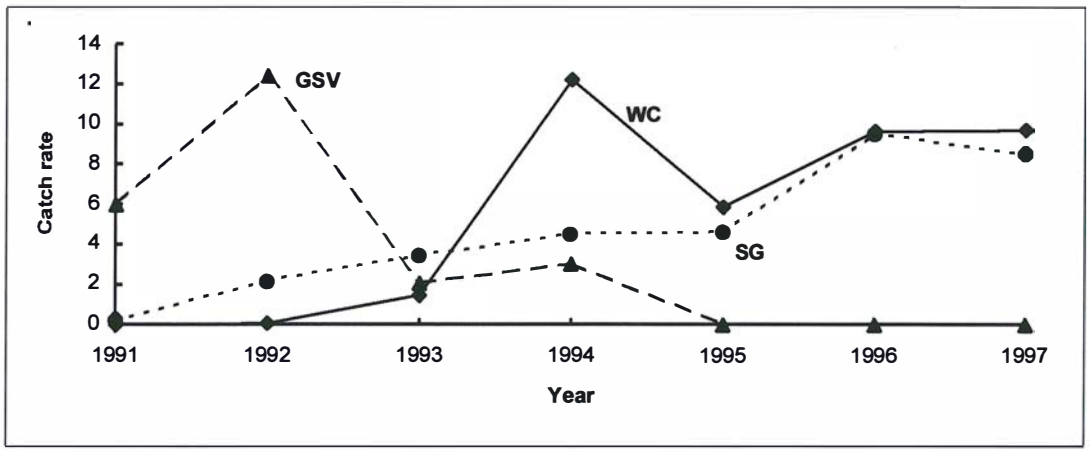


Figure 4.4 Fluctuations in pilchard catch rates (t per boat-day) between Gulf St Vincent (GSV), the West Coast (WC) and Spencer Gulf (SG) between 1991 and 1997.

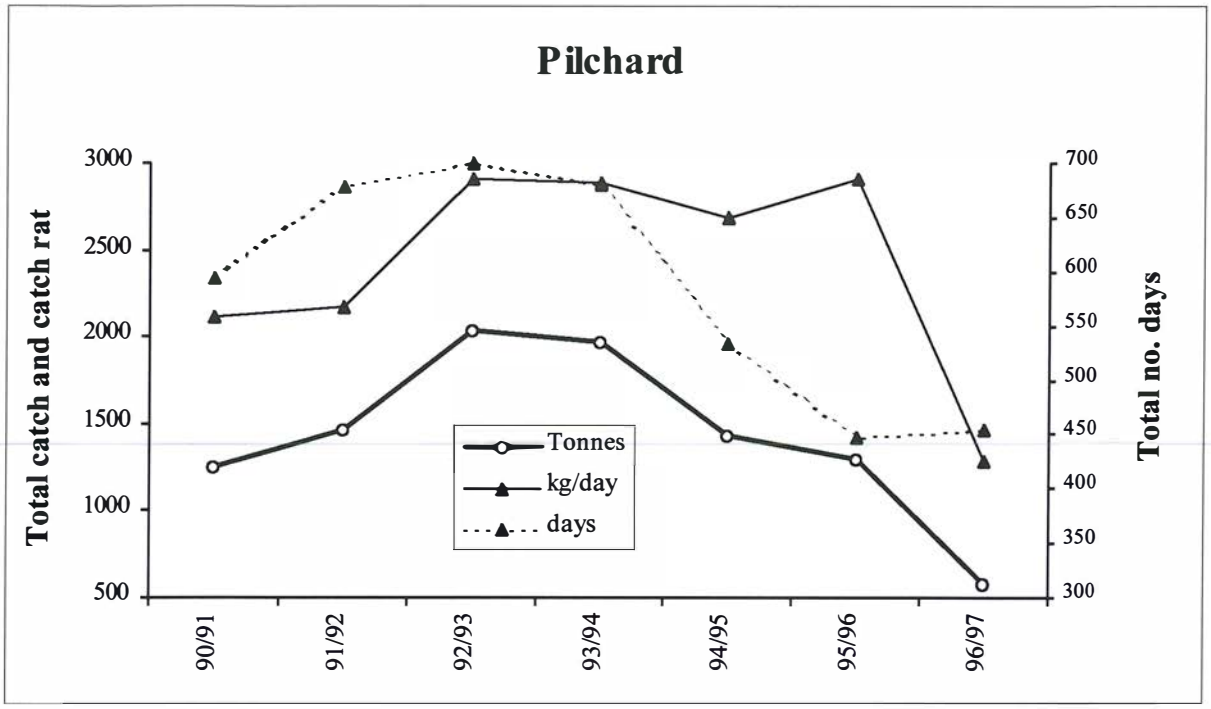


Figure 4.5 Total catch (t), effort (days) and catch rate (kg/day) for the pilchard fishery in Port Phillip Bay between 1990/91 and 1996/97.

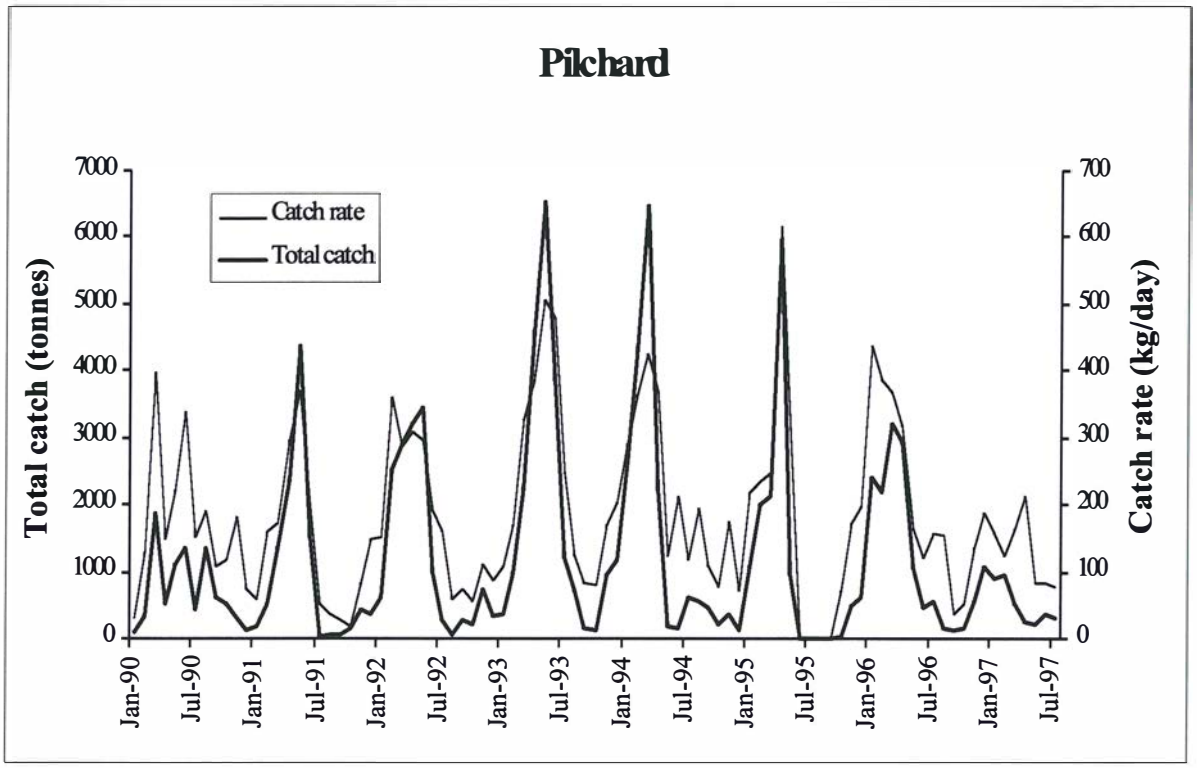


Figure 4.6 Monthly catches (t) and catch rates (kg/day, purse-seine only) for the pilchard fishery in Port Phillip Bay between January 1990 and July 1997.

Table 4.3 Commercial pilchards catches (t) from different areas in Victoria between 1978/79 and 1995/96 (Victorian Fisheries Catch and Effort Information Bulletin 1997).

Year	Port Phillip Bay	Westernport Bay	Gippsland Lakes	Malacoota Inlet	Bass Strait	Total
1978/79	220.2	0	0	0	742.3	962.5
1979/80	466.3	6.6	0	0	109	581.9
1980/81	496.8	0	0	0	2.9	499.7
1981/82	506.4	0	0	0	0.1	506.6
1982/83	522.4	1.4	0	0	20	543.8
1983/84	587.5	0.1	0.6	0	4841.1	5429.3
1984/85	447.7	0	0.9	0	1859.8	2308.5
1985/86	605.9	0.4	0.5	0	1.8	608.7
1986/87	1089.1	2.8	4.9	0	87.5	1184.4
1987/88	1104.5	0	6.7	0	2.5	1113.7
1988/89	1443.3	1.2	0	0	798.9	2243.4
1989/90	835.8	0	17.4	0	701.4	1554.6
1990/91	1362.7	0.1	0	0	956.4	2319.1
1991/92	1485.3	0	0	0	956	2441.3
1992/93	2058.4	0	0	0	1171.6	3230
1993/94	2034	0	0	0	847.7	2881.8
1994/95	1451.2	7.8	0	0	1075.6	2534.9
1995/96	1338.9	0	0	0	1004.1	2343
1996/97	596.0	0.0	0.0	0.0	177.0	773.0
5 yr mean	1495.7	1.6	0.0	0.0	855.2	2352.5
10 yr mean	1371.0	0.9	2.4	0.0	769.1	2143.5

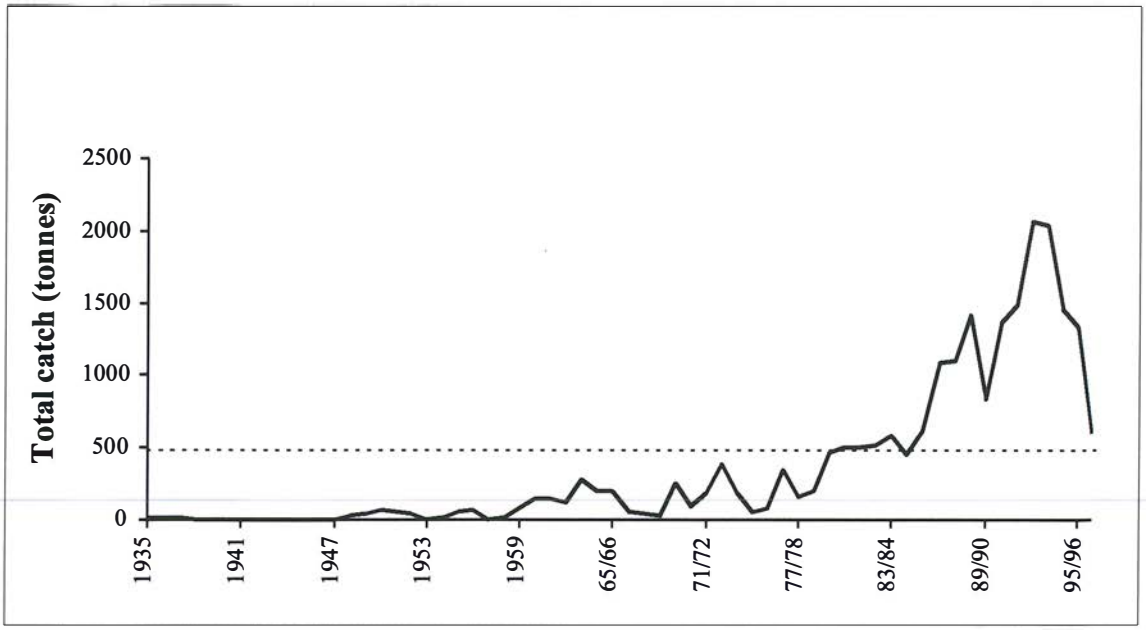


Figure 4.7 Commercial pilchard catches in Port Phillip Bay between 1935 and 1996/97.

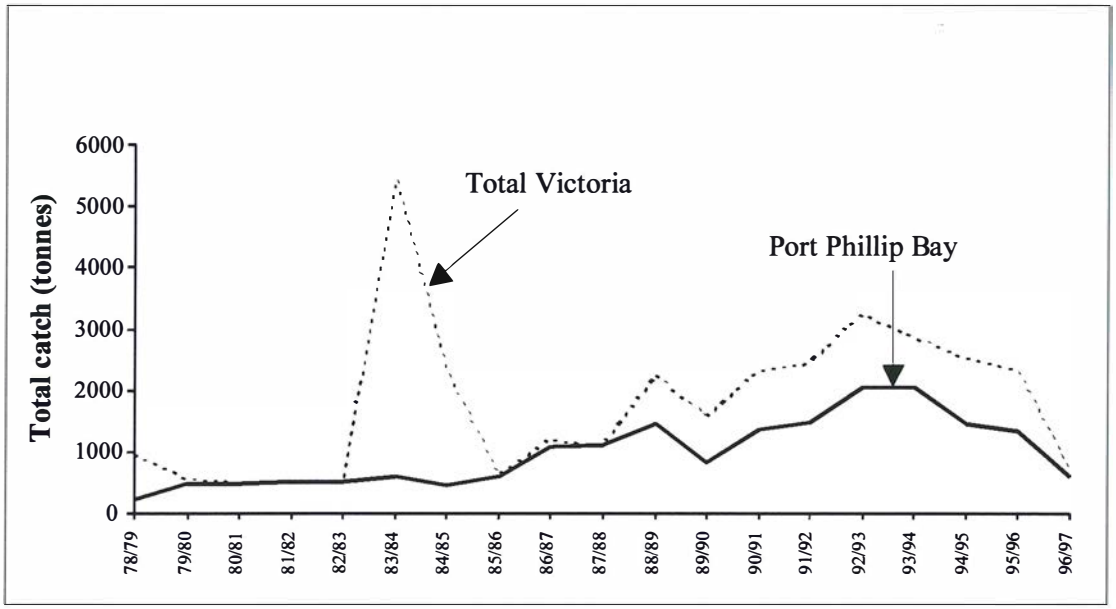


Figure 4.8 Annual commercial pilchard catches in Victoria and in Port Phillip Bay between 1978/79 and 1996/97.

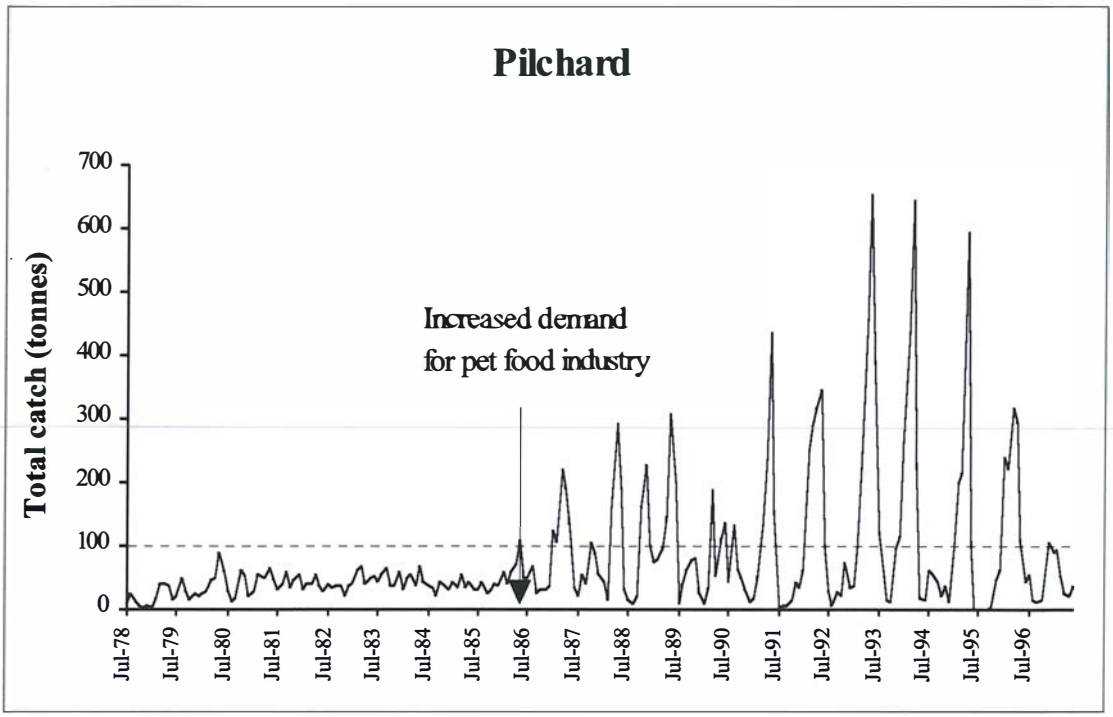


Figure 4.9 Monthly pilchard catches in Port Phillip Bay between July 1978 and June 1997.

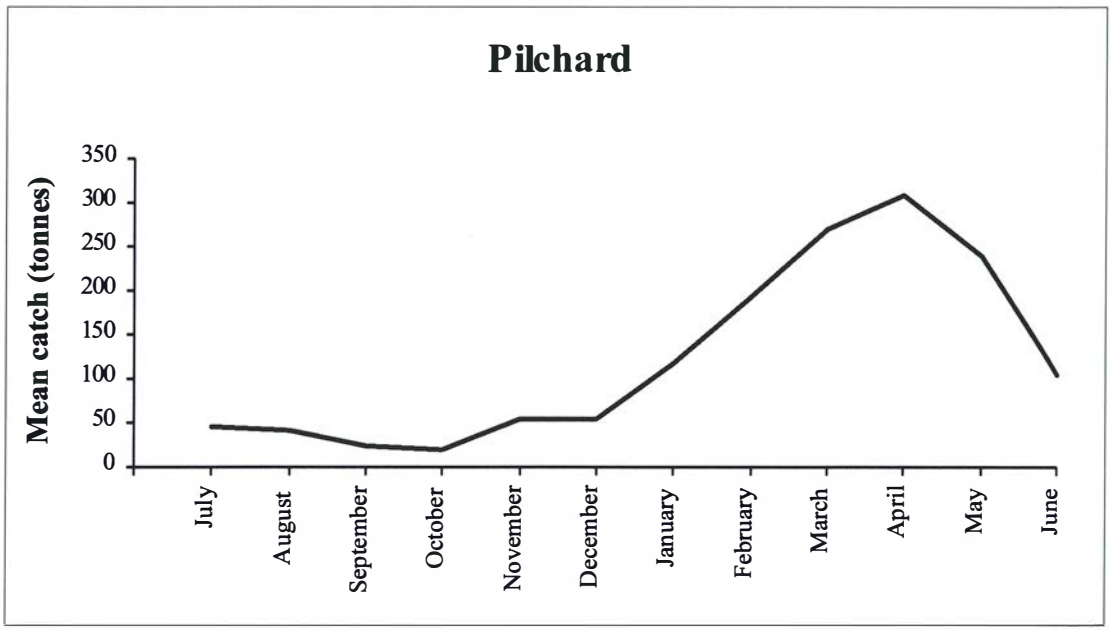


Figure 4.10 Mean monthly pilchard catches in Port Phillip Bay between 1990/91 and 1996/97

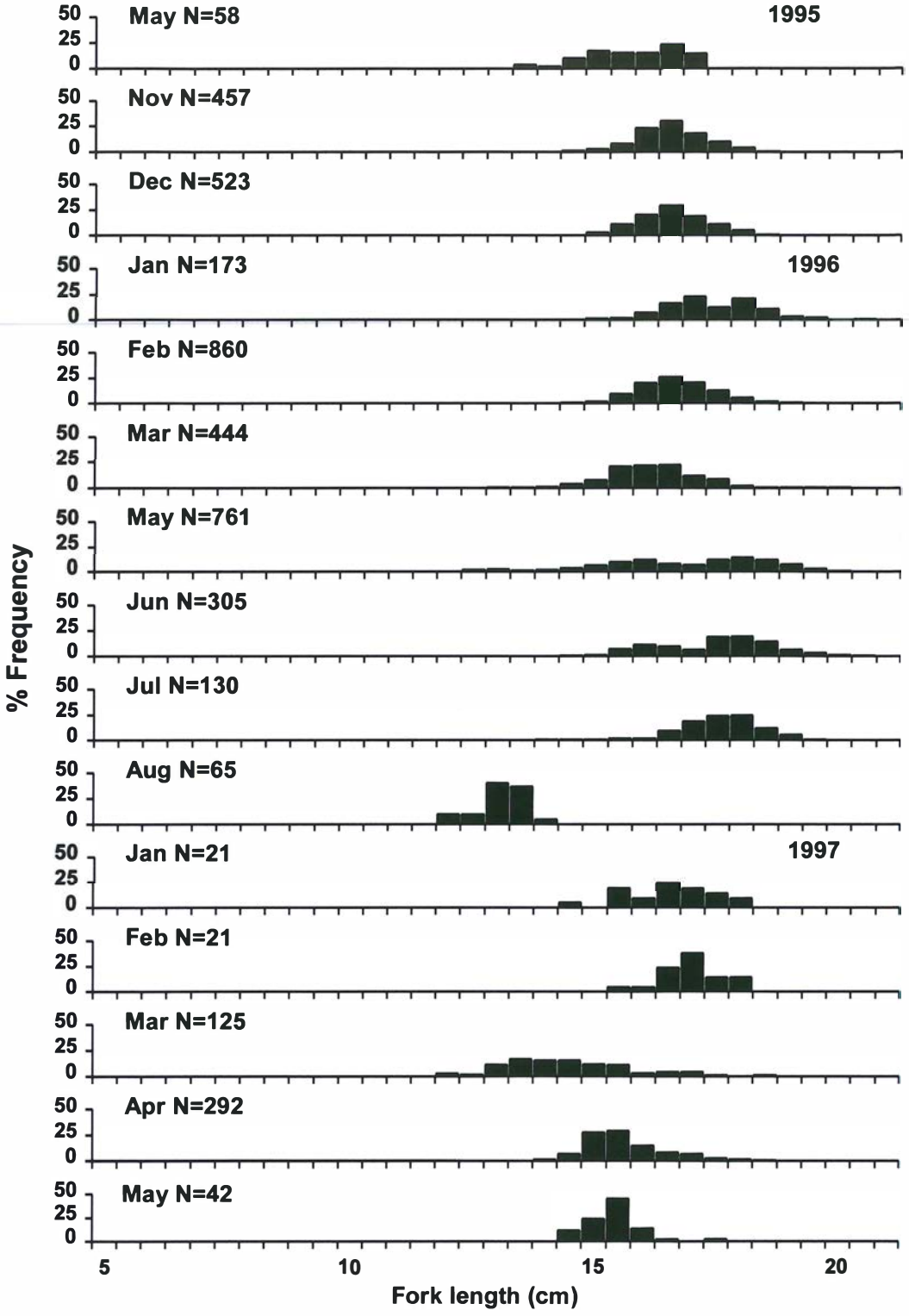


Figure 4.11 Length frequency distributions of pilchards sampled from Coffin Bay between May 1995 and May 1997.

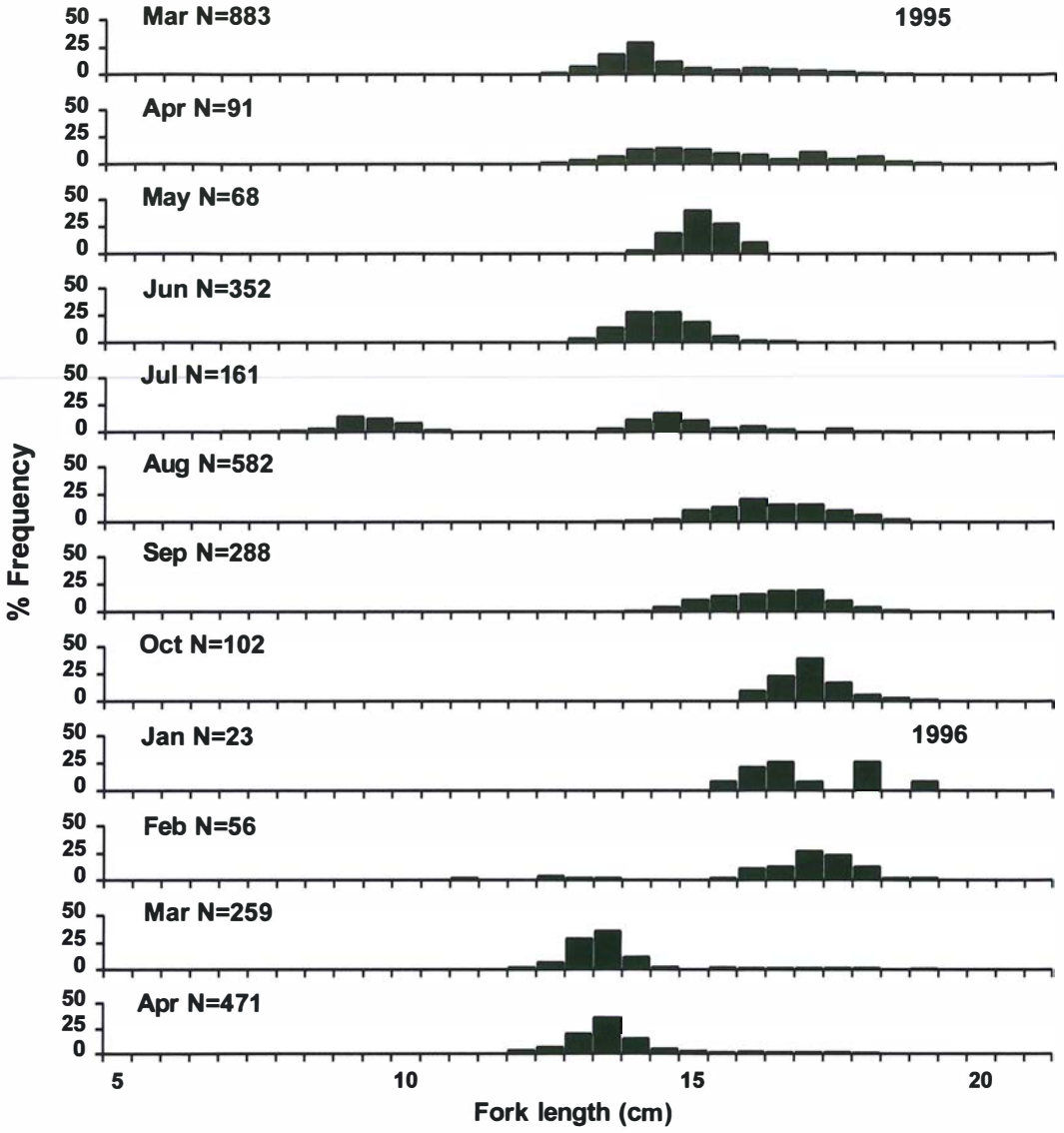


Figure 4.12 Length frequency distributions of pilchards sampled from Spencer Gulf in between May 1995 and April 1996.

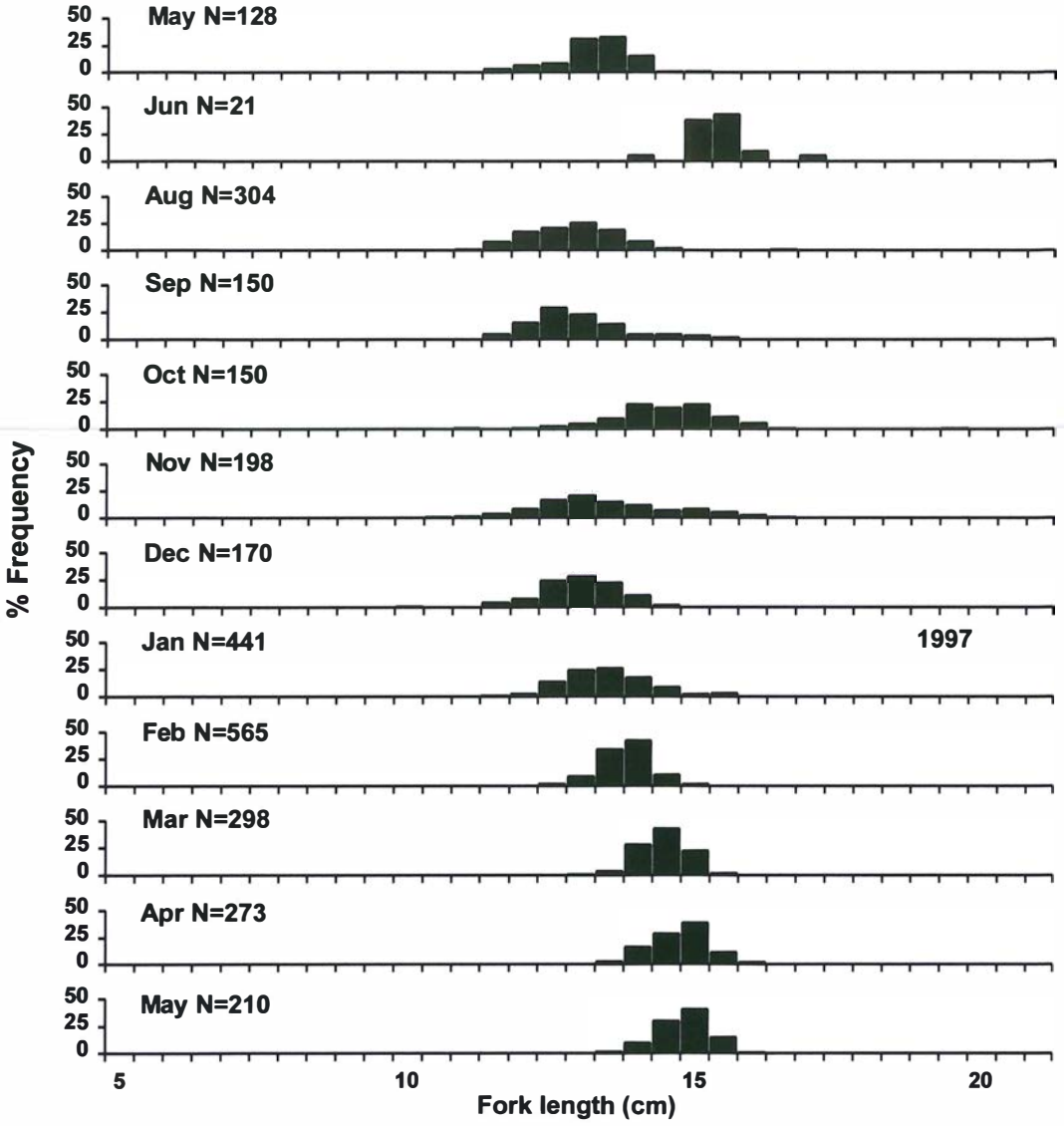


Figure 4.13 Length frequency distributions of pilchards sampled from Spencer Gulf between May 1996 and April 1997.

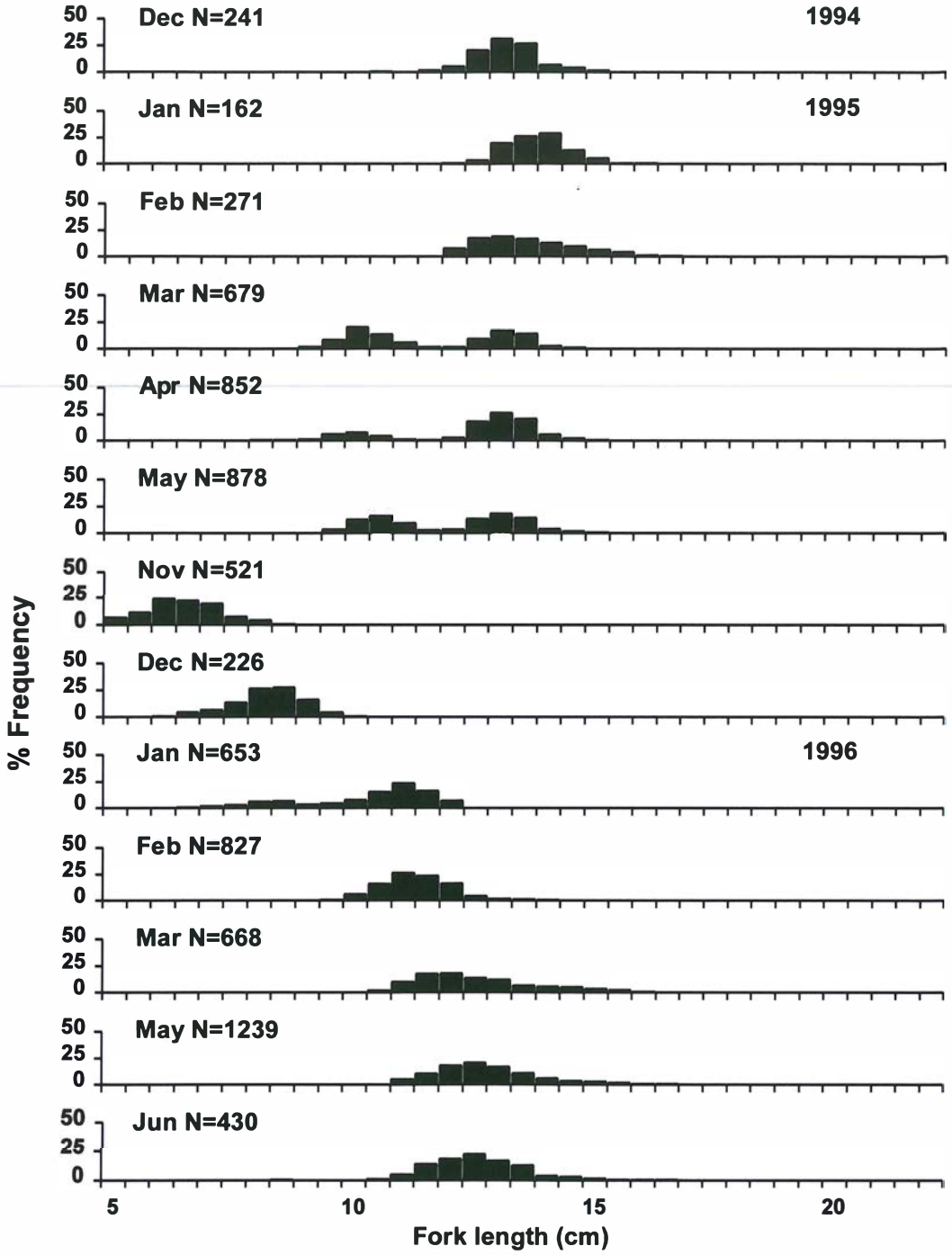


Figure 4.14 Length frequency distributions of pilchards sampled from Port Phillip Bay between December 1994 and June 1996.

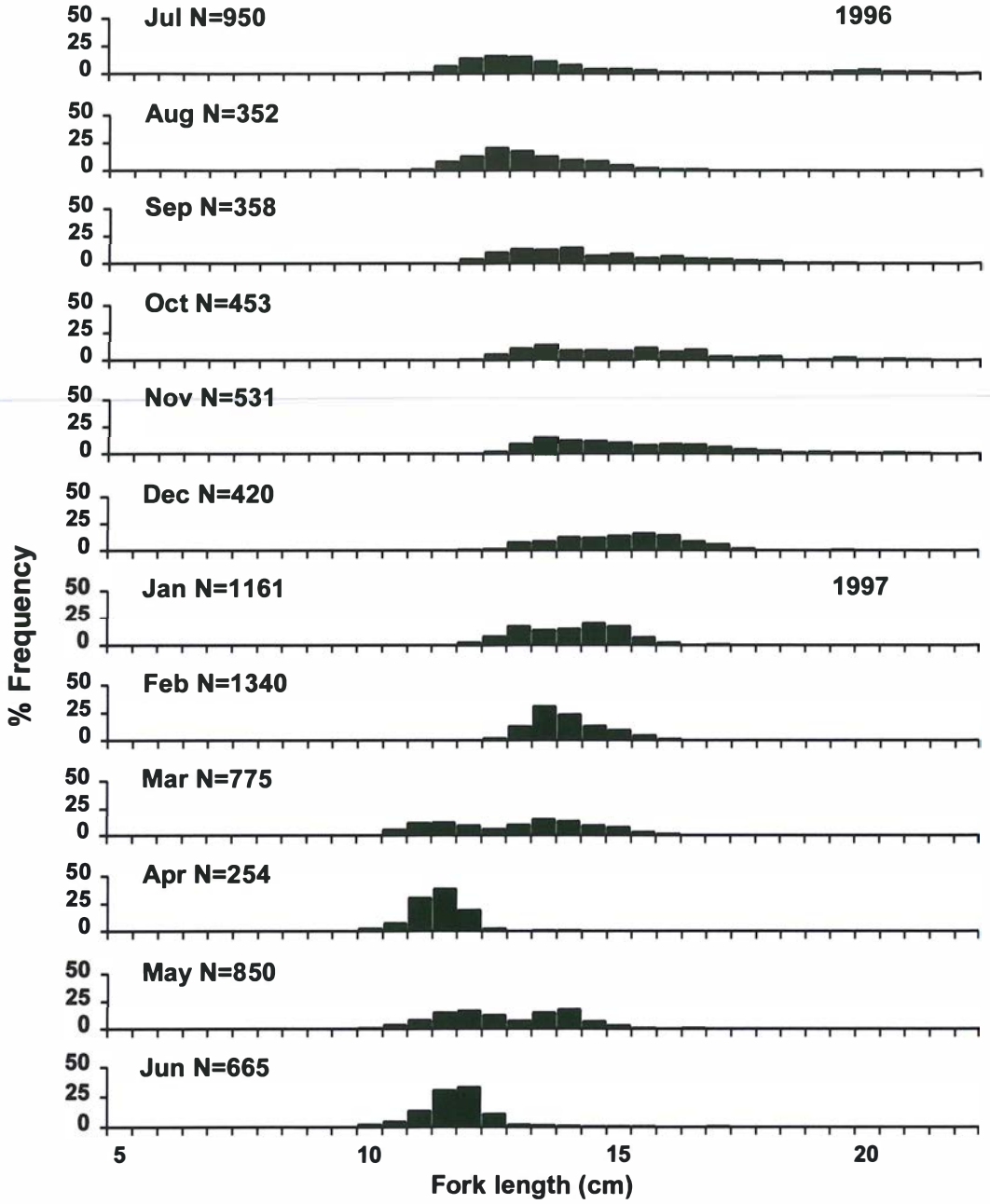


Figure 4.15 Length frequency distributions of pilchards sampled from Port Phillip Bay between July 1996 and June 1997.

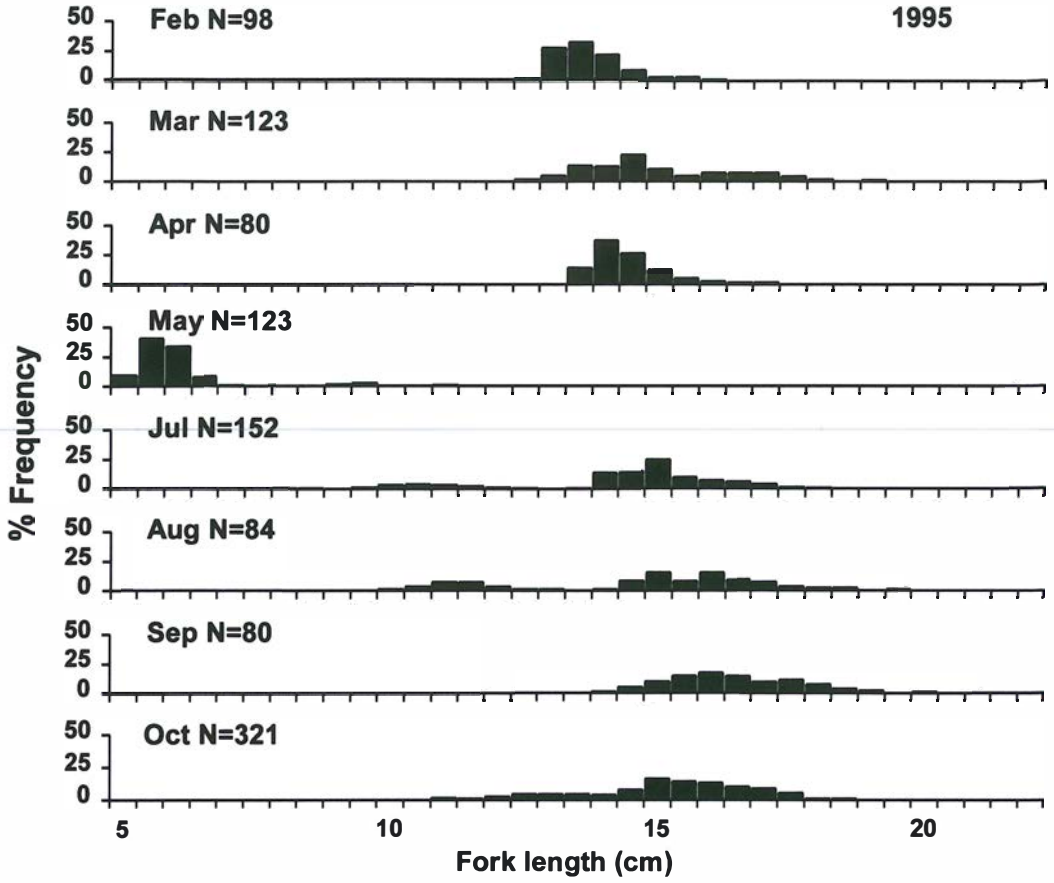


Figure 4.16 Length frequency distributions of pilchards sampled from Lakes Entrance between February 1995 and October 1995.

Table 4.4 Percentage contribution (%) of 0+ to 3+ age classes of pilchard in main South Australian fishing areas between 1995 and 1997.

	Spencer Gulf				**Coffin Bay**			
Age class	**0+**	**1+**	**2+**	**3+**	**0+**	**1+**	**2+**	**3+**
1995	7	50	25	12	2	22	45	22
1996	20	65	8	7	2	20	35	23
1997	10	78	10	1	5	40	39	10

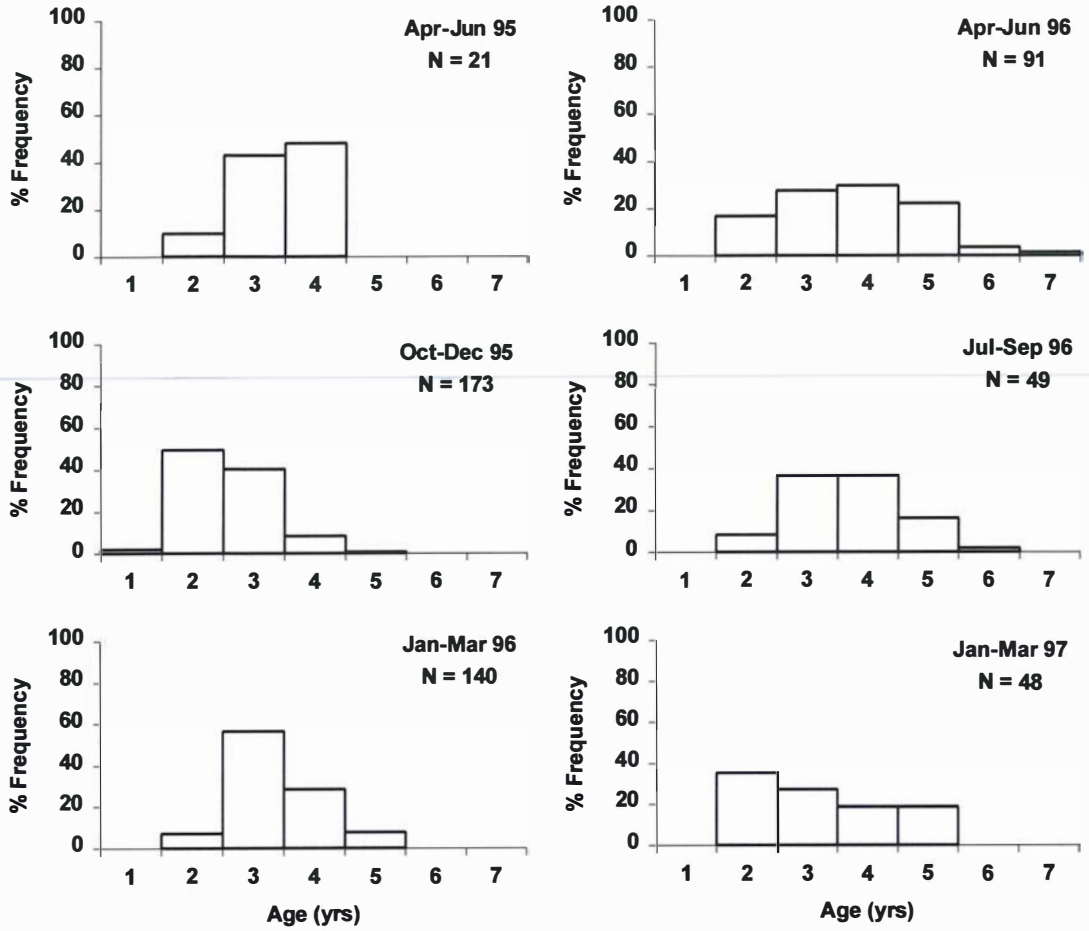


Figure 4.17 Age frequency distributions of samples of pilchards from Coffin Bay between May 1995 and March 1997, by quarter (sexes combined).

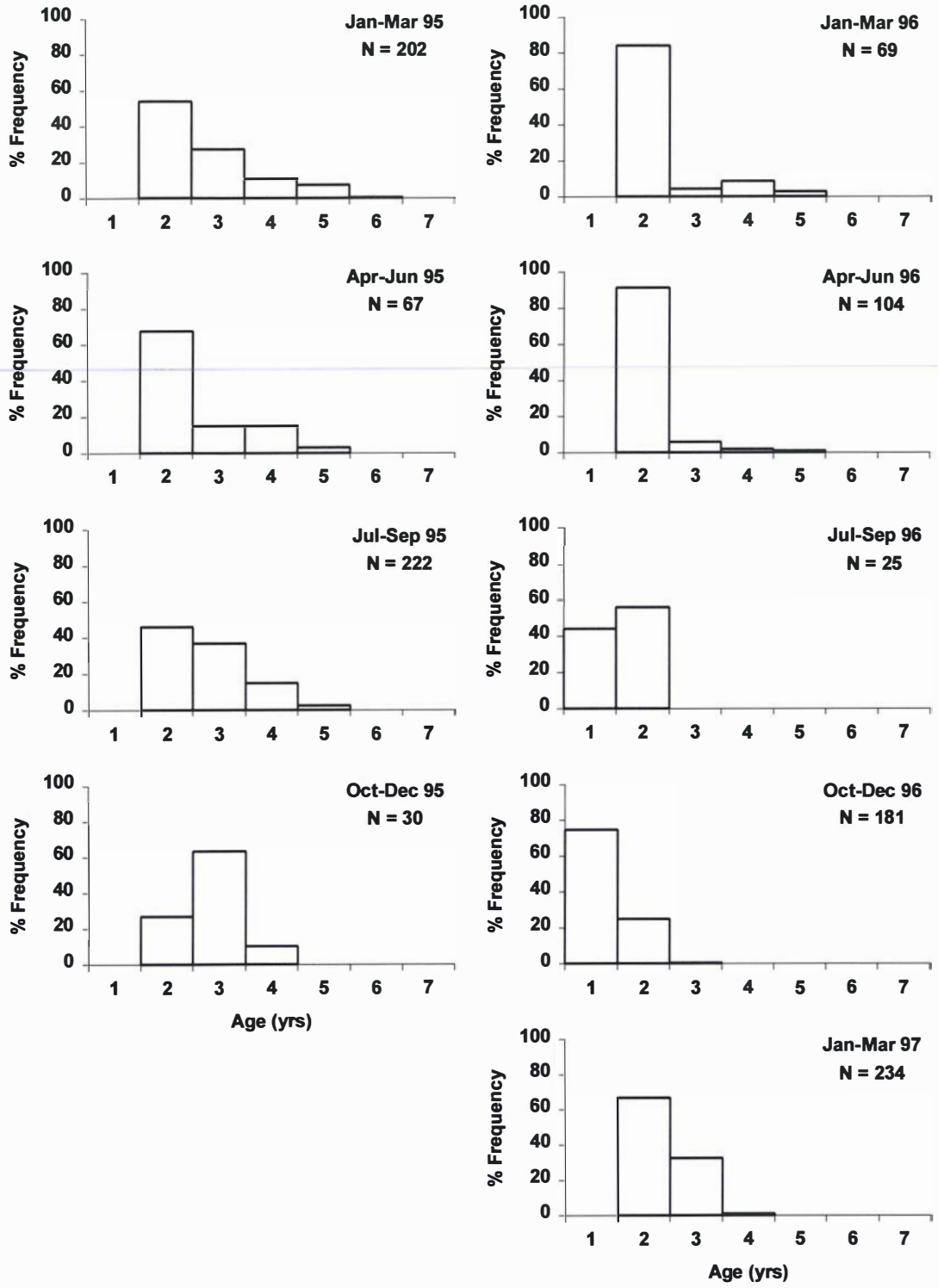


Figure 4.18 Age frequency distributions of samples of pilchards from Spencer Gulf between March 1995 and March 1997, by quarter (sexes combined).

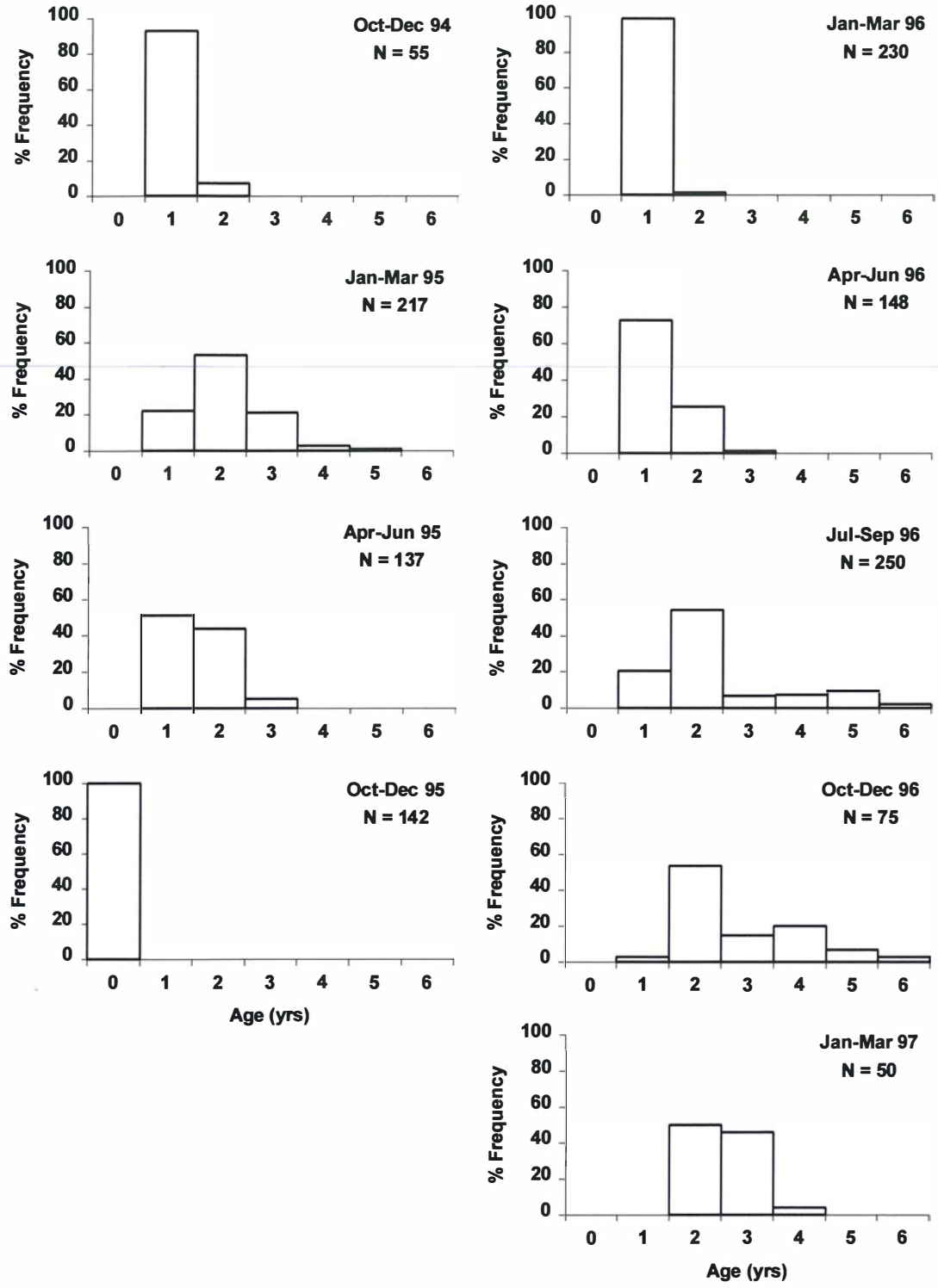


Figure 4.19 Age frequency distributions of samples of pilchards from Port Phillip Bay between December 1994 and February 1997, by quarter (sexes combined).

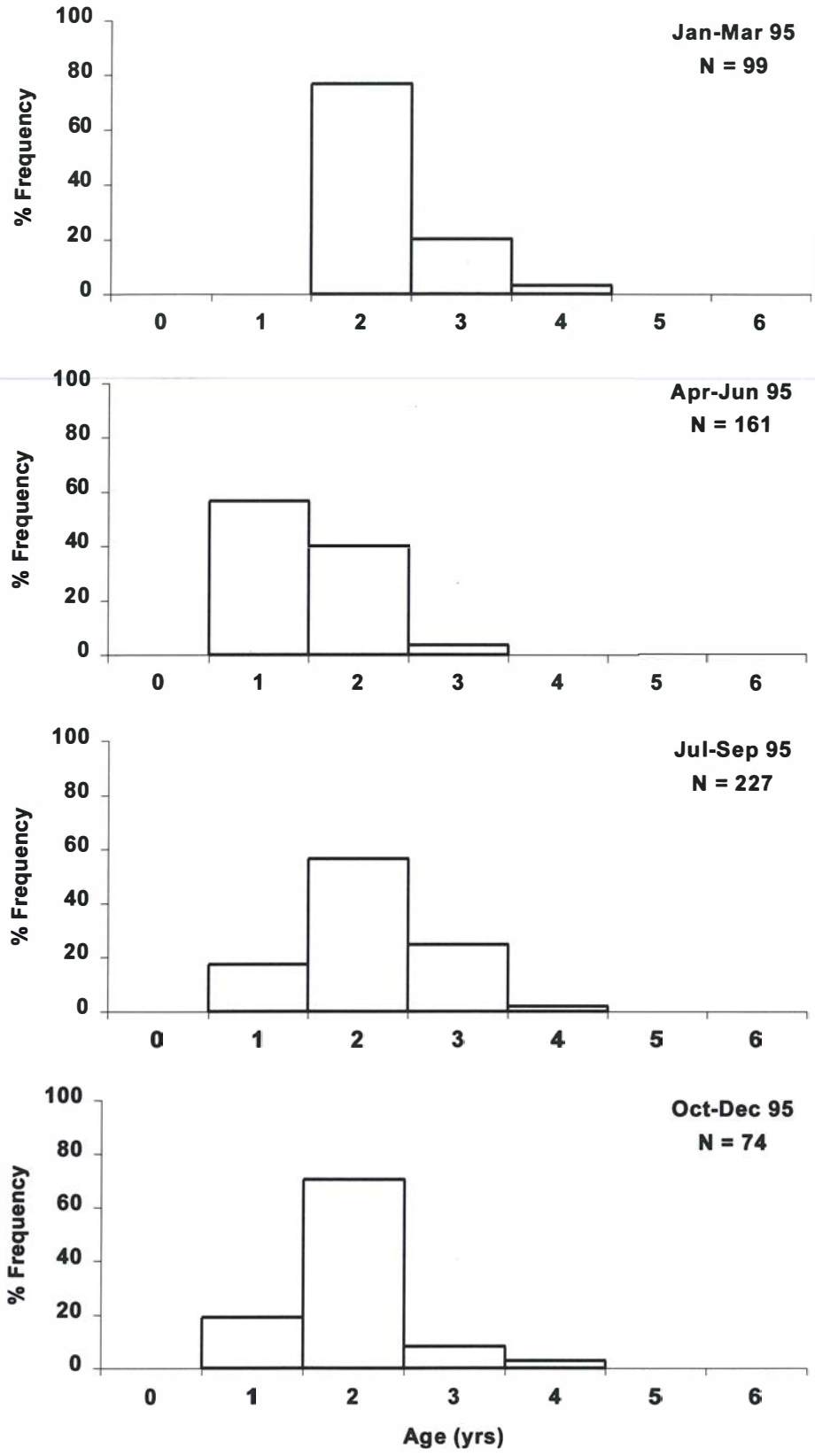


Figure 4.20 Age frequency distributions of pilchards from Lakes Entrance between Febraury and October 1995 (sexes combined).

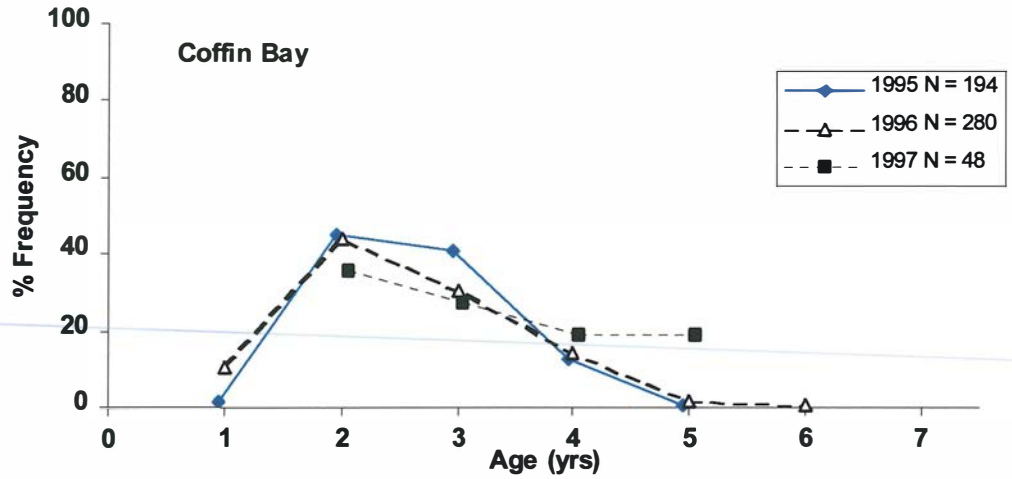


Figure 4.21 Age percentage frequency for pilchards caught in Coffin Bay by year (sexes combined).

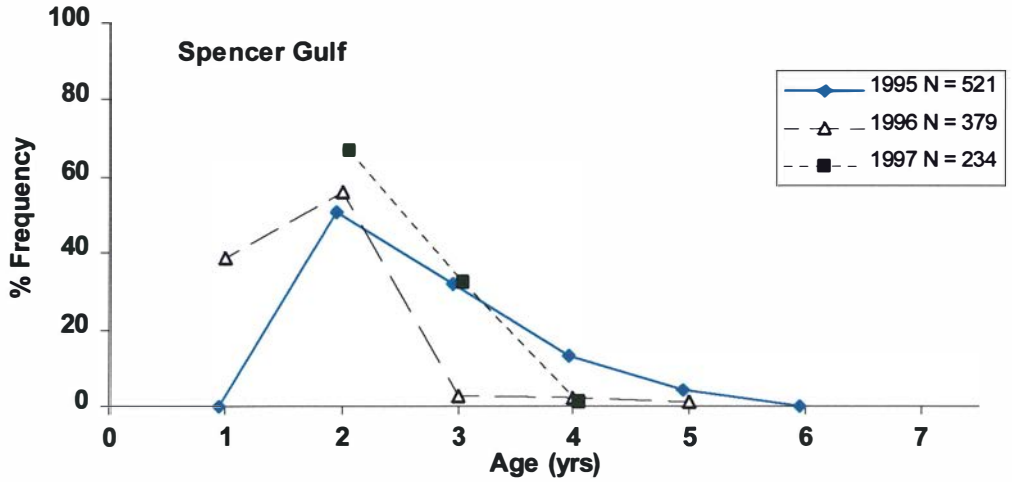


Figure 4.22 Age percentage frequency for pilchards caught in Spencer Gulf by year (sexes combined).

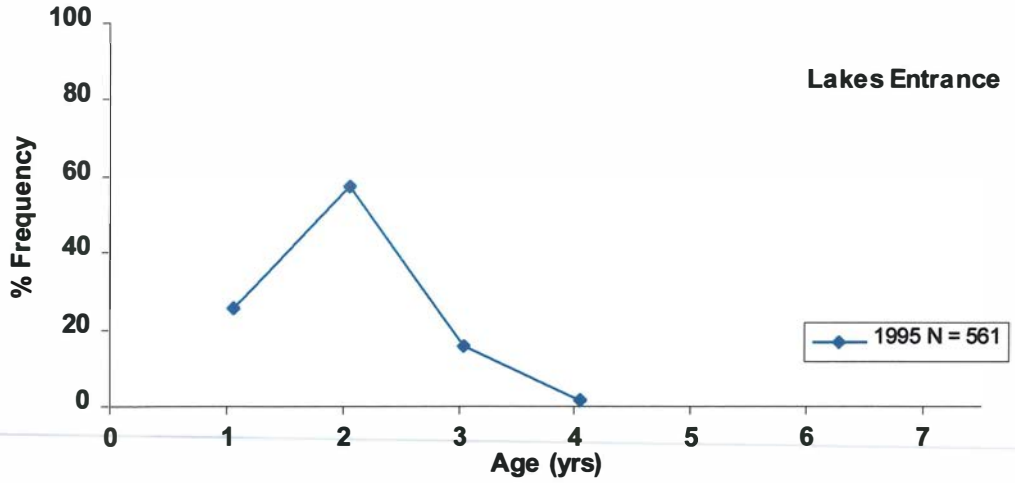


Figure 4.23 Age percentage frequency for pilchards caught in Lakes Entrance (sexes combined)

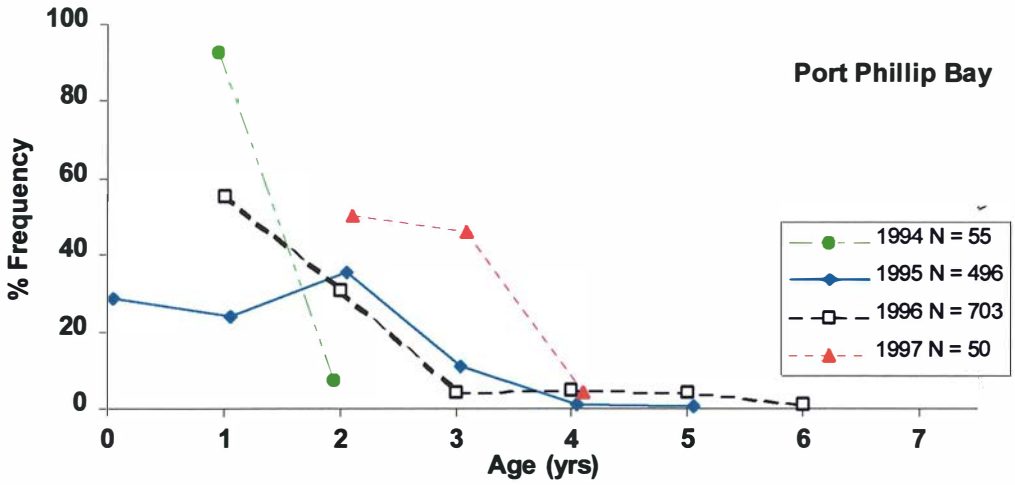


Figure 4.24 Age percentage frequency for pilchards caught Port Phillip Bay samples (sexes combined).

Table 4.5 Commercial anchovy catches (t) from different areas in Victoria between 1978/79 and 1996/97 (Victorian Fisheries Catch and Effort Information Bulletin 1997).

Year	Port Phillip Bay	Westernport Bay	Gippsland Lakes	Bass Strait	Total
1978/79	141.8	5.1	21.3	38.5	206.7
1979/80	175.6	0.0	0.0	473.5	649.1
1980/81	138.1	3.0	52.5	16.8	210.5
1981/82	147.1	1.4	5.4	1.0	154.9
1982/83	197.7	1.0	9.0	0.2	207.9
1983/84	128.7	1.1	27.3	11.8	169.0
1984/85	69.6	2.8	84.4	2.9	159.7
1985/86	69.2	1.4	13.8	3.5	87.9
1986/87	138.1	2.8	40.3	9.8	191.0
1987/88	104.0	10.7	0.3	34.0	149.1
1988/89	32.9	8.1	28.0	0.5	69.5
1989/90	16.3	0.0	18.3	33.8	68.4
1990/91	43.9	0.0	0.0	5.4	49.2
1991/92	45.2	0.1	0.0	34.1	79.4
1992/93	89.2	0.0	0.0	85.2	174.4
1993/94	104.8	0.0	1.0	552.6	658.4
1994/95	86.0	0.5	9.5	360.5	456.5
1995/96	165.0	0.1	12.0	468.0	645.0
1996/97	491.0	0.0	11.0	366.0	868.0
5 yr mean	187.2	0.1	6.7	366.4	560.5
10 yr mean	117.8	2.0	8.0	194.0	321.8

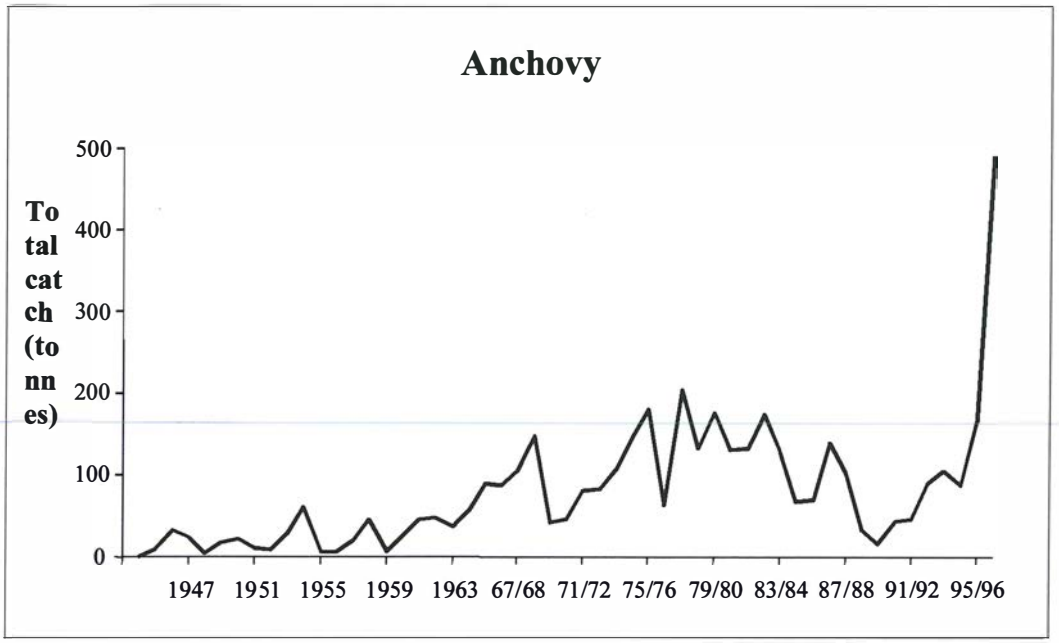


Figure 4.25 Anchovy catches (tonnes) in Port Phillip Bay between 1944 and 1996/97.

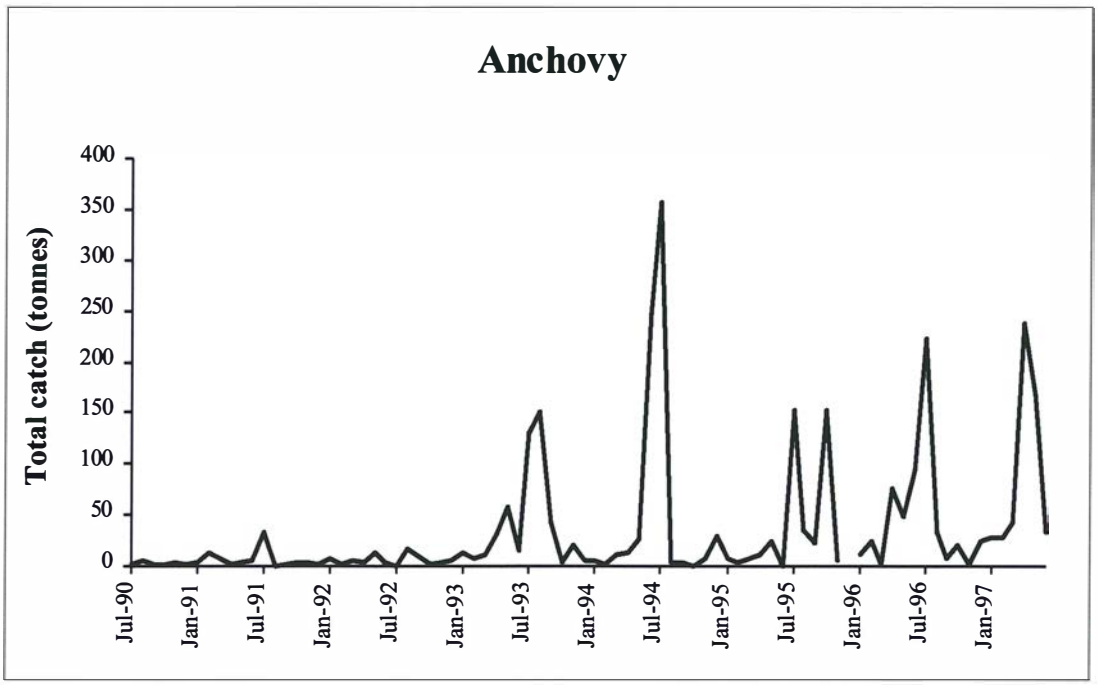


Figure 4.26 Monthly anchovy catches (t) in Victorian waters between 1990/91 and 1996/97.

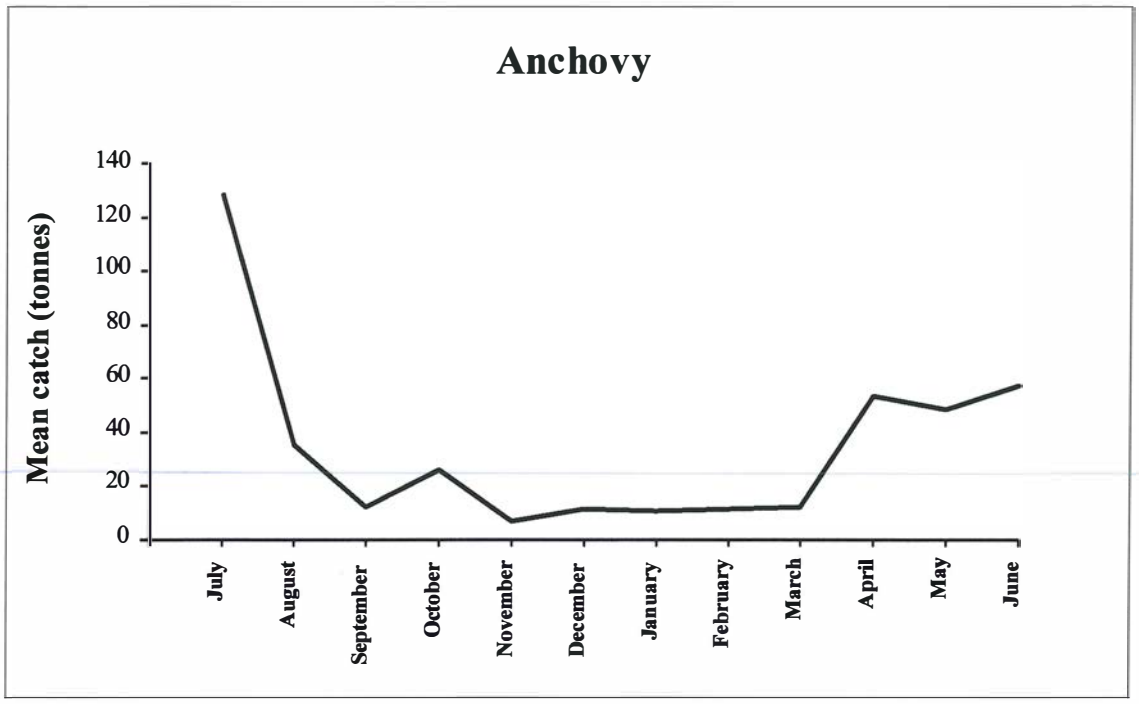


Figure 4.27 Mean monthly anchovy catch (t) in Victorian waters between 1990/91 and 1996/97.

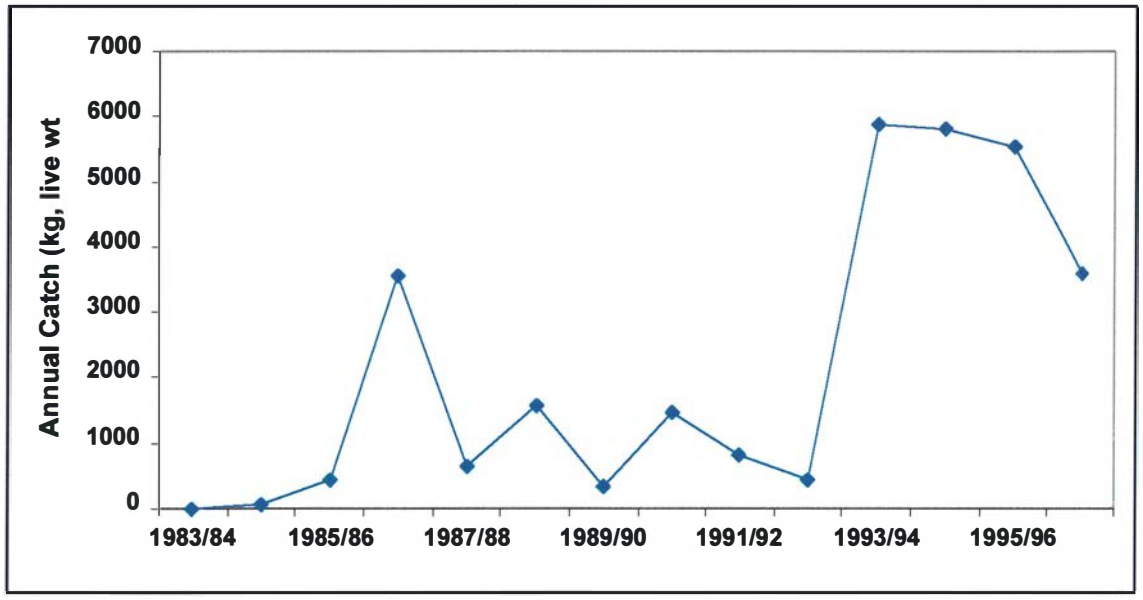


Figure 4.28 Commercial catch (live weight) of blue mackerel in South Australia between 1983/84 and 1996/97.

CHAPTER 5. STOCK DISCRIMINATION OF *SARDINOPS SAGAX* IN SOUTH EASTERN AUSTRALIA

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Objective: To investigate whether Australian pilchards occur as either one large interbreeding population or two or more discrete stocks. Results suggest a complex population structure. The extent of genetic differentiation among populations was relatively high, and the effective number of migrants per generation (N_em) was 1.7 at the macrogeographic scale along the eastern and southern coasts of Australia. When analysed separately from the south coast sample sets, the east coast populations showed a lower level of differentiation among populations than with the combined sample sets. The south coast population showed a higher degree of differentiation than the east coast samples. The genetic variance was as high as that of the entire pilchard population along the eastern and southern coasts combined. It is possible that several stocks of fish exist along the eastern and south eastern coastline with considerable degree of overlapping across boundaries. There appears to be large-scale mixing among populations with temporal, rather than spatial associations of some groups of individuals. The morphometric and otolith microchemistry methods may reflect environmental effects and as such may not be appropriate to delineate stock structures.

5.2 Methods

In any study aimed at stock discrimination, it is essential that more than one approach be taken. In this study we used four different approaches: allozyme electrophoresis, mtDNA analysis, morphometrics and otolith microchemistry.

Collection of Samples

Specimens were collected from Queensland (5 sample sets), New South Wales (5 sample sets), Victoria (9 sample sets), South Australia (9 sample sets), and an additional sample set was obtained from New Zealand (Whangarei, North Auckland), for comparison with the Australian pilchards (Figure 5.1). Appendix 1 presents the collection site and date, number of individuals, sex ratio, size range and breeding status of each sample set investigated in this study.

The pilchard samples were collected from 1995 to early 1998 using purse seine nets and frozen prior to refrigerated transport to the laboratory. Upon arrival, all specimens were stored whole at -20^{0}C, until dissection. Several tissue samples were dissected out (liver, heart,otoliths, gonads, gills and red muscle tissue along the lateral line above the anal region) and kept at -70^{0}C until analysed.

Allozyme Studies

Tissue preparation

The frozen liver tissue samples were homogenized on ice with an equal volume of cold homogenizing buffer (see Appendix 2.2) using a perspex rod. The slurry was then centrifuged using an Heraeus Sepatech 17RS Biofuge at 4^{0}C, at 5000rpm for 10 minutes. The supernatant containing cellular proteins was subsequently used for electrophoresis.

Allozyme electrophoresis

The laboratory techniques for the electrophoresis population study follow the same technical methods and buffer and staining recipes as described in Dixon *et al.*, (1993). Three different buffer systems were used: Citrate aminopropyl morpholine, pH 6.1, Tris-citrate, pH 5.8, and Tris-Maleate, pH 7.8. Six marker loci were investigated: Peptidase B (Leucyl leucyl glycine) (*Pep*-B*) E.C. No 3.4.11 or 3.4.13.9, Aconitate Hydratase (*AH**) E.C. No 4.2.1.3, Phosphoglucomutase (*Pgm**) EC 5.4.2.2, Esterase (*Est*-4*) E.C. No 3.1.1.1, Mannose Phosphate Isomerase (*Mpi**) E.C. No 5.3.1.8, Aspartate aminotransferase (*Aat*-1*) E.C. No 2.6.1.1. In most cases only three enzyme loci (*Pep*-B*, *Aat*-1*, and *Est*-4*) were active, possibly due to denaturation of samples prior to arrival. These enzymes were therefore used as markers in the analyses.

Data Analysis

The results were analysed using four different approaches: 1) combined data across the east and south eastern range of the species, 2) comparison among sample sets collected within the same site, 3) comparison within region and, 4) comparison between adjoining regions. Since mixing occurs during non-spawning periods, it is critical that only spawning fish be examined to establish geographical relationships (Kornfield *et al.*, 1982). In this research, due to the reliance on commercial fishers to obtain fish samples, only five sample sets conprising of spawning fish were obtained for electrophoretic analyses. The sample numbers

were low and ranged between 13 and 54 individuals. The results may be biased due to the small sample sizes.

Measures of genetic variability

The genotype for each individual, at each of the enzyme loci was recorded and analysed using the BIOSYS computer program by Swofford and Selander (1989). Allele frequencies were calculated to determine genetic diversity. The following measures of genetic variability (and their standard errors) indicative of any population shifts in allele frequencies were computed to estimate any differences in allele frequencies among and within populations, under the assumption that each population analysed was in Hardy-Weinberg equilibrium at each variable locus:

1. The mean number of alleles per locus
2. The mean heterozygosity per locus, as a measure of protein polymorphism, was calculated in two ways:
    a) the biased estimate (based on Hardy-Weinberg expectations)
    b) the unbiased estimate based on conditional expectations (Levene 1949, Nei 1978)

Departures from random mating

Each polymorphic marker locus was tested for agreement of genotype frequencies to those expected under Hardy-Weinberg equilibrium, using goodness-of-fit chi-square tests (Wright 1969). Levene (1949) correction for small sample sizes was employed in the chi-square analyses. As more than two alleles were present in the populations, and as pointed out by Sokal and Rohlf (1969), the chi-square test is suspect in cases where the expected frequencies of some classes is low, certain genotypic classes were pooled and the tests were performed one more time. It should be pointed out that pooling may result in failure to detect real deviations from Hardy-Weinberg expectations (Swofford and Selander 1989). A test for non-random association of phenotypes between loci (linkage disequilibrium) was also carried out.

Levels of differentiation among populations

To describe the levels of genetic differentiation within populations F-statistics (Wright 1951, 1978) were used. F_{st} is interpreted as the variance of allele frequencies among populations. It is commonly used as a measure of population subdivision. It provides a convenient approach for estimating interpopulational gene flow. F_{is} describes the departure from random meeting within populations (local inbreeding) whereas F_{it} describes this departure in the total populations (total inbreeding coefficient). These two values are positive when there

is a deficiency of heterozygotes and a negative value indicates an excess of heterozygotes. The significance of the genetic variance for each locus was tested using chi-square analysis (Waples 1987, Chesser 1983, Workman and Niswander 1970). A significant chi-square value indicates an F-coefficient value significantly greater than zero. The statistics used were as follows:

F_{is}: chi-square= F_{is}^2 (k-1); df=[k(k-1)]/2

Fst: chi-square= $2NF_{st}$(k-1); df=(k-1)(s-1)

where N is the total number of individuals sampled, k is the number of alleles and s the number of subpopulations analysed for each locus.

The number of migrants per generation N_em was calculated by the method of Wright (1978) and modification of Crow and Aoki (1984). The relationship is:

N_em= [$(1/F_{st})$-1]/4 α

where α =$[n/n-1)]^2$ and n=the number of populations.

Test for temporal and spatial homogeneity of genotype frequencies

The distribution of genotype frequencies among populations was evaluated using log-likelihood ratio (G) for contingency tables analyses under the null hypothesis of homogeneity of genotype frequencies among populations. The chi-square contingency analyses may lead to suspect results in cases where expected values are low in each cell (Cochran 1954).The G test is considered statistically more robust than the chi-square as it does not suffer from these limitations. It has been recommended by Williams (1976) in preference to the chi-square.

Estimation of genetic distance

In order to estimate the level of divergence between populations the arc genetic distance coefficient of Cavalli-Sforza and Edwards (1967) were used. A matrix of genetic distance was produced for each distance coefficient, together with the corresponding geographic distance. The relationship between those two values was evaluated by plotting geographic distance against genetic distance.

Cluster analysis

To determine the relative genetic differences among geographic populations dendograms based on the genetic distances of Cavalli-Sforza and Edwards arc distance (1976) were constructed using two methods of analysis: (1) cluster analysis (Sneath and Sokal 1973) using unweighted pair group method (UPGMA), (2) distance Wagner procedure (Farris 1972).

The UPGMA algorithm is based on the computation of the average similarity or dissimilarity of an operational taxonomic unit (OTU) to existing cluster on the basis of its average distance to the members of that cluster (Sneath and Sokal 1973).

Swofford's modified Wagner network (Swofford 1981; Swofford and Selander 1989), in which tree optimization was achieved with branch-length optimization, was used. The tree was rooted at the midpoint of the greatest patristic (path length) distance separating a pair of populations.

Comparison of distance matrix and tree building methods

Comparison of the distance matrix methods was evaluated by "goodness-of-fit" which is a measure of the fit of the inferred distances in the tree to the empirical distance values in the original matrix (see Avise 1994). The statistics used were (1) the method of Prager and Wilson (1976), "F", and (2) Farris (1972) " f " value. The smaller the value of these statistics, the better the fit.

Mitochondrial DNA analyses

Pilot study

Initially a pilot study was performed which aimed to:

1) Identify and optimise the method for isolation of the mtDNA
2) Evaluate the use of restriction enzymes in the population study

Five different methods to isolate mtDNA were used. The first method was a combination of large scale and small scale methods (Tamura and Aotsuka, 1988, Sambrook *et al.,* 1989, and modified by Karyn Davis, 1996). A major problem encountered was the large amount of mucus present in the lysed tissue, in particular active ripe gonads (stage 4 and higher). This problem had not been encountered in earlier analyses. Pipetting of extracts was severely hampered as mucus was carried over in "strings". Most of the DNA was lost during the extraction process. DNA concentration was quantified using the 260/280 ratio. The other

tissues used contained less mucus. The tissues that had the highest yield of total DNA were the heart and gills. However, the concentration of mtDNA that could be extracted from these tissues was very low.

The second method used was a total DNA extraction method on a microscale (Stacey *et al.*, 1986, Kidd *et al.*, 1983). This method uses smaller amounts of tissue than the first method. Even though the mucus problem was still present, larger yields of DNA were obtained than with the previous method. However large DNA smears were found after the use of restriction enzymes, indicating that nuclear DNA contamination and possible interference in restriction enzyme activity by the presence of mucus in the extracted DNA sample.

The third method of DNA extraction used was the Chelex-100 resin (BioRad) following the methods of Nielsen *et al.*, (1994). This method was the least successful (about 1/10 of the DNA yield of the other 2 methods was achieved)

The fourth method used and the most successful of the four is the CTAB method (Sambrook *et al.*, 1989). This procedure is particularly suitable for the isolation of DNA from plant and animal tissues that are rich in mucopolysaccharides. This method eliminated most of the mucus problems and yielded high concentrations of DNA in all tissues. Preliminary results of restriction enzyme digestions were smeary (probably due to some mucopolysaccharides bound to the DNA molecules). The use of 3 different commercially available DNA cleaning columns as a final cleaning step prior to using restriction enzymes has been investigated. This was not successful.

Another approach was investigated. The Nucleon ST extraction products (Amersham Life Science) have been used as an alternative extraction method without the use of phenol. The Nucleon resin contained within the Nucleon genomic extraction kits is designed to give high yields of pure DNA from mucus-containing tissues. This method eliminated the mucus problem in approximately 80% of the samples and has been used in this research as the method of choice to isolate DNA from pilchards.

The quantity of DNA was estimated by measuring the absorbance of an appropriate dilution at 260nm using a Beckman DU 7500 spectrophotometer. One absorbance unit was taken as approximately 50mg/ml of double-stranded DNA (Sambrook *et al.*, 1989). The purity of

DNA was estimated using the A_{260}:A_{280} ratio. A ratio of 1.8 is representative of a pure DNA solution.

Due to the presence of mucopolysaccharides in some of the DNA extracts, and possible interference with the restriction enzymes, a different approach was adopted. This was initiated to increase the DNA to mucus concentration using an amplification method. Initial amplification of 710-bp of the mitochondrial control region (D-loop) was carried out with PCR (Saiki, 1990) conditions using a light strand primer (5'TCACCCTTAACTCCCAAAGC 3') (Kessing *et al.*, 1989) and a heavy strand primer (5'ATGACCCTGAAGAAAGAACCAG 3') designed by J.C. Patton at Ecological Genetics, Bryan, Texas (Lamb *et al.*, 1994). The thermal profile was 94^{o}C/30 sec, 55^{o}C/1 min, and 72^{o}C/2 min, for 32 cycles.

Ten ml of a 50ml PCR reaction were loaded onto a 1% agarose gel (1x TBE) and the products separated by electrophoresis. The gel was subsequently soaked for 10-15min in a solution of ethidium bromide and destained for a further 15min in ice cold water. The gels were then placed on a U.V transilluminator and photographed using a Polaroid camera. The amplified product size was estimated using a 100-base pair marker (Promega). A series of restriction enzymes were then tested on the remainder of the amplified DNA solution.

Morphometrics

Pilchard specimens were measured by hand using vernier calipers prior to dissection. Seventeen morphometric characters were used for the analyses.

Multivariate analyses were applied to analyse the morphometric measurements using the statistical package Statistica for Windows, Release 4.5, StatSoft Inc. (1993), and Jumpin (SAS 1997). The stepwise discriminant function analysis (SDFA) was carried out to classify individual fish into groups. The data was standardised to the overall mean standard length of all samples using the formula by Reist (1984),

$e=\log Y-b(\log X-\log 0)$

where e= adjusted measurement

Y= observed measurement in mm

b= the slope of relationship between logY and log X

X= standard length in mm

0= the grand mean of standard length (all specimens)

This method of standardisation was adopted by Syahailatua (1992) in his study on Australian pilchards. The morphometric characters of SDFA were analysed after transformation using the Z-score. Then the cluster to determine the similarity of each group was done using the group centroids on the five possible discriminant functions of the SDFA by the Mahalanobis method.

Otolith microchemistry

Otolith Preparation

Sagittal otoliths from pilchard samples obtained for the genetic study (details of capture method, time and site are given in Chapter 5a) were cleansed and rinsed in milli-Q water, air dried at 37^0C, weighed and recleansed with Milli-Q water. The otoliths were then stored in sterile plastic bags until used in the microchemistry study. Samples were stored and analysed together to remove the analytical variation that can complicate interpretation (Edmonds *et al.*, 1995). Otoliths of similar weight within each site were pooled to provide a sample weight suitable for analysis. In most cases, samples contained at least 0.03 g of pooled otoliths. Sample sizes were small due to the small number of fish obtained for the genetic study. However, samples sizes as low as two (Rieman *et al.*, 1994) and four (Gunn and Ward, 1994) have been included in studies elsewhere.

Microwave Digestion

All containers and measuring equipment used in this section of the study were soaked in a 5% nitric acid bath and rinsed three times with Milli-Q water before use. The pooled otolith samples were placed in Teflon digestion vessels, to which 10 mls of 5% spectroscopic grade nitric acid was added. Vessels were then capped and attached to the microwave digestor carousel. Twelve vessels were placed in the carousel for each digestion run. A batch of twelve samples included one digestion (method) blank and one sample of certified reference material (CRM 422, cod muscle, Graham B. Jackson Pty Ltd). The digestion blank contained 5% nitric acid. It was also used to assess laboratory contamination and to characterise spectral background from the reagents used in the sample processing. The microwave digestor (model MDS-81D, CEM Corporation, Carolina 28079, USA) was programmed following the United States Environmental Protection Agency microwave methods for fish tissue. Digested samples were forced through a 0.45μm pore size and 25mm diameter filter, under vacuum pressure. The vessel caps and pressure relief disks were washed with Milli-Q water into the Teflon vessels and the washings were transferred to the

volumetric flasks. Washing was repeated three times. The solution was then diluted to 25 mL with five percent nitric acid and transferred to plastic containers.

Analysis of Chemical Composition

The digested samples were analysed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) using a Perkin Elmer Optima 3000. A total of 11 elements (Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Sr) were analysed for each sample. The wavelengths used for detection are given in Table 1. Element concentrations were standardised for pooled otolith sample weights and were adjusted to account for the digestion blank values. The minimum detection limits of the ICP-AES instrument for the eleven elements was determined by multiplying the mean standard deviation of the five percent nitric acid blank by three. Calibration standards consisted of four multi-element standards containing Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr, and four multi-element standards containing S and P (Alpha Resources). The standards were prepared to concentration levels of 0.5, 1.0, 1.5, and 2.0 ppm respectively. The instrument was calibrated by running the calibration standards to create analytical calibration lines. A rinse blank (5% nitric acid in Milli-Q water) was used to flush the instrument between standards and samples in order to reduce memory interferences. The calibration blank (5% nitric acid in Milli-Q water) was run prior to analysis. Calibration standards (treated as samples) were interspersed regularly between otolith samples to check for instrument drift and the sample assay sequence was randomised (Campana *et al.*, 1994).

Data Analysis

The otolith chemical composition of pilchard samples caught from different areas were analysed for evidence of separate phenotypic stocks. Multivariate analysis of variance (MANOVA) and univariate analysis of variance (one-way ANOVA) were used to test for differences in elemental concentrations between samples taken from the same site at different times. This was done in order to assess the validity of pooling samples to increase sample size. Hotelling's T^2, Roys and Wilks tests were used in the MANOVA model, however Pillais' tests was considered the most powerful (Norusis, 1992). ANOVA and MANOVA models were used to test for differences in otolith elemental concentrations between sites. This was done on all sites (except those with n=1) and on a subset of sites which contained sample sizes greater than five.

Forward step-wise discriminant analysis was used to discriminate sampling sites on the basis of otolith chemical composition. The stepwise method was used in order to determine the

combination of elements which contributed most to the discrimination model. For purposes of comparison, with the morphometric component of this study, Mahalanobis' distance method was used. The assumption of equality of group covariance matrices was tested using Box's M test (Norusis, 1994). The accuracy of the discriminant function was assessed using the jackknifing (leaving-one-out) method (Norusis, 1994) which gives an almost unbiased estimate of the expected actual error rate (Lachenbruch, 1975). This provides a measure of the general utility of the discriminant function when extended to the whole population (Castonguey *et al.*, 1991).

The group centroids for both discriminant analyses were clustered to determine the relationship between sites. The unweighted pair group method using arithmetic averages (UPGMA) was used. Two dendrograms were constructed using the results of the cluster analyses. All statistical analyses were undertaken using SPSS for Windows (version 6.1).

5.3Results

Allozyme Studies

Allele frequencies

Preliminary examination of genotype frequencies revealed divergence from Hardy-Weinberg (H-W) equilibrium in the form of heterozygote deficits at all loci and a high degree of inter-population differentiation in allele frequencies. The allele frequencies observed in all populations are listed in

Genetic variability measures

The mean number of alleles per locus for mixed populations (Table 5.4) was 3.2 along the species distribution on the east and southeastern regions of Australia. The populations showed a lower than expected level of heterozygosity , i.e there are significant deficiencies in heterozygote proportions in the populations. The only population that had a higher observed heterozygosity than that expected under H-W was CB4.

Deviations from Hardy-Weinberg expectations were observed in all spawning populations. The mean number of alleles per locus was 3.06. Lower than expected numbers of heterozygotes were observed in all populations (Table 5.5).

F-statistics

The F_{is} value, which is the fixation index coefficient within populations, was positive over all populations at all loci (Table 5.7). A positive F_{is} value indicates a deficiency of heterozygotes. F_{is} also describes the level of inbreeding within populations. This value, though relatively high here was not significant ($p>>0.05$). The moderately high F_{st} value among all populations (0.116, $p\leq0.000$) provides evidence of departures from panmixia, i.e. there are significant differences among populations. These results indicate population structuring at the geographic and time-scales sampled. The effective number of migrants per generation N_em was 1.7. However when these statistics were examined on a shorter time-scale, e.g. 1997-1998 (Table 5.8), the F_{st} value was lower (0.088) than over the four-year period, with an N_em value of 2.4. The F_{st} value on the east coast of Australia, including New Zealand, was 0.076 (Table 5.9), while that obtained when only the north eastern populations (Queensland, NSW border, and Gosford) were examined was 0.066 (Table 5.12). The results indicate a larger degree of mixing among populations on the eastern coast of Australia even though there is evidence of some level of population structuring. The south coast populations (Table 5.10), on the other hand, provide a more complex picture than those on the eastern coast of Australia (Table 5.9). The F_{st} values within and between the two regions of Victoria and South Australia (Tables 5.15-5.20) , respectively were as high as that obtained over the four-year sampling on the combined eastern and southern regions of Australia. There appears to be a certain degree of mixing between the Lakes Entrance populations and the Eden population, $Fst=0.078$, and $N_em=1.9$. The number of migrants exchanged per generation among the spawning samples was 1.2, with an Fst value of 0.114 (Table 5.13). This result, however, may be biased due to the small sample sizes.

Departures from random mating

Using the observed genotype frequencies, expected genotype frequencies were predicted under H-W equilibrium assumptions. Of the twenty five populations examined, only LE4 was in equilibrium at all three loci examined. Significant deviations from H-W expectations were observed in all populations (Table 5.21). Most loci were in deficits of heterozygotes in all populations. The results for the spawning populations are shown in Table 5.22. The STF and BB95 populations were out of equilibrium at the Est-4* and Pep-B* loci with deficiencies in heterozygotes. CO1 and NZ1 were out of equilibrium at the 3 loci examined, with heterozygote deficiencies at Est-4* and Pep-B* , and excess of heterozygotes at the Aat-1* locus. FIS was in equilibrium at Est-4* and Aat-1* with deficiencies in heterozygotes at the Pep-B* locus.

Test for temporal and spatial homogeneity of genotype frequencies

Log likelihood tests for each population pair among regions and groups within regions, each with their respective degrees of freedom, are shown in Table 5.6. A high degree of allelic heterogeneity was found at each locus and over all loci among all populations as shown by the small p values. The only two sample set pairs that were not significantly different in gene frequencies were MO1 and M37. These two sample sets were collected within a week of each other.

Cluster Analysis

Cluster analysis (UPGMA) (Figure 5.2) method of tree building, using Cavalli-Sforza and Edwards arc distance coefficient shows a complex picture of population structure. A temporal, rather than spatial, structure of genotypic similarity among and within regions emerges. The goodness of fit statistics, which represent how well the dendogram fit the data, were Farris (1972) "f" 12.768, and Prager and Wilson (1976) "F" 16.234 respectively. While the three south Australian sample sets caught in 1998 grouped together, there appears to be considerable mixing of genotypes among the other sample sets along the eastern and south eastern coast of Australia. However, the genetic distance of close to 0.4 may indicate strong divergence in gene frequencies among groups of individuals within the pilchard population. The dendogram obtained using the Wagner method of tree building, with rooting at the midpoint of the longest patristic distance (Figure 5.5), grouped the Lakes Entrance 16 Aug 97 sample set with the three South Australian sample sets caught in 1998. The tree obtained by this method was not as good a fit to the data as that obtained by the UPGMA method, as shown by the larger “f” and “F” values respectively.

Genetic distance

The genetic distance of Cavalli-Sforza and Edwards were plotted against geographic distance. The results show no pattern of isolation by distance but rather a discrete population structure (Figures 5.2 and 5.3).

Spawning populations

Cluster analyses using UPGMA (Figure 5.6) and Distance Wagner method of tree building (Figure 5.7) show 2 groups comprising of STF and FI, and BB95, NZ1, and CO1 respectively.

Mitochondrial DNA analyses

The restriction enzymes used in this study were *Hpa* II, *Tru*9 I, *Rsa* I, *Aat* 2, *Taq* I, *Ban* II, *Mbo* I, *Dde* I, *Dra* I, *Hinc* II, *Pst* I, *Pvu* II, *Alu* I, *Bgl* I, *Sau*3AI, *Bam* HI, *Hae* III, *Xba* I GQ, *Hin*d III, *Xho* I, *Hin*f I. The following restriction sites were found to be polymorphic: *Hpa* II, *Tru*9 I, *Dde* I, *Pvu* II. However star reactions, i.e. non-specific reactions were observed for most of these polymorphic enzymes, thus causing problems in the interpretation of the fragment lengths. The use of restriction enzymes in this research was thus abandoned and the amplified products of the d-loop region of the pilchard mt-DNA were cycle sequenced using the light strand primer. The results of this research are being analysed and will be reported elsewhere.

Morphometric studies

Twenty nine pilchard populations, sampled over a four year period, were analysed with 16 morphometric characters to determine how many groups of pilchards occur in Australian waters. The results show the possible existence of two or more major groups consisting of several overlapping temporal groups based on the characteristics of morphometric variables (Figure 5.8). In this study, logarithmic transformation for morphometric data was used to adjust the data variation. This transformation is widely used in the statistical analysis of morphometric data (e.g. Claytor and MacCrimmon 1987; Sokal and Rohlf 1981; Winans 1984, 1987; Dixon et al., 1987). The data was further divided into the eastern and southern sample sets. The results of the east coast analysis show the New Zealand sample is more similar in morphometric chracters to two Lakes Entrance sample sets, one Eden sample and a Coolum sample (Figure 5.9). The interesting result is that the Lakes Entrance 1995 sample (LE0), which was caught during the early 1995 pilchard kill, comes out as a separate group from the rest of the other south coast sample sets (Figure 5.10). It is found to cluster with some of the northeastern populations. This study included a much larger number of sample sets than the previous work of Suahailatua (1992) which showed the possible existence of 4 groups of pilchard on the eastern and southeastern coasts of Australia. Our study contrasts with the previous, in that a more complex picture of population structure, which is influenced by temporal rather than spatial components, emerges.

Multivariate discriminant function analyses were performed on 16 morphometric characters of 29 sample sets. The results showed that the correct classification of specimens according to the geographical locality was between 11.54% for Flinders Island and 100% for Gosford, Lakes Entrance (1995) and Boston Bay (1995) respectively. The overall mean, broken up by

year, was 83.63%, and 82.39% for 1998 and 1997 respectively (Table 5.23, 5.24). The mean for 1996, and 1995 were 82% and 100% respectively (Table 5.25).

Following the discriminant function analyses, canonical analyses were performed and the means of the canonical scores were plotted, using roots 1 and 2, for the 1997 (divided into south and east coasts) (Figures 5.11 and 5.12). The raw canonical scores were plotted for the fish caught in 1998. Examination of the east coast populations for 1997, reveal the separation of a Queensland group comprising of two Mooloolaba sample sets and one Coolum sample set, from the other east coast sample sets. The other group included the two Eden sample sets, two Clarence River sample sets, a Coolum sample set and a Mooloolaba sample. The New Zealand sample clustered with that large group. The south coast populations appear to be divided into 2 or three overlapping sets. Two of the Lakes Entrance sample sets separated out from the Port Philip Bay and the south Australian samples. The 1998 samples which comprised of one New South Wales sample, Gosford (G98), and South Australian and Victorian samples, shows the clear separation of the Gosford sample from the soth coast samples. A high degree of mixing is evident among the south coast samples.

Otolith Microchemistry

Elemental Concentrations and Sample Organisation

The minimum detection limits of the ICP-AES (ppm) were: Ba (0.00290), Ca (0.01002), Cu (0.00351), Fe (0.00397), K (0.08069), Mg (0.00030), Mn (0.00052), Na (0.00147), P (0.05134), S (0.00958), Sr (0.00034). The elemental concentrations of sagittal otoliths taken from *S. sagax* are shown in Table 5.26 and 5.27. The sample size for each site was small. The ANOVA and MANOVA results showed that replicate samples from each site were significantly different ($p<0.001$ in all cases) therefore they were not pooled for analysis.

Spatial and Temporal Differences in Elemental Composition

The MANOVA (for all sites with $n \geq 2$) indicated significant differences between sample sets ($p<0.001$) and the one-way ANOVA results showed significant differences in the levels of Ba, Cu, Fe, Na, S and Sr ($p<0.05$). The step-wise discriminant analysis including all sample sets correctly classified 40.90% of the samples. However, samples from Port Phillip Bay (21.3.97), Anxious Bay (23.4.97) and New Zealand showed a one hundred percent classification success. The Coolum (8.11.97) and Lakes Entrance (26.4.95) samples showed a classification success of 66.7%. The elements included in the discriminant functions were: Mg, Na and Sr. The jackknifing method showed a classification success of 11.67%. Box's

M test was significant (137.95449, df=24, $p<0.001$). The dendrogram obtained from the cluster analysis showed two major branches separating the southern samples from those located more to the east and north.

The MANOVA testing for differences in trace element concentrations between samples with sizes greater than 5 was significant ($p<0.01$). Significant differences were found in the levels of Ba and Mg (ANOVA, $p<0.05$).

The step-wise discriminant analysis including the sample sets with sample sizes greater than five correctly classified 82.14 % of the samples. The New Zealand sample showed a 100% classification success. The Port Phillip Bay samples (22.11.97 and 11.2.98) showed classification levels of 72.7% and 85.7% respectively with the highest levels of misclassification being into each other. The Coffin Bay sample showed a classification success of 80%. No samples were misclassified into the New Zealand group. The elements included in the discriminant functions were: Mg, Ba and Na. The jackknifing method showed a classification level of 17.86%. Box's M test was not significant (21.65498, df=18, $p=0.6218$). The dendrogram obtained from the cluster analysis showed the Coffin Bay sample to branch off separately from the Port Phillip Bay and New Zealand samples.

5.4 Discussion

Genetic analyses

In many marine species, with large fecundities such as those with a pelagic larval stage and with high rates of mortality, especially in the earlier stages of their life cycles, the resulting number of offspring is highly variable (Hedgecock, 1994). According to theoretical population genetics the resulting effective population size is considerably lower than actual population sizes (Crow and Kimura, 1970; Crow and Denniston, 1988). Moreover, it is now widely accepted that the complex oceanic patterns that affect the reproduction and survival of marine organisms both at a spatial and a temporal scale are strongly correlated with population structure (e.g. Parrish *et al.*, 1981) and with the overall or regional recruitment of these organisms (e.g. Roughgarden *et al.*, 1988). These processes may result in a small minority of offspring replacing the adult population as most individuals fail in the "sweepstake" of reproductive activity and success. Hedgecock (1994) postulated that the resulting large variance in Ne/N ratios (N stands for actual population size and Ne is the effective population size) accounts for local differentiation in many marine populations despite the potential for high gene flow (e.g. Burton 1983; Hedgecock *et al.*, 1982;

Hedgecock, 1986). Paradoxically, similarity of allelic frequencies throughout the ranges of such species is often observed (Palumbi, 1992).

In the Australian pilchard, *S. neopilchardus*, genetic analyses revealed a high degree of genetic heterogeneity among populations and between temporally separated samples from the same location. Only two sample sets of populations tested for similarity of allele frequencies showed no significant differences in genotype frequencies. These two populations were collected at Mooloolaba, Queensland, within a week from each other. This indicates that pilchard groups may spend some time in the same location before moving on to new grounds. In the other regions replicated sample sets collected at different times of the year showed marked differences in gene frequencies. Significant departures from Hardy-Weinberg proportions and deficiencies of heterozygotes were observed at many loci in most populations, indicating some degree of population subdivision and /or inbreeding.

We could not, however, establish whether this population structuring is due to homing behaviour to spawning estuaries or embayments. Such spatial and temporal patterns may be the product of various ecological and genetic processes. Populations may not be a random mix of individuals but, rather, an aggregate of genetically similar individuals. Alternatively, limited dispersal of gametes may reflect in restricted gene flow and subsequent isolation by distance and differentiation among groups of recruits by the process of genetic drift. This type of spatial pattern would reflect degrees of association within populations. Selection processes also affect the distribution of genotypes within and among populations. This process operates on populations subjected to variable environmental conditions and may be observed at both a macro- and microgeographic scale. Genetic distribution may also be influenced by the association of life history traits with environmental factors, for example, reproductive strategies combined with disturbance patterns may result in non-random association of genotypes (Soulé, 1987). In many marine populations, reproductive success and survival rely upon factors such as proximity, survival and compatibility of gametes, ecological and environmental conditions/disturbances such as complex oceanic current patterns, salinity, temperature, wind movement, predation, parasitism, etc. Survival of larvae and subsequent recruits may thus depend upon their ability to pass through windows of opportunity through space and time and may be partly governed by the surrounding localities and the interactions between them.

The degree of divergence from equilibrium in *S.sagax* may be attributed to several factors. First of all, the presence of null alleles causes a deficiency in heterozygotes scored. However, Koehn *et al.*, (1976) and Zouros *et al.*, (1980) showed that the proportion of nulls had to be unusually high to cause such deviations from equilibrium. In this study, no evidence of null alleles was observed and therefore this effect could not be attributed to the presence of a null allele. Secondly, selection through differential viability, may affect the number of each genotype present in a population (e.g. Cook, 1971). However, selection pressures have to be very intense to have any effects on changes in relative genotype frequencies between conception and sampling (Richardson *et al.*, 1986). As shown by Adamkewicz *et al.*, (1984), at the Lap locus, recruits of the clam *Mercenaria mercenaria* that were younger than five months old showed no signs of heterozygote deficiencies as compared to year old recruits. They suggested that selection against heterozygotes, through differential viability, may be occurring in the second half of the first year. It seems unlikely however, that such an effect would occur for all loci and alleles studied in the project. Thus selection may be an unlikely explanation.

Thirdly, sex-linkage may be a frequent cause of deviation from Hardy-Weinberg expectations (Pasteur *et al.*, 1988). In this study this explanation may be discarded as heterozygotes occurred in both sexes at all loci, proving that the marker loci were not sex-linked.

A fourth explanation that will result in deviation from equilibrium is the occurrence of bottlenecks or crashes in population numbers. Hardy-Weinberg equilibrium assumes large numbers of individuals mating randomly in a population. When the population size is small, such as after a population crash, chance events (genetic drift) have a greater effect in sampling of gametes than when population size is large. This means that the smaller the population size, the larger the drift. In the case of sardine populations that are known to undergo massive kills, large reductions in population numbers will occur. It is expected that such huge reductions in numbers will affect the amount of drift in the following generations and also the mating structure of the population.

However, bottlenecks result in loss of alleles and not in heterozygosity in a population (e.g. Malécot, 1964; McCommas and Bryant, 1990). In the populations of *S. neopilchardus*, the mean number of alleles per locus was relatively low (3.2); heterozygosity levels were also lower than predicted in most populations. These severe deficiencies in heterozygotes may partly be attributed to bottleneck effects. Founder effects, on the other hand, give rise to

genetic drift which in turn result in a 65% reduction in heterozygosity (Nei *et al.*, 1975) and a simultaneous reduction in the average number of alleles per locus (Systma and Schaal, 1985). A fifth explanation, is the presence of gametic phase disequilibrium. This is the non-random association of alleles of different loci. In this case no evidence of linkage disequilibrium was found in any of the populations sampled and between pairs of loci. Therefore, this explanation may be rejected.

A sixth, and most likely explanation here is the presence of two or more genetically distinct groups of individuals in the population, causing a Wahlund effect. This process occurs when sampling is done across apparent panmictic populations which are actually composed of several sub-populations with different gene frequencies. This Wahlund effect could be due to gene-frequency differences between the sexes, age or year classes within the sample sets. Alternatively it could be due to the presence of distinct subpopulations in the same area. Pilchards are a highly schooling species that form large shoals (Pitcher, 1983). These aggregations usually consist of fish of approximately the same size, however, mixed schools are not uncommon (Fletcher *et al.*, 1997).

This observation is also supported by this study where mixed schools were observed in many sample sets. These aggregations are themselves ephemeral as they appear and disappear within short time scales (hours to days). Therefore it is highly likely that catches consist of many groups of individuals with different gene frequencies.

From the results obtained in this study, it appears that the pilchard population on the eastern and south eastern coast of Australia is made up of three or more major stocks, comprising of several quasi subgroups, with overlapping boundaries. The fact that there were similarities in gene frequencies between some northeastern populations and the southern ones may either be coincidental or a reflection of past patterns of gene flow among those populations.

Morphological Studies

The material for the Australian pilchard only came to hand intermittently, as shown in our collection data. This is due to the limited fishery in some regions of New South Wales and fluctuations in abundance and availability in other parts of the country. Due to the different spawning seasons among sampling sites and our reliance on commercial fishers to provide the fish samples, it was not possible to collect fish of the age at the same time or season.

Although there were differences in sizes (Appendix 2) within and among samples (hence age classes), adjustment of the variables using the standardisation coefficient (Reist, 1985) should have reduced any effects caused by size differences. This method is also suggested by Claytor and MacCrimmon (1987). Two previous morphological studies (Blackburn, 1951; Syahailatua, 1992) have described the possible existence of four regional populations of pilchards in Australia: an eastern group (New South Wales), a south-eastern group (Victoria), a south-western group (southern Western Australia), and the western group (West coast, Western Australia). The study reported here contrasts with the previous study of Syahailatua (1992), in that a larger sample set was studied.

Vertebral counts were not examined in this research as any such variations among and between individuals or localities may be partly non-genetic in pilchards (e.g. Blackburn, 1951) and in other fish (e.g. Gabriel, 1944; Tåning, 1952) and thus are of limited value in stock discrimination studies. In addition, since sexual differences in morphological characters were not found in the previous study of Syahailatua (1992), it was assumed that sexual dimorphism is rare in this fish. Accordingly, the sexes were combined in the morphological analyses.

Geographical grouping in the Australian pilchard is evident from this research. A highly complex population structure emerges where regional stocks are ephemeral and a high degree of population mixing occurs between the eastern and south eastern regions. There may in fact exist several stocks of fish that could not be delineated here, due to the intrinsic nature of their schooling abilities.

Otolith microchemistry

Based on the results obtained in the genetic studies and from what is known about the schooling patterns of Australia pilchards (Pitcher, 1983; Fletcher *et al.*, 1987), interpretation of population structure using trace element composition of otoliths should be approached with caution. First of all, the necessity to combine otoliths of similar weight from the same sample batch may have introduced considerable bias in the results. Secondly, due to the limitations of the method, variability in elemental composition of pairs of otoliths taken from the same fish were not examined. These differences, if any, may not be discounted here. In addition, most studies have assumed that otoliths, when analysed, retain their composition prior to handling, i.e. in live fish. This assumption may be far from the truth. Proctor and Thresher (1998) reported that most elements were affected to various degrees by handling and

storage methods, with Sr and Ca being the most stable. Based on the relative stability to handling artefacts of the elements they tested, they postulated that Mg and Ba based on their chemical similarity to Ca and Sr are also expected to demonstrate similar lability levels. Sodium, however, is apparently labile and this behaviour in other biological aragonite structures such as corals has been documented (Amiel *et al.*, 1973).

However, post-mortem artefacts that may be present have not been tested in this research and hence their significance here cannot be commented upon. It is indeed not our intention to suggest that this research, despite obvious reservations about its interpretation, is without merit. Although the results were limited due to small sample sizes, they did show geographical variations in otolith elemental composition. Different elemental concentrations were found in New Zealand pilchards as compared with those found in fish caught in Australian waters. There are some similarities among southern Australian samples while the northern and south-eastern samples show differences when compared with the southern samples. Temporal differences are also evident, especially within Port Phillip Bay.

Temporal differences may be due to: sampling of different stocks which move within the bay at different times, movement between stocks, or differences due to exposure to different environmental conditions over time.

Elemental concentrations of pilchard otoliths caught from the lower west coast and western south coast of Western Australia showed limited migration of a few major populations (Edmonds *et al.*, 1995). Temporal differences sometimes exceeded spatial differences. The reasons for temporal variation were thought to include migration and the possibility of different life stages being represented unevenly in replicate samples.

Specific Elements which may be Important

Comparisons of the elements common to this study and that of Edmonds *et al.* (1995) showed similar concentrations. Similar trace element concentrations across several species, eg. yellow-eye mull*et Aldrichetta forsteri* (Edmonds *et al.*, 1992), orange roughy *Hoplostethus atlanticus* (Edmonds *et al.*, 1991, 1995) have been noted . However, strontium concentrations in pilchards were found to be a magnitude lower than those found in other species. This study also found relatively lower concentrations of strontium in pilchard sagittae. Sodium, strontium and phosphorus were the most important elements for distinguishing pilchards caught from within Western Australia (Edmonds *et al.* 1995). This

study also found that sodium and strontium were important, along with barium and magnesium. The finding, in both studies, that sodium varied between sites is unusual, given that it is considered to be tightly regulated (Campana *et al.*, 1995). This could be due to post-treatment lability of sodium (Proctor and Thresher, 1998).

Differences Between Ages/Sizes

Spatial differences between fish in their otolith microchemistry can only be effectively used to discriminate phenotypic stocks if fish of the same age are compared (Begg, 1997; Milton *et al.*, 1997). Potential bias from size related factors was found in *Arripus trutta* samples from Tasmanian waters (Kalish, 1989) and samples of school and spotted mackerel from northern Queensland (Begg, 1997).

This study did not compare otolith weight groups due to the small numbers of fish obtained from each site and the necessity of pooling otoliths for analysis. The data was not standardised for fish length, age or otolith weight. This may have introduced some level of bias. Marked differences in sodium concentration with otolith weight occurs in pilchard otoliths (Edmonds *et al.*, 1995), suggesting that the differences in sodium concentrations detected in this study would be especially size biased.

Differences in trace element concentrations in the otoliths of fish of different ages (inferred from length or otolith weight) from the same site are not unexpected, especially if element deposition is not irreversible. Older fish are likely to have different concentrations to younger fish as a result of older fish migrating to spawning or feeding grounds, or younger fish inhabiting nursery grounds discrete from adult habitats. The age groups would, thereby, be exposed to different environmental conditions. Differences may also be the result of temporal variations in environmental parameters, so that older fish may be exposed to environmental conditions that are not present in the next year.

Age class differences may be unimportant if regional differences are genetically determined (Thresher *et al.*, 1994). If differences do occur between year classes then environmental influences may be more important, or more plausible, as an explanation.

The reasons for differences in the trace element levels of sagittae from different locations are currently unknown (Gauldie *et al.*, 1993). Elemental composition of otoliths appears to vary with environmental conditions, including: temperature (Radtke, 1989; Townsend *et al.*,

1992), salinity (Kalish, 1990; Hoff and Fuiman, 1995) and water chemistry (Edmonds *et al.*, 1989; Rieman *et al.*, 1994). There is also evidence that elemental composition reflects intrinsic conditions such as: growth rate (Edmonds *et al.*, 1995; Kalish, 1989; Sadovy and Severin, 1992), body size (Gauldie *et al.*, 1986), age (Casselman *et al.*,1981; Gallahar and Kingsford, 1996; Hoff and Fuiman, 1993), stress (Townsend *et al.*, 1992) and reproductive state (Fuiman and Hoff, 1995; Kalish, 1991).

Not all factors affecting the deposition of trace elements in otoliths are strictly environmental, nor do they necessarily act in a simplistic manner (Campana *et al.*, 1994; Edmonds *et al.*, 1995; Kalish, 1989). Elemental concentrations in otoliths are likely to be the result of complex interactions between genomic and physiological control, and environmental conditions. As previously mentioned, post-mortem artefacts due to the lability of the different elements may also affect the results.

Results of stock identification studies using otolith microchemistry have commonly been in general agreement with genetic studies (Edmonds *et al.*, 1989, 1991, 1992; Gauldie and Nathan, 1977; Kalish, 1990). Environmental and genetic factors have been shown to be responsible for chemical deposition in *Oncorhynchus nerka* vertebrae (Behrens Yamada *et al.*, 1987). Thresher *et al.* (1994) suggested that the chemical composition of southern temperate groundfish (*Nemadactylus macropterus*) otoliths is much less sensitive to environmental conditions than previously thought and suggested that regional differences in composition either have a genetic basis or are set by environmental influences early in life and are then maintained throughout subsequent life history.

The validity of using stock and site specific fingerprints does not rest upon the mechanism underlying their formation (Campana *et al.*, 1994). Under Ihssen *et al.*'s (1981) definition of a stock as an "inter-specific group of randomly mating individuals with temporal or separate spatial integrity", the progeny of a single spawning aggregation would grow up in a range of different environments with little intermixing after hatch (Campana *et al.*, 1994). Such stocks would likely be characterised by different whole otolith elemental fingerprints just as they would be by physiological differences such as growth rate (Campana *et al.*, 1994).

General conclusion

Biogeographic results derived from allozymes, morphometrics and otolith microchemistry were not congruent for this study of pilchard populations. These data suggest that these

markers do not reflect the same evolutionary architecture in *S. neopilchardus*. However, what is clear is that a high degree of heterogeneity exists. The morphometric and otolith microchemistry data are more likely to reflect environmental factors than genetic effects and thus, if used separately may be of little value to separate genetic stocks.

The genetic heterogeneity that was found in the Australian is most likely due to the presence of several groups or stocks of fish in Australian waters. Since no association of phenotypes was detected in this fish, it is impossible to define the genetic characteristics of these populations. However, the observed divergences are due to gene frequency differences and not to fixed differences among populations. In spite of this apparent heterogeneity, a high degree of mixing is evident. This is not surprising as pilchards are believed capable of travelling distances in excess of 30 km per day (Fletcher *et al.*, 1997). This hypothesis is supported by the fact that the rate of spread of the disease causing the 1995 pilchard kill was estimated as having a median rate of 30.4 km per day.

The pilchard population on the south coast appears to be made up of two or three stocks, as the coefficient of genetic differentiation among populations was as large as that obtained when the entire eastern and southern populations were analysed. It was not possible, however to separate the groups according to their breeding grounds. On the eastern coast, there appears to be one or two northern populations with overlapping boundaries, a central population in the Gosford and a southeastern population with overlapping boundaries with the Lakes Entrance region, in Victoria. In terms of fisheries management, this research could not establish clear stock boundaries. We however recommend that the stocks be managed on a state by state basis and that fisheries managers collaborate with each other.

Figure 5.1. Map of Australia showing sampling sites for *Sardinops sagax.*

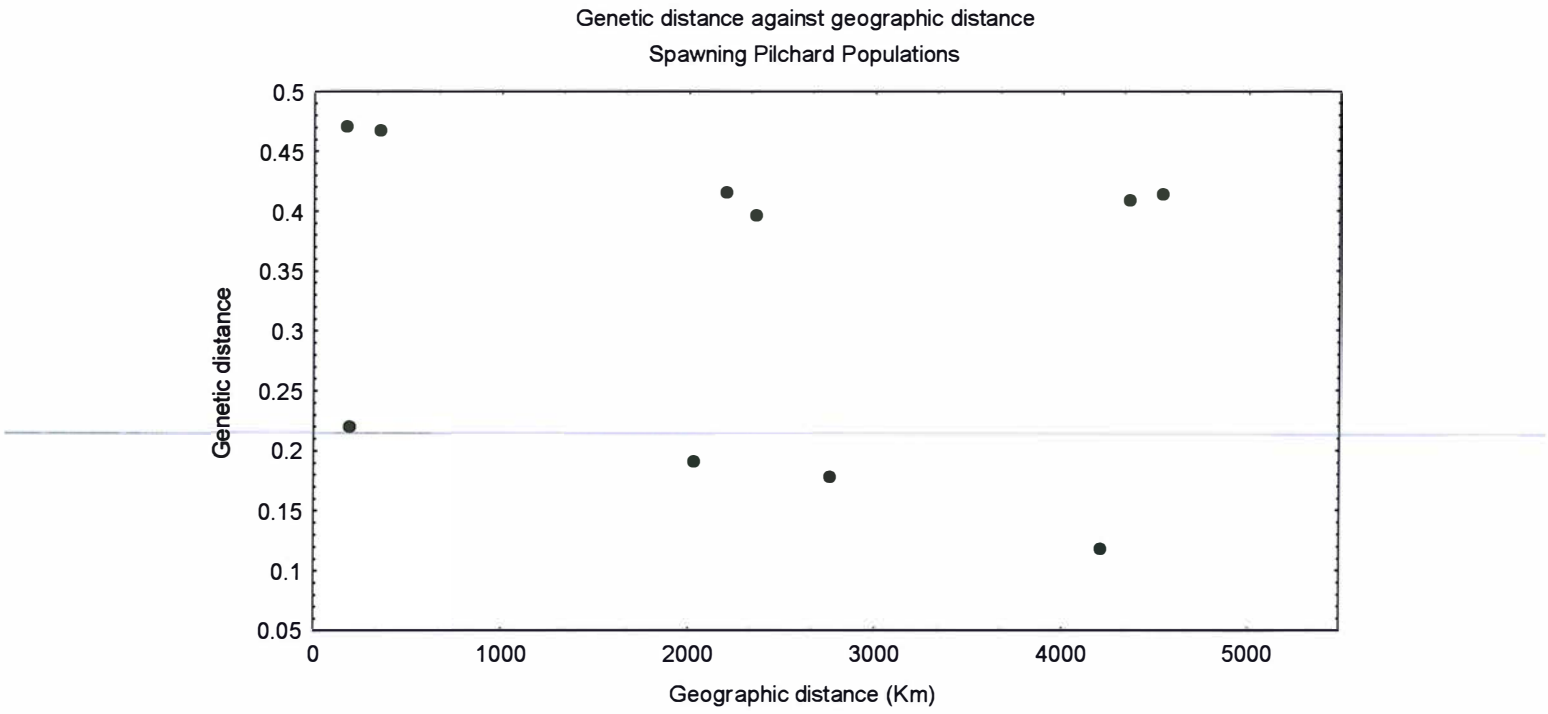


Figure 5.2 Genetic Distance plotted against geographic distance (spawning populations).

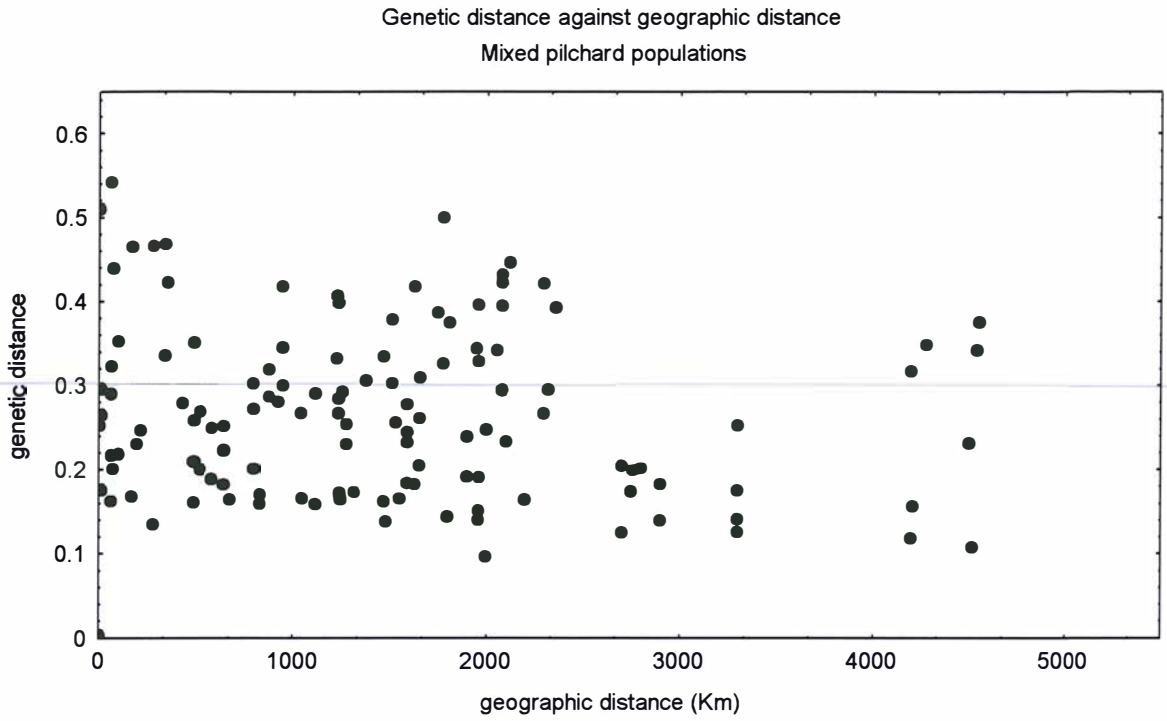


Figure 5.3 Genetic distance plotted against geographic distance (mixed populations).

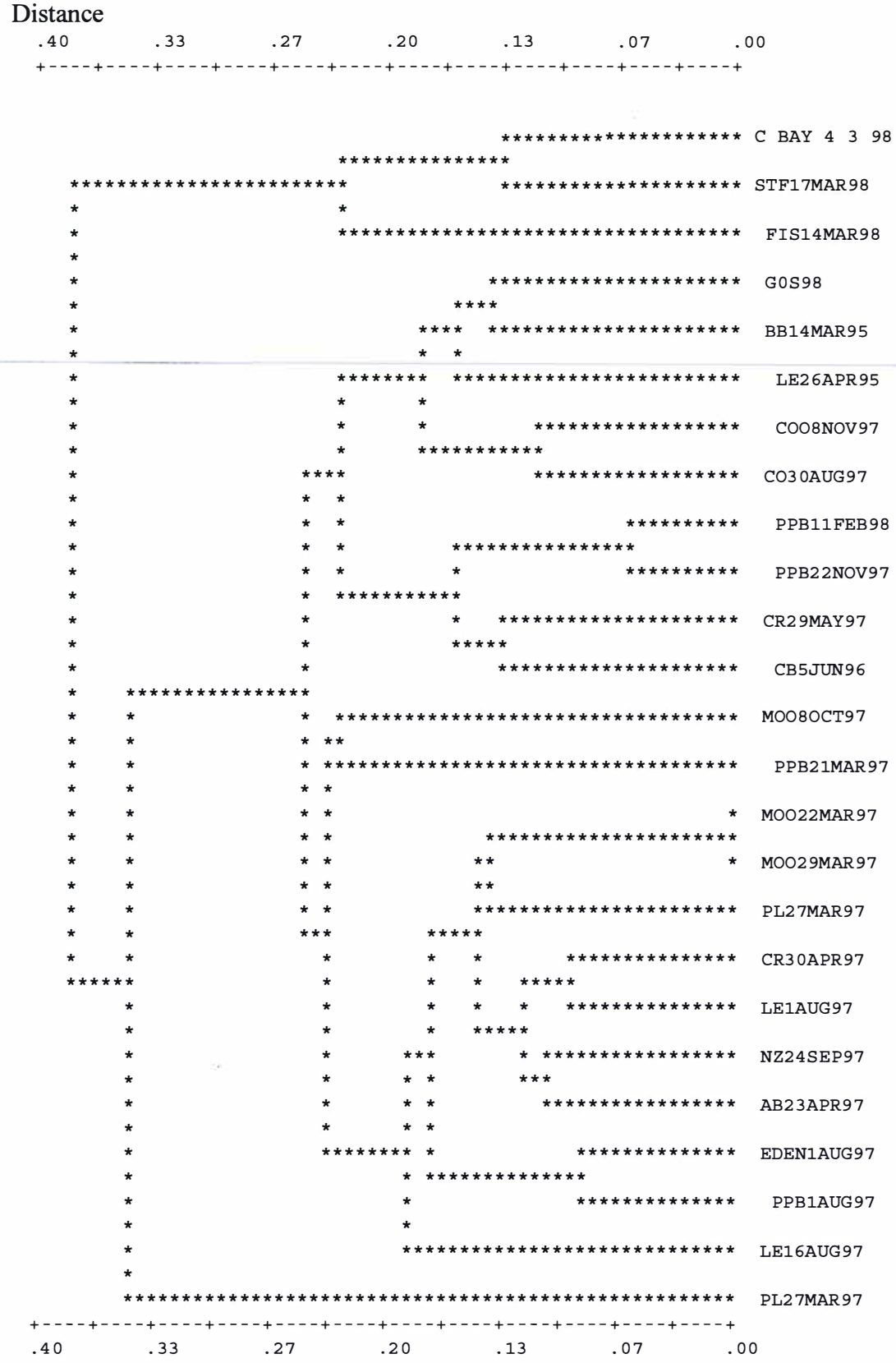


Figure 5.4 UPGMA of Australian Pilchards on the eastern and south eastern coast of Australia Goodness of fit statistics. Farris (1972) "f" = 12.768; Prager and Wilson (1976) "F" = 16.234

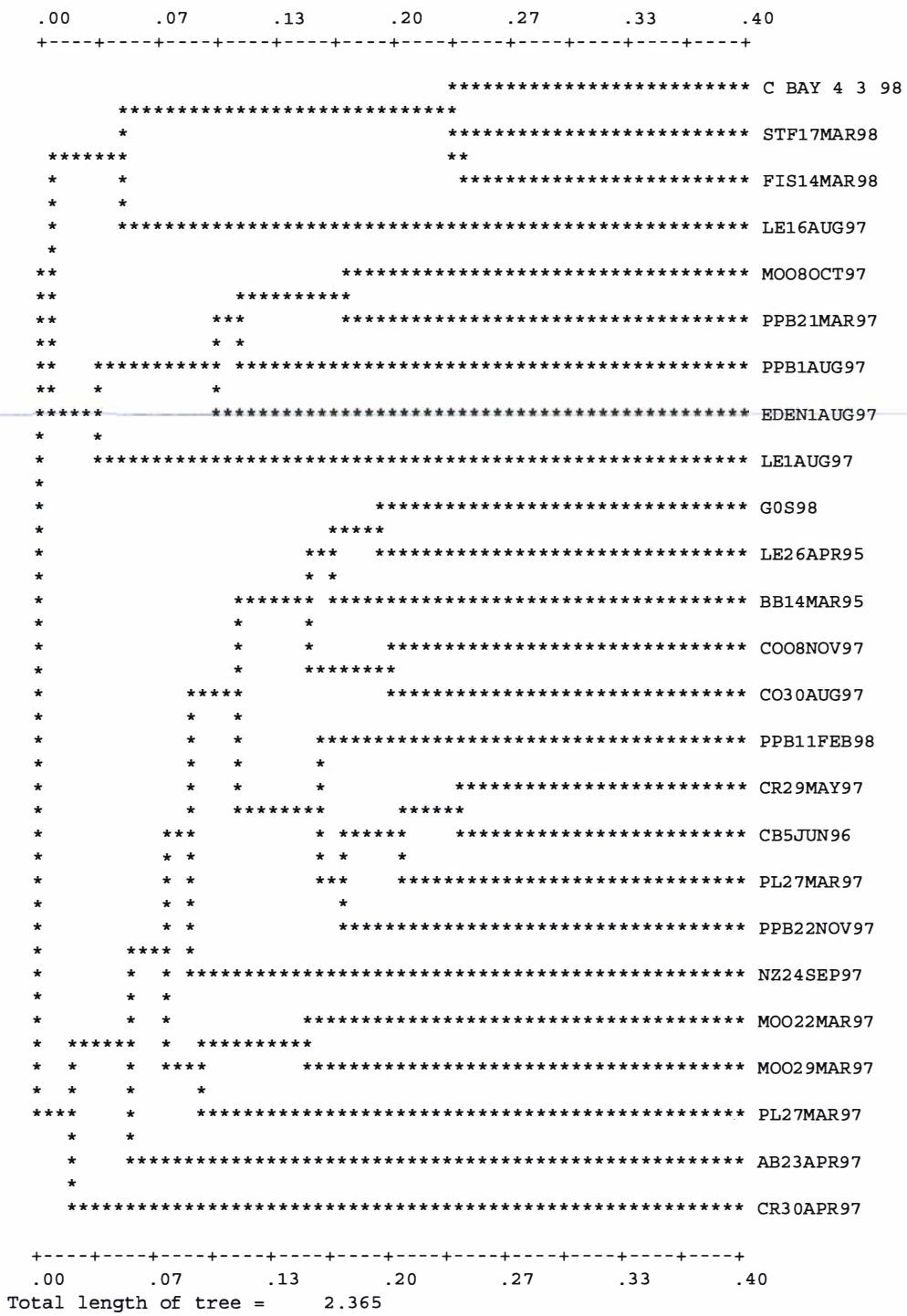


Figure 5.5 Cluster analysis of the Australian Pilchard on the eastern and south eastern coast of Australia, using the Wagner tree method produced by rooting at midpoint of longest path. Goodness of fit statistics: Farris (1972) "f" = 25.052; Prager and Wilson (1976) "F" = 31.854 Distance from root

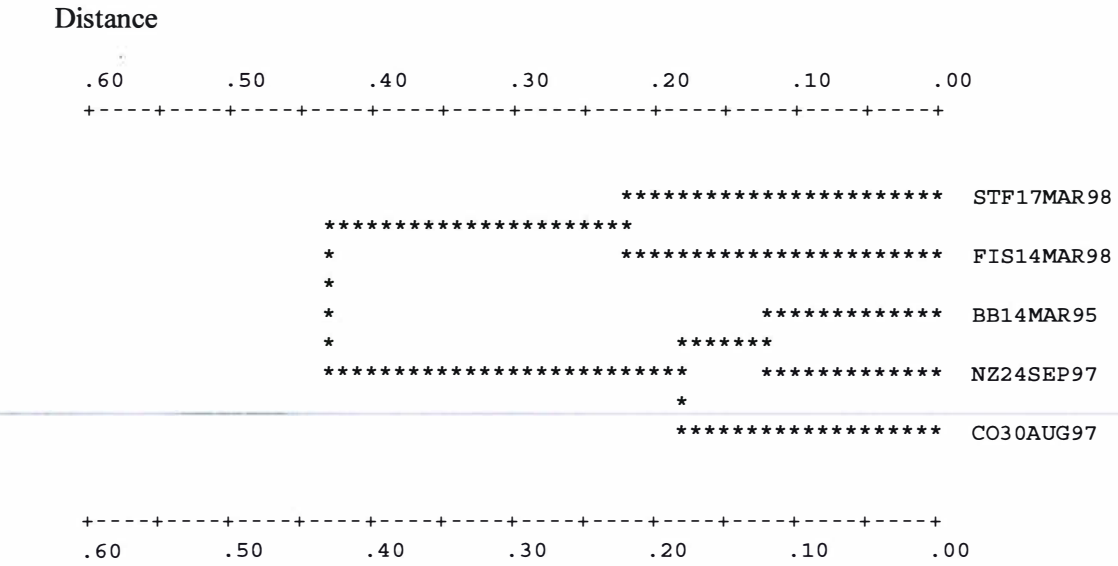


Figure 5.6 UPGMA cluster analyses in spawning populations Goodness of fit statistics Farris (1972) "f" = .174 Prager and Wilson (1976) "F" = 5.283 Percent standard deviation (Fitch and Margoliash,1967) = 5.632 Cophenetic correlation = .984

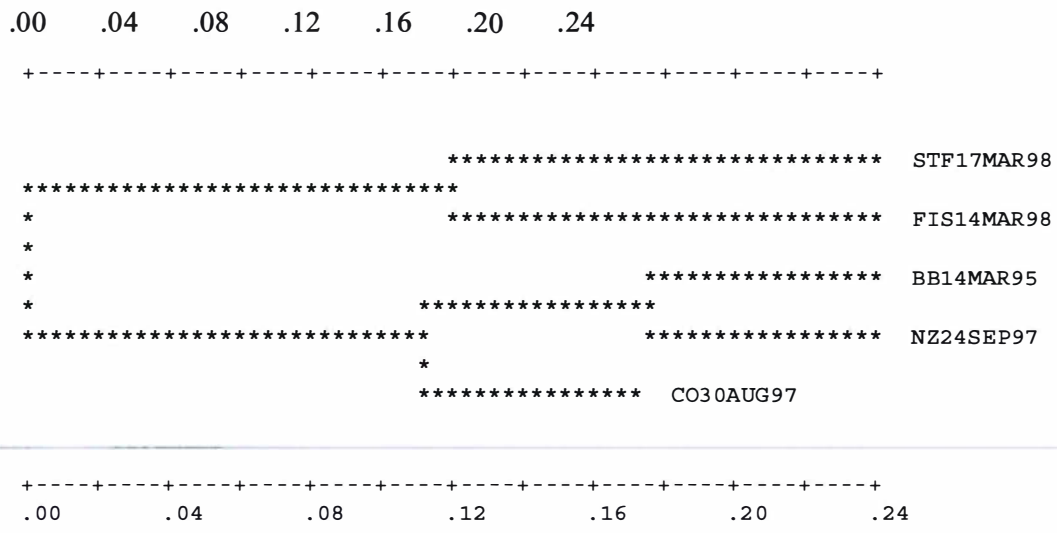


Figure 5.7 Spawning populations Distance Wagner method of tree-building Goodness of fit statistics Farris (1972) "f" = .121 Prager and Wilson (1976) "F" = 3.693 Percent standard deviation (Fitch and Margoliash,1967) = 6.317 Cophenetic correlation = .990 Wagner tree produced by rooting at midpoint of longest path. Distance from root Total length of tree = .701

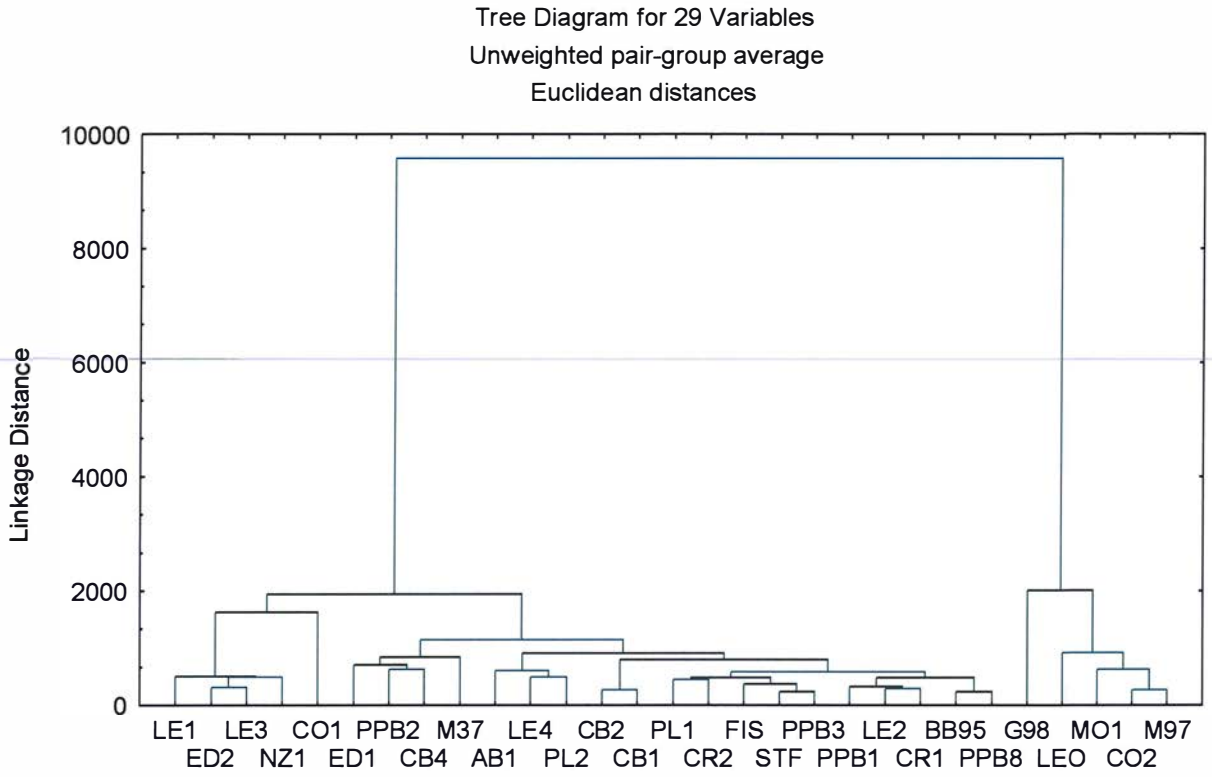


Figure 5.8 Cluster analysis of 29 sample sets of pilchards collected between 1995 to 1998 on the eastern and southern coasts of Australia

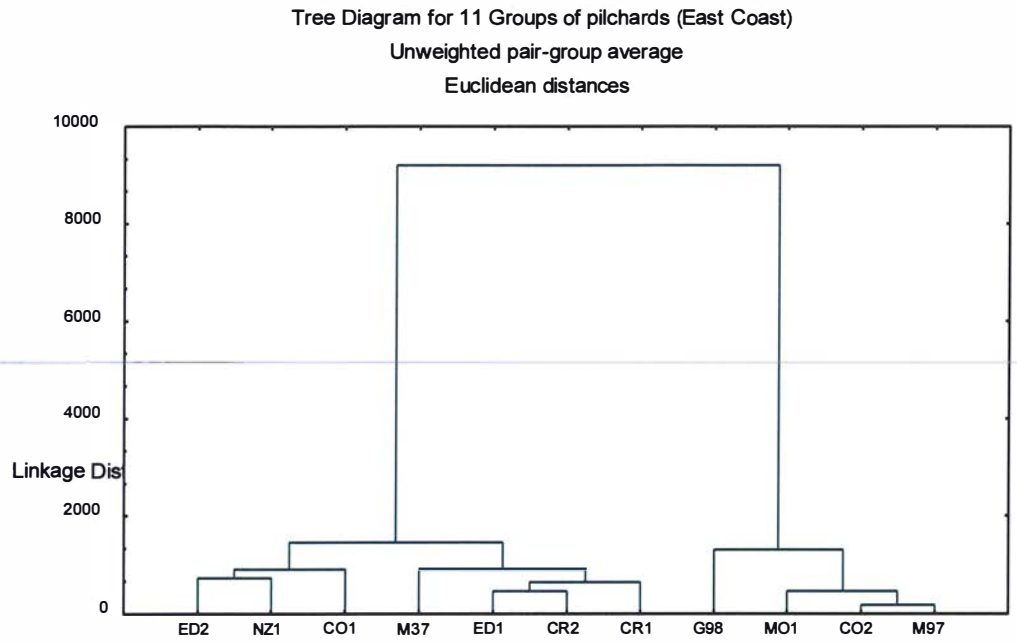


Figure 5.9 Cluster analysis based on morphometric characteristics of samples of pilchards caught between 1996 and 1998 on the east coast of Australia

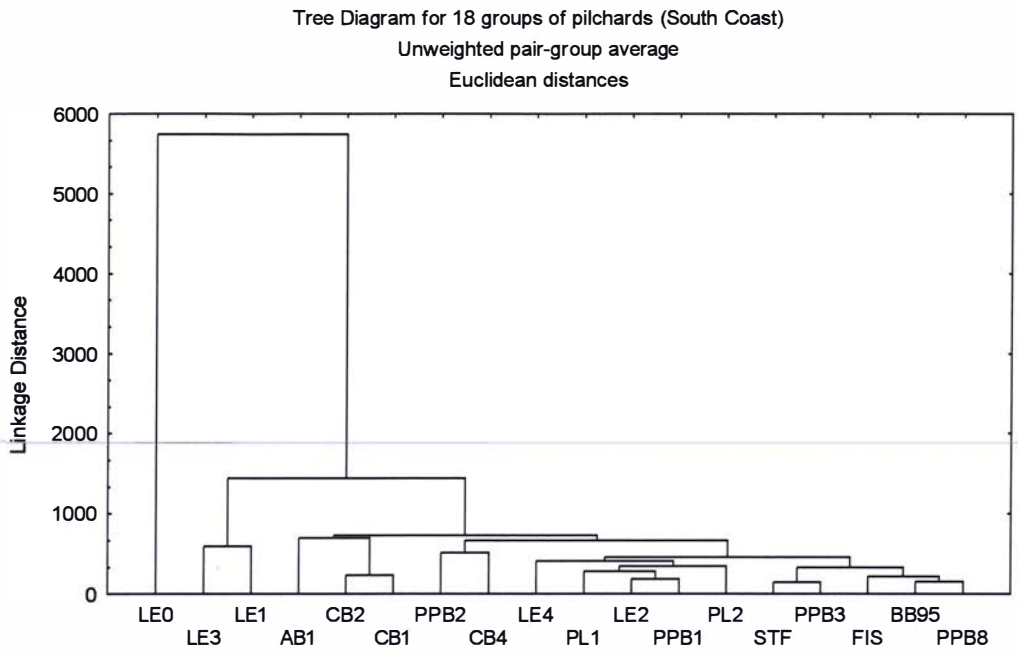


Figure 5.10 Cluster analysis of morphometric characters of the south coast samples of pilchards collected between 1995 and 1998.

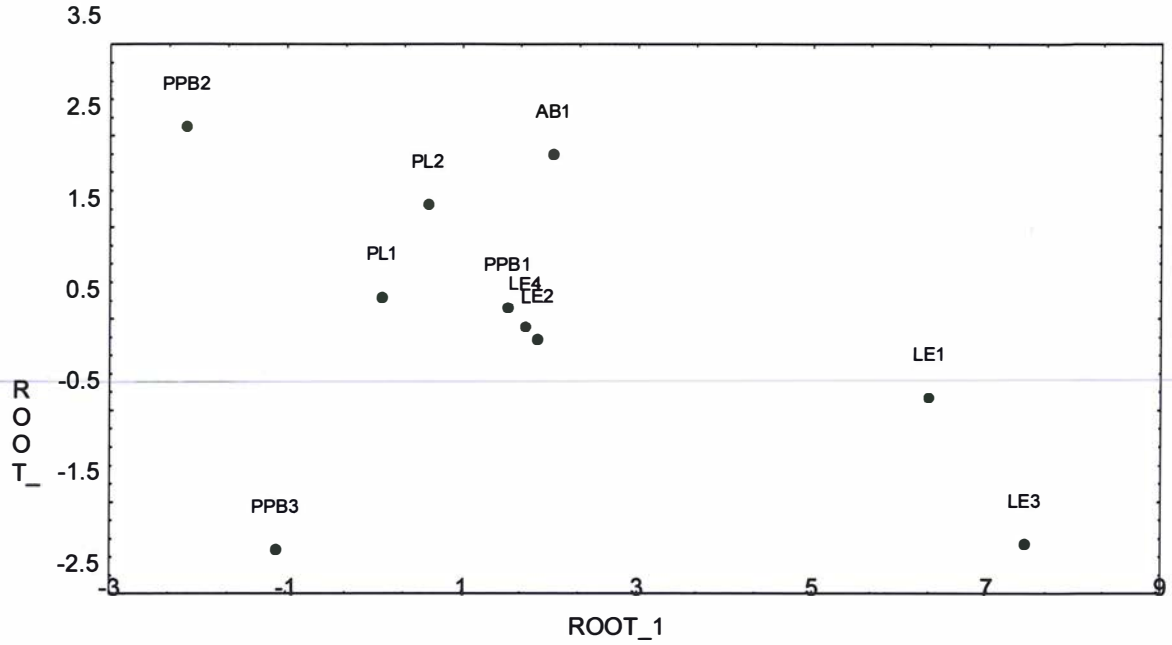


Figure 5.11 Means of Canonical Scores of morphometric characters of south coast pilchard populations.

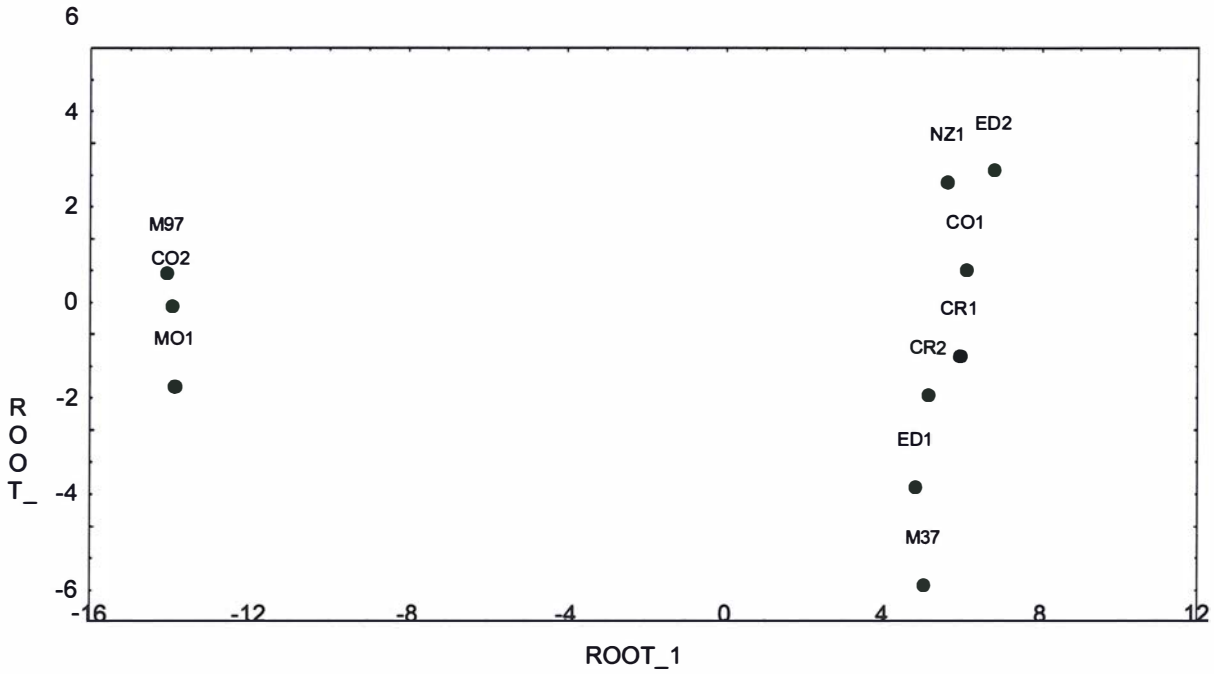


Figure 5.12 Means of Canonical Scores of morphometric characters of east coast pilchard populations.

Table 5.1 Operating conditions of ICPMS for the analysis of otolith microchemistry of *S. sagax*.

Elements	Detection Wavelength (nm)
Ba	233.53
Ca	317.93
Cu	324.75
Fe	238.20
K	766.49
Mg	279.55
Mn	279.55
Na	589.59
P	214.91
S	180.67
Sr	421.55

Table 5.2 Allele frequencies at six marker loci in east and south Australian mixed populations of *S.sagax*. The spawning populations allele frequencies are listed in Table 5.3.

Locus	Pop CB4	STF	G98	BB95	M97	M37	MO1	LE0	CO2	PPB8	CR2	CO1	CB1	CB2	CR1	PPB3
Est-4																
(N)	99	65	45	43	62	92	92	62	80	100	36	49	19	35	41	100
A	.106	.054	.133	.151	.137	.190	.190	.113	.063	.255	.347	.031	.079	.371	.354	.220
B	.273	.254	.000	.000	.000	.033	.033	.000	.000	.000	.000	.000	.000	.000	.000	.015
C	.621	.692	.378	.267	.516	.576	.576	.331	.456	.505	.417	.602	.553	.300	.524	.475
D	.000	.000	.411	.581	.347	.201	.201	.556	.480	.240	.208	.367	.289	.329	.122	.290
E	.000	.000	.078	.000	.000	.000	.000	.000	.000	.000	.028	.000	.079	.000	.000	.000
PEP-B																
(N)	99	87	37	43	63	64	64	62	80	100	33	50	19	35	41	64
A	.222	.477	.230	.174	.008	.242	.242	.331	.169	.040	.091	.220	.263	.157	.073	.055
B	.000	.000	.000	.000	.167	.000	.000	.000	.031	.000	.000	.000	.000	.000	.000	.000
C	.778	.500	.554	.477	.675	.578	.578	.589	.531	.460	.273	.400	.474	.171	.683	.414
D	.000	.023	.216	.349	.151	.180	.180	.081	.269	.500	.606	.370	.263	.671	.244	.531
E	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.030	.010	.000	.000	.000	.000
AAT-1																
(N)	65	85	45	19	63	85	85	62	80	100	36	50	19	35	46	100
A	.000	.006	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.515	.459	.389	.447	.540	.347	.353	.252	.237	.295	.083	.260	.263	.200	.489	.300
D	.408	.382	.511	.395	.341	.329	.324	.476	.394	.500	.528	.280	.263	.486	.391	.430
E	.077	.153	.100	.158	.119	.324	.324	.282	.369	.205	.389	.460	.474	.314	.120	.270
PGM-1																
(N)	11	19	12	43	32	-	-	24	65	-	9	20	-	7	-	18
A	.136	.211	.125	.174	.078	-	-	.042	.138	-	.278	.275	-	.357	-	.139
B	.000	.105	.000	.000	.000	-	-	.000	.038	-	.000	.000	-	.000	-	.000
C	.864	.684	.042	.477	.781	-	-	.208	.746	-	.722	.100	-	.571	-	.444
D	.000	.000	.833	.349	.141	-	-	.750	.077	-	.000	.625	-	.071	-	.417
MPI-1																
(N)	-	55	-	19	-	-	-	-	-	-	8	-	-	-	-	-
A	-	.336	-	.237	-	-	-	-	-	-	.063	-	-	-	-	-
B	-	.064	-	.026	-	-	-	-	-	-	.000	-	-	-	-	-
C	-	.536	-	.658	-	-	-	-	-	-	.938	-	-	-	-	-
D	-	.064	-	.079	-	-	-	-	-	-	.000	-	-	-	-	-
AH-1																
(N)	-	58	-	-	10	-	-	-	-	-	-	-	-	-	-	-
A	-	.000	-	-	.000	-	-	-	-	-	-	-	-	-	-	-
B	-	.267	-	-	.000	-	-	-	-	-	-	-	-	-	-	-
C	-	.733	-	-	.950	-	-	-	-	-	-	-	-	-	-	-
D	-	.000	-	-	.050	-	-	-	-	-	-	-	-	-	-	-
E	-	.000	-	-	.000	-	-	-	-	-	-	-	-	-	-	-
F	-	.000	-	-	.000	-	-	-	-	-	-	-	-	-	-	-

Table 5.3 Allele frequencies in spawning populations of pilchards STF: St Francis Island, FIS: Flinders Island, BB95: Boston Bay 95, CO1: Coolum, NZ1: New Zealand.

Locus	STF	Fis	BB95	CO1	NZ1
Est-4*					
(N)	24	13	21	34	54
A	0.042	0.154	0.095	0.044	0.213
B	0.333	0.269	0.000	0.000	0.000
C	0.625	0.577	0.286	0.544	0.444
D	0.000	0.000	0.619	0.412	0.343
Pep-B*					
(N)	32	13	21	34	52
A	0.547	0.154	0.071	0.221	0.087
B	0.000	0.000	0.000	0.000	0.000
C	0.438	0.846	0.571	0.426	0.558
D	0.016	0.000	0.357	0.338	0.356
E	0.000	0.000	0.000	0.015	0.000
Aat-1*					
(N)	31	13	11	34	56
A	0.000	0.000	0.000	0.000	0.018
B	0.000	0.000	0.000	0.000	0.000
C	0.387	0.115	0.455	0.294	0.411
D	0.387	0.462	0.409	0.250	0.393
E	0.226	0.423	0.136	0.456	0.179
Pgm-1*					
(N)	-	-	21	14	20
A	-	-	0.071	0.214	0.275
B	-	-	0.000	0.000	0.000
C	-	-	0.571	0.143	0.700
D	-	-	0.357	0.643	0.025
Mpi-1*					
(N)	22	13	-	-	29
A	0.250	0.077	-	-	0.103
B	0.045	0.000	-	-	0.000
C	0.636	0.769	-	-	0.345
D	0.068	0.154	-	-	0.552

Table 5.4 Genetic variability at 3 loci in all non-spawning population (standard errors in parentheses) ** Unbiased estimate (see Nei, 1978).

Population	Mean sample size per locus	Mean number of alleles per locus	Mean heterozygosity Direct count	H-W expected**
1. CB4	87.7 (11.3)	2.7 (0.3)	0.537 (0.119)	0.482 (0.068)
2. STF	79.0 (7.0)	3.3(0.3)	0.510 (0.126)	0.535 (0.048)
3. G98	42.3 (2.7)	3.3 (0.3)	0.377 (0.068)	0.619 (0.027)
4. BB95	35.0 (8.0)	3.0 (0.0)	0.463 (0.242)	0.613 (0.019)
5. M97	62.7 (0.3)	3.3 (0.3)	0.308 (0.152)	0.560 (0.031)
6. M37	80.3 (8.4)	3.3 (0.3)	0.408 (0.172)	0.614 (0.028)
7. MO1	80.3 (8.4)	3.3 (0.3)	0.404 (0.168)	0.614 (0.028)
8. LE0	62.0 (0.0)	3.3 (0.0)	0.419 (0.164)	0.585 (0.029)
9. CO2	79.0 (0.0)	3.3 (0.3)	0.418 (0.122)	0.613 (0.028)
10. PPB8	100.0 (0.0)	3.0 (0.0)	0.463 (0.067)	0.596 (0.028)
11. CR2	34.0 (1.0)	3.7 (0.3)	0.357 (0.166)	0.596 (0.041)
12. CO1	49.7 (0.3)	3.3 (0.3)	0.442 (0.119)	0.606 (0.050)
13. CB1	35.0 (0.0)	3.0 (0.0)	0.362 (0.038)	0.603(0.052)
14. CR1	42.7 (1.7)	3.0 (0.0)	0.191 (0.061)	0.556 (0.041)
15. PPB3	88.0 (12.0)	3.3 (0.3)	0.384 (0.091)	0.616 (0.034)
16. NZ1	108.3 (1.5)	3.3 (0.3)	0.405 (0.164)	0.596 (0.037)
17. FIS	26.0 (0.0)	2.7 (0.3)	0.436 (0.156)	0.516 (0.078)
18. AB1	113.3 (3.7)	3.3 (0.3)	0.327 (0.011)	0.605(0.029)
19. ED2	74.0 (2.1)	3.0 (0.0)	0.394 (0.173)	0.454 (0.086)
20. LE2	47.0 (0.0)	3.0 (0.0)	0.291 (0.093)	0.454 (0.039)
21. LE4	42.7 (0.9)	3.0 (0.0)	0.131 (0.051)	0.455 (0.095)
22. PL1	33.0 (0.0)	2.8 (0.2)	0.302 (0.133)	0.530 (0.087)
23. PL2	43.0 (1.0)	3.0 (0.0)	0.346 (0.170)	0.520 (0.093)
24.PPB1	30.7 (3.4)	2.3 (0.7)	0.387 (0.241)	0.408 (0.204)
25. PPB2	79.0 (8.1)	3.0 (0.0)	0.326 (0.137)	0.433 (0.141)

Table 5.5 Genetic variability at 3 loci in spawning populations of *S.sagax* (standard errors in parentheses) ** Unbiased estimate (see Nei, 1978).

Population	Mean sample size per locus	Mean number of alleles per locus	Mean heterozygosity Direct count	H-W expected**
STF	29 (2.5)	3.0 (0.0)	0.541 (0.115)	0.561 (0.049)
BB95	17.7 (3.3)	3.0 (0.0)	0.430 (0.240)	0.576 (0.030)
CO1	34.0 (0.0)	3.3 (0.3)	0.412 (0.133)	0.619 (0.040)
NZ1	54.0 (1.2)	3.3 (0.3)	0.413 (0.171)	0.619 (0.029)
FIS	13.0 (0.0)	2.7 (0.3)	0.359 (0.185)	0.494 (0.112)

Table 5.6 Log-Likelihood Tests (G-Statistics) for spatial differences in allele frequencies between and among sample set of *S. sagax* along the eastern and southern coasts of Australia.

Populations	G-stat	Degrees of freedom	Probability
Year 1995			
BB95vsLE0	79.82	6	<0.001
Year 1996			
CB1vsCB2	28.28	8	<0.001
Year 1997			
PPB3vsPPB1vsPP	146.96	14	<0.001
M97vsM37vsMO1	521.81	16	<0.05
CO2vsCO1	18.33	7	<0.001
CR1vsCR2	120.59	8	<0.001
PL1vsPL2	128.21	8	<0.001
ED2vsAB1	127.44	7	<0.001
PPB3vsPPB2	127.11	7	<0.001
PPB3vsPPB1	94.48	7	<0.001
PPB2vsLE2	63.26	6	<0.001
PPB1vsLE2	85.83	7	<0.001
PPB1vsCR1	54.54	6	<0.001
PPB1vsED2	36.82	6	<0.001
PPB2vsED2	300.27	7	<0.001
PPB2vsM37	275.96	8	<0.001
PPB3vsED2	201.23	7	<0.001
PPB1vsPL1	153.69	6	<0.001
PPB1vsPL2	27.15	6	<0.001
LE4vsLE2	20.38	6	<0.001
LE2vsCO2	160.39	7	<0.001
LE4vsPL2	25.77	2	<0.001
M97vsMO1	97.49	8	<0.001
M97vsM37	97.87	16	<0.001
MO1vsM37	0.02	8	>0.250
MO1vsCO2	57.73	8	<0.001
M97vsCO2	84.19	7	<0.001
ED2vsM97	174.21	7	<0.001
ED2vsM37	87.62	7	<0.001
ED2vsMO1	96.23	7	<0.001
M37vsCR1	56.93	7	<0.001
M37vsCR2	80.67	9	<0.001
M97vsCR1	215.61	8	<0.001
M97vsCR2	210.56	8	<0.001
ED2vsCO1	70.38	6	<0.001
ED2vsCO2	180.47	7	<0.001
CO2vsMO1	57.73	8	<0.001
AB1vsPL2	40.97	7	<0.001
CO1vsNZ1	80.08	7	<0.001
M97vsNZ1	285.41	9	<0.001
LE2vsNZ1	59.89	7	<0.001
LE4vsNZ1	31.27	7	<0.001
ED2vsNZ1	118.27	7	<0.001
AB1vsNZ1	33.31	8	<0.001
PL1vsNZ1	115.28	7	<0.001

PL2vsNZ1	21.82	7	<0.005
M37vsNZ1	37.30	8	<0.001
MO1vsNZ1	37.25	8	<0.001
CO2vsNZ1	75.60	8	<0.001
CR1vsNZ1	45.47	7	<0.001
CR2vsNZ1	83.96	9	<0.001
PPB1vsNZ1	57.32	7	<0.001
PPB2vsNZ1	72.84	7	<0.001
PPB3vsNZ1	47.97	8	<0.001
PPB3vsLE2	121.73	7	<0.001
PPB3vsLE4	74.72	7	<0.001
PPB3vsM97	88.38	8	<0.001
PPB3vsMO1	53.62	7	<0.001
PPB3vsCO2	70.42	8	<0.001
PPB3vsCO1	65.86	7	<0.001
PPB3vsCR1	93.84	7	<0.001
PPB3vsCR2	33.05	9	<0.001
PPB3vsPL1	72.85	7	<0.001
PPB3vsPL2	63.60	7	<0.001
PPB3vsAB1	75.67	8	<0.001
PPB1vsLE4	40.45	6	<0.001
PPB1vsM97	51.81	7	<0.001
PPB1vsM37	61.68	7	<0.001
PPB1vsMO1	61.07	7	<0.001
PPB1vsCO2	83.36	7	<0.001
PPB1vsCO1	92.06	7	<0.001
PPB1vsCR2	133.96	8	<0.001
PPB1vsAB1	68.54	8	<0.001
PPB2vsLE4	16.77	6	<0.05
PPB2vsM97	98.63	7	<0.001
PPB2vsMO1	45.04	7	<0.001
PPB2vsCO2	141.08	7	<0.001
PPB2vsCO1	137.23	6	<0.001
PPB2vsCR1	31.06	6	<0.001
PPB2vsCR2	149.87	8	<0.001
PPB2vsPL1	195.26	6	<0.001
PPB2vsPL2	35.65	6	<0.001
PPB2vsAB1	483.38	7	<0.001
LE2vsM97	96.90	7	<0.001
LE2vsM37	81.72	7	<0.001
LE2vsMO1	81.35	7	<0.001
LE2vsCO1	167.01	6	<0.001
LE2vsCR1	27.14	6	<0.001
LE2vsCR2	148.61	7	<0.001
LE2vsPL1	158.68	6	<0.001
LE2vsPL2	51.51	6	<0.001
LE2vsED2	87.76	6	<0.001
LE2vsAB1	60.68	7	<0.001
LE4vsM97	73.43	7	<0.001
LE4vsM37	39.86	7	<0.001
LE4vsCO1	109.26	6	<0.001
LE4vsCO2	109.68	6	<0.001
LE4vsMO1	39.34	7	<0.001
LE4vsED2	224.66	7	<0.001
LE4vsCR1	20.93	6	<0.005
LE4vsCR2	110.52	8	<0.001
LE4vsPL1	130.16	6	<0.001
M97vsCO1	247.27	8	<0.001

M97vsPL1	252.15	8	<0.001
M97vsPL2	184.77	8	<0.001
M97vsAB1	285.65	9	<0.001
M37vsCO2	57.20	8	<0.001
M37vsCO1	56.03	8	<0.001
M37vsPL1	115.45	7	<0.001
M37vsPL2	28.42	7	<0.001
M37vsAB1	55.35	8	<0.001
MO1vsCO1	54.46	7	<0.001
MO1vsCR1	56.80	7	<0.001
MO1vsCR2	81.52	9	<0.001
MO1vsPL1	115.22	7	<0.001
MO1vsPL2	28.43	7	<0.001
MO1vsAB1	54.89	8	<0.001
CO2vsCR1	101.93	8	<0.001
CO2vsCR2	79.93	9	<0.001
CO2vsPL1	115.86	6	<0.001
CO2vsPL2	41.70	7	<0.001
CO2vsAB1	120.11	8	<0.001
CO1vsCR1	135.45	6	<0.001
CO1vsCR2	72.62	8	<0.001
CO1vsPL1	77.09	6	<0.001
CO1vsPL2	58.76	6	<0.001
CO1vsAB1	118.23	7	<0.001
CR1vsPL1	165.42	6	<0.001
CR1vsPL2	28.10	6	<0.001
CR1vsED2	70.38	6	<0.001
CR1vsAB1	40.59	7	<0.001
CR2vsPL1	81.91	8	<0.001
CR2vsPL2	98.58	8	<0.001
CR2vsED2	170.25	8	<0.001
CR2vsAB1	93.61	8	<0.001
PL1vsED2	215.51	6	<0.001
PL2vsED2	69.88	6	<0.001
ED2vsLE2	87.76	6	<0.001
ED2vsLE4	46.03	6	<0.001
Year 1998			
CB4vsPPB8	363.58	8	<0.001
PPB8vsG98	74.43	7	<0.001
STFvsFIS	61.87	6	<0.001
CB4vsSTF	27.12	6	<0.001
CB4vsFIS	83.44	6	<0.001
PPB8vsFIS	161.49	7	<0.001
PPB8vsSTF	343.79	7	<0.001
G98vsCB4	184.89	8	<0.001
G98vsFIS	147.56	8	<0.001
G98vsSTF	161.75	8	<0.001

Table 5.7 Summary of F-statistics at all loci among the eastern and southern populations of pilchards in Australia from 1995 to 1998 Effective number of migrants per generation (N_em)= 1.7. N.S.=non-significant χ^2 value ***=significant χ^2 value ($p \leq 0.000$).

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.401 $^{N.S}$	0.458	0.096***
*Pep-B**	0.547 $^{N.S}$	0.637	0.199***
*Aat-1**	0.032 $^{N.S}$	0.095	0.065***
Mean	0.304 $^{N.S}$	0.385	0.116***

Table 5.8 Summary of F-statistics among the populations of pilchards on the east and south coasts of Australia in 1997-1998 N_em =2.4

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.223	0.301	0.099
*Pep-B**	0.543	0.592	0.108
*Aat-1**	-0.004	0.056	0.060
Mean	0.237	0.304	0.088

Table 5.9 Summary of F-statistics among the populations of pilchards on the east coast of Australia N_em =2.5

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.477	0.508	0.060
*Pep-B**	0.661	0.694	0.097
*Aat-1**	-0.001	0.072	0.073
Mean	0.364	0.412	0.076

Table 5.10 Summary of F-statistics among the populations of pilchards on the south coast of Australia N_em =1.4

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.356	0.429	0.112
*Pep-B**	0.463	0.602	0.259
*Aat-1**	0.052	0.103	0.054
Mean	0.364	0.366	0.136

Table 5.11 Summary of F-statistics among the populations of pilchards on the south eastern coast of Australia (Eden, Lakes Entrance and New Zealand) N_em =1.9

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.391	0.454	0.103
*Pep-B**	0.408	0.438	0.049
*Aat-1**	0.028	0.101	0.075
Mean	0.255	0.313	0.078

Table 5.12 Summary of F-statistics among the populations of pilchards on the north eastern coast of Australia (Queensland, New South Wales border and Gosford) N_em =2.7

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.475	0.503	0.053
*Pep-B**	0.686	0.711	0.080
*Aat-1**	0.027	0.090	0.065
Mean	0.386	0.427	0.066

Table 5.13 Summary of F-statistics among the spawning populations of pilchards in Australia N_em =1.2.

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.320	0.424	0.153
*Pep-B**	0.607	0.662	0.139
*Aat-1**	-0.145	-0.083	0.055
Mean	0.233	0.320	0.114

Table 5.14 Summary of F-statistics among the populations of pilchards in Queensland and the New South Wales border region N_em =2.8

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.493	0.520	0.052
*Pep-B**	0.701	0.727	0.088
*Aat-1**	0.005	-0.083	0.055
Mean	0.233	0.067	0.062

Table 5.15 Summary of F-statistics among the populations of pilchards in Victoria N_em =1.6

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.341	0.391	0.076
*Pep-B**	0.391	0.518	0.208
*Aat-1**	0.085	0.136	0.056
Mean	0.251	0.328	0.104

Table 5.16 Summary of F-statistics among the populations of pilchards in South Australia N_em =0.95

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.344	0.439	0.146
*Pep-B**	0.494	0.640	0.288
*Aat-1**	0.046	0.101	0.058
Mean	0.272	0.390	0.162

Table 5.17 Summary of F-statistics among the populations of pilchards in Port Philip Bay and South Australia N_em =1.2

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.352	0.424	0.111
*Pep-B**	0.475	0.637	0.308
*Aat-1**	0.037	0.086	0.051
Mean	0.260	0.371	0.150

Table 5.18 Summary of F-statistics among the populations of pilchards in South Australia excluding Port Lincoln N_em =1.0

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.239	0.360	0.159
*Pep-B**	0.473	0.574	0.192
*Aat-1**	-0.042	0.030	0.069
Mean	0.205	0.315	0.139

Table 5.19 Summary of F-statistics among the populations of pilchards in South Australia excluding Flinders Island and St Francis Island N_em =0.9

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.447	0.518	0.130
*Pep-B**	0.484	0.635	0.292
*Aat-1**	0.114	0.142	0.032
Mean	0.332	0.432	0.150

Table 5.20 Summary of F-statistics among the populations of pilchards in Port Phillip Bay and Port Lincoln N_em =1.05

Locus	F_{is}	F_{it}	F_{st}
Est-4*	0.467	0.482	0.027
*Pep-B**	0.479	0.692	0.408
*Aat-1**	0.112	0.138	0.029
Mean	0.321	0.417	0.142

Table 5.21 Departures from H-W expectations in mixed pilchard populations on the eastern and southern coasts of Australia Key: * =significant at $\rho<0.05$; ** = significant at $\rho<0.01$; *** = significant at $\rho<0.001$. F= inbreeding coefficient; D= Selander's (1970) coefficient for heterozygote deficiency or excess

Population	locus	χ^2	ν	ρ	F	D
CB4	Est-4*	9.173	1	0.002***	-0.166	0.160
	*Pep-B**	1.649	1	0.199^{NS}	0.123	-0.128
	*Aat-1**	9.297	1	0.002***	-0.231	0.222
STF	*Est-4**	8.683	1	0.003**	-0.289	0.280
	*Pep-B**	19.799	1	0.000***	0.493	-0.496
	*Aat-1**	4.341	1	0.037*	-0.101	0.094
G98	*Est-4**	2.406	1	0.121^{NS}	0.365	-0.372
	*Pep-B**	10.070	1	0.002**	0.590	-0.596
	*Aat-1**	2.001	1	0.157^{NS}	0.192	-0.201
BB95	*Est-4**	17.067	1	0.000***	0.590	-0.595
	*Pep-B**	15.086	1	0.000***	0.663	-0.667
	*Aat-1**	6.177	1	0.013*	-0.530	0.490
M97	*Est-4**	19.172	1	0.000***	0.539	-0.543
	*Pep-B**	51.212	1	0.000***	0.872	-0.873
	*Aat-1**	1.272	1	0.259^{NS}	-0.016	0.008
M37	*Est-4**	20.452	1	0.000***	0.356	-0.359
	*Pep-B**	36.187	1	0.000***	0.783	-0.784
	*Aat-1**	0.004	1	0.953^{NS}	-0.077	0.071
MO1	*Est-4**	20.452	1	0.000***	0.356	-0.359
	*Pep-B**	36.187	1	0.000***	0.783	-0.784
	*Aat-1**	0.063	1	0.802^{NS}	-0.060	0.054
LE0	*Est-4**	9.305	1	0.002**	0.471	-0.465
	*Pep-B**	20.533	1	0.000***	0.610	-0.613
	*Aat-1**	3.966	1	0.046*	-0.168	0.158
CO2	*Est-4**	11.578	1	0.001***	0.416	-0.419
	*Pep-B**	18.376	1	0.000***	0.574	-0.577
	*Aat-1**	1.621	1	0.203^{NS}	-0.015	0.009
PPB8	*Est-4**	2.101	1	0.147^{NS}	0.229	-0.233
	*Pep-B**	16.405	1	0.000***	0.367	-0.370
	*Aat-1**	0.203	1	0.653^{NS}	0.082	-0.087
CR2	*Est-4**	16.250	1	0.000***	0.664	-0.669
	*Pep-B**	19.2	1	0.000***	0.669	-0.674

	*Aat-1**	1.617	1	0.203 NS	-0.233	0.216
CO1	*Est-4**	6.774	1	0.009**	0.390	-0.396
	*Pep-B**	5.908	1	0.015*	0.481	-0.486
	*Aat-1**	3.870	1	0.049*	-0.059	0.048
CB1	*Est-4**	4.661	1	0.031*	0.472	-0.486
	*Pep-B**	12.662	1	0.000***	0.752	-0.759
	*Aat-1**	1.092	1	0.296 NS	-0.157	0.126
CR1	*Est-4**	18.414	1	0.000***	0.708	-0.712
	*Pep-B**	25.603	1	0.000***	0.792	-0.794
	*Aat-1**	9.131	1	0.003**	0.487	-0.493
PPB3	*Est-4**	14.706	1	0.000***	0.283	-0.287
	*Pep-B**	30.844	1	0.000***	0.626	-0.629
	*Aat-1**	3.575	1	0.059 NS	0.249	-0.252
NZ1	*Est-4**	25.357	1	0.000***	0.545	-0.547
	*Pep-B**	33.113	1	0.000***	0.603	-0.605
	*Aat-1**	4.366	1	0.037*	-0.125	0.120
FIS	*Est-4**	1.572	1	0.210 NS	0.232	-0.246
	*Pep-B**	9.248	1	0.002**	0.567	-0.575
	*Aat-1**	2.188	1	0.139 NS	-0.233	0.209
AB1	*Est-4**	36.580	1	0.000***	0.478	-0.481
	*Pep-B**	25.590	1	0.000***	0.442	-0.444
	*Aat-1**	36.855	1	0.000***	0.448	-0.450
ED2	*Est-4**	15.450	1	0.000***	0.496	-0.499
	*Pep-B**	5.475	1	0.019*	0.294	-0.299
	*Aat-1**	16.307	1	0.000***	-0.234	0.226
LE2	*Est-4**	0.720	1	0.396 NS	0.161	-0.170
	*Pep-B**	0.048	1	0.827 NS	0.027	-0.038
	*Aat-1**	29.241	1	0.000***	0.784	-0.787
LE4	*Est-4**	3.092	1	0.079 NS	0.258	-0.266
	*Pep-B**	3.792	1	0.051 NS	0.333	-0.341
	*Aat-1**	0.028	1	0.867 NS	0.051	-0.063
PL1	*Est-4**	11.427	1	0.001***	0.602	-0.608
	*Pep-B**	28.562	1	0.000***	0.893	-0.894
	*Aat-1**	19.711	1	0.000***	0.700	-0.704
PL2	Est-4*	30.193	1	0.000***	0.764	-0.767
	*Pep-B**	6.467	1	0.011*	0.340	-0.349
	*Aat-1**	1.050	1	0.305 NS	-0.079	0.067
PPB1	*Est-4**	3.792	1	0.052 NS	0.421	-0.429

	*Aat-1**	6.140	1	0.013*	-0.316	0.297
PPB2	*Est-4**	29.478	1	0.000***	0.589	-0.592
	*Pep-B**	0.520	1	0.471 ^{NS}	-0.070	0.062
	*Aat-1**	1.992	1	0.158 ^{NS}	0.057	-0.062

Table 5.22 Departures from H-W expectations in mixed pilchard populations on the eastern and southern coasts of Australia Key: * =significant at $\rho<0.05$; ** = significant at $\rho<0.01$; *** = significant at $\rho<0.001$. F= inbreeding coefficient; D= Selander's (1970) coefficient for heterozygote deficiency or excess

Population	locus	χ^2	ν	ρ	F	D
STF	Est-4*	3.883	1	0.049*	-0.343	0.315
	*Pep-B**	6.439	1	0.011*	0.386	-0.396
	*Aat-1**	0.158	1	0.691^{NS}	0.006	-0.022
BB95	Est-4*	8.142	1	0.004**	0.638	-0.647
	*Pep-B**	8.504	1	0.004**	0.648	-0.656
	*Aat-1**	1.939	1	0.164^{NS}	-0.497	0.429
CO1	Est-4*	7.884	1	0.005**	0.503	-0.510
	*Pep-B**	7.645	1	0.006**	0.551	-0.557
	*Aat-1**	4.135	1	0.042*	-0.052	0.036
NZ1	Est-4*	12.675	1	0.000***	0.537	-0.541
	*Pep-B**	19.995	1	0.000***	0.653	-0.657
	*Aat-1**	5.708	1	0.017*	-0.163	0.153
FIS	Est-4*	0.831	1	0.362^{NS}	0.192	-0.223
	*Pep-B**	16.762	1	0.000***	1.000	-1.000
	*Aat-1**	0.511	1	0.474^{NS}	-0.035	-0.005

Table 5.23 The percentage of pilchard samples, collected in 1998, correctly classified to actual site based on stepwise discriminant function analysis of morphometric characters.

		No. of fish classified				
Site	Correctly classified%	PPB8	G98	STF	CB4	FIS
PPB8	96.15	125	0	4	0	1
G98	100.00	0	53	0	0	0
STF	68.97	19	0	60	8	0
CB4	90.53	0	0	9	86	0
FIS	11.54	13	0	8	2	3
Total	83.63	157	53	81	96	4

Table 5.24 The percentage of pilchard samples, collected in 1997, that were correctly classified to actual site based on stepwise discriminant function analysis of morphometric characters.

Sites	Correctly classified																	
%	M97	CO2	MO1	PPB3	CR1	CO1	CR2	PL2	M37	PPB1	NZ1	LE1	LE2	LE3	LE4	ED1	ED2	PL1
AB1	PPB2																	
M97	87.10	54	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0															
CO2	69.44	19	50	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0															
MO1	93.15	5	0	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0															
PPB3	93.86	1	0	0	214	0	0	1	2	1	1	0	0	1	0	1	4	0
	0	1	1															
CR1	71.15	0	0	0	0	37	0	0	2	0	5	4	0	0	0	2	0	0
	1	0	1															
CO1	97.67	0	0	0	0	1	42	0	0	0	0	0	0	0	0	0	0	0
	0	0	0															
CR2	71.43	0	0	0	3	2	0	25	0	0	1	0	0	0	0	0	1	0
	2	0	1															
PL2	62.22	0	0	0	1	1	0	2	28	1	5	0	0	3	0	0	0	0
	2	0	2															
M37	78.57	0	0	0	1	3	0	3	2	88	1	0	0	0	0	0	7	0
	1	0	6															
PPB1	63.64	0	0	0	0	2	0	2	5	0	21	1	0	0	0	0	0	0
	0	0	2															
NZ1	91.96	0	0	0	0	0	0	0	0	0	1	103	0	1	1	0	0	6
	0	0	0															
LE1	20.00	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	7
	0	0	0															
LE2	69.05	0	0	0	0	3	0	0	1	0	1	0	0	29	0	1	1	0
	3	2	1															
LE3	33.33	0	0	0	0	0	0	0	0	0	0	4	0	0	5	0	0	6
	0	0	0															
LE4	80.00	0	0	0	0	0	0	0	0	0	0	0	0	5	0	32	1	1
	0	1	0															
ED1	82.35	0	0	0	2	0	0	1	0	2	0	0	0	0	0	2	42	0
	0	0	2															
ED2	94.12	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	96
	0	0	0															
PL1	45.45	0	0	0	1	1	0	0	1	0	5	0	0	4	0	4	1	0
	15	0	1															
AB1	82.35	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0
	2	28	0															
PPB2	87.13	0	0	0	0	0	0	1	4	4	0	0	0	0	0	0	4	0
	0	0	88															
Total	82.39	79	57	72	222	51	43	35	45	96	41	118	2	45	6	43	61	116
	26	32	105															

Table 5.25 The percentage of pilchard samples, collected in 1996 and 1995 respectively, that were correctly classified to actual site based on stepwise discriminant function analysis of morphometric characters.

Year 1996

Groups	Percent	CB1	CB2
CB1	66.67	12	6
CB2	91.43	3	32
Total	83.02	15	38

Year 1995

Groups	Percent	LE0	BB95
LE0	100	84	0
BB95	100	0	43
Total	100	84	43

Table 5.26 Elemental concentrations of sagittal otoliths in *S. sagax* from the eastern and southern coasts of Australia. Key: BB=Boston Bay, LE=Lakes Entrance, CB=Coffin Bay, AB=Anxious Bay, Mool=Mooloolaba, PL=Port Lincoln, CR=Clarence River, NZ=New Zealand, PPB=Port Phillip Bay, St.F=St Francis Island, Fl.Is=Flinders Island. Trace element composition (ppm) of sagittal otoliths. Ca and Na expressed as ppt.

	Site	BB	LE	CB	PPB	AB	Mool.	PL	CR	Eden
		14.3.95	26.4.95	17.5.96	21.3.97	23.4.97	22.3.97	27.3.97	30.4.97	1.8.97
	Element						29.3.97	23.4.97		
		n=3	n=3	n=1	n=4	n=2	n=3	n=2	n=2	n=3
Ba	Mean	1.07	2.18	2.14	5.99	3.44	8.72	0.99	2.5	1.93
	SD	1.85	3.08		1.40	4.86	7.07	1.41	0.10	1.80
	Range	0-3.20	0-3.15		4.66-7.97	0-6.87	1.77-15.90	0-1.99	2.43-2.57	0-3.56
Ca	Mean	353.09	446.96	319.03	466.31	408.69	378.95	384.62	346.52	491.53
	SD	94.16	135.88		64.71	34.62	28.98	28.06	41.18	184.74
	Range	246.18-423.71	353.90-410.96		408.24-552.08	384.21-433.17	351.87-409.52	364.78-404.46	317.41-375.64	354.84-701.71
Cu	Mean	0.49	30.54	0	46.13	34.93	26.86	2.88	14.85	21.86
	SD	0.85	43.19		19.09	49.4	23.36	4.07	10.52	31.38
	Range	0-1.48	1.34-29.87		21.21-61.63	0-69.86	0-42.41	0-5.76	7.41-22.29	1.31-57.98
Fe	Mean	0	3540.52	0	282.88	106.84	122.17	71.94	99	483.45
	SD	0	4924.83		246.27	7.14	107.12	101.74	120.72	602.58
	Range	-	0-28.20		70.02-598.29	101.79-111.89	0-200.01	0-143.89	13.64-184.36	0-1158.55
K	Mean	546.82	712.33	561.17	833.44	600.51	315.98	483.20	356.42	630.08
	SD	208.57	312.00		399.34	38.48	12.28	75.69	41.06	280.63
	Range	355.74-769.32	392.93-443.79		470.61-1354.19	573.31-627.72	305.96-329.68	429.68-536.72	327.39-385.45	403.21-943.89
Mg	Mean	27.4	41.21	36.9	46.26	23.11	33.70	25.29	45.23	44.99
	SD	19.95	44.69		23.67	1.33	4.31	17.51	21.79	59.85
	Range	10.61-49.46	25.80-45.17		25.63-74.80	22.17-24.05	29.46-38.08	12.90-37.67	29.82-60.64	1.12-113.17
Mn	Mean	0	15.53	0.89	8.79	3.24	3.51	0.63	0.81	2.99
	SD	0	20.79		2.09	0.69	2.42	0.89	1.15	2.28
	Range	-	-		5.67-10.02	2.75-3.73	0.73-5.17	0-1.26	0-1.62	0.92-5.43
Na	Mean	2.64	3.81	2.77	4.54	3.16	3.49	3.12	2.96	4.49
	SD	0.76	1.21		1.18	0.28	.47	0.08	0.54	2.03

	Range	1.76-3.15	2.60-3.07		3.55-6.13	2.98-3.36	2.96-3.87	3.06-3.17	2.58-3.34	
P	Mean	76.84	100.91	122.01	130.65	88.46	70.03	74.92	133.25	72.14
	SD	45.60	10.69		32.52	59.54	31.15	27.52	82.79	44.04
	Range	44.91-129.06	100.86-130.91		96.30-164.89	46.36-130.56	45.84-105.17	55.46-94.38	74.71-191.79	3.11-6.82
S	Mean	600.11	633.97	527.23	692.43	647.66	579.92	628.95	803.19	723.28
	SD	179.78	74.23		85.57	60.73	47.06	4.47	252.74	128.18
	Range	409.06-765.97	613.38-661.02		620.62-815.70	604.72-690.56	533.54-627.63	625.78-632.11	624.47-981.90	26.81-114.77
Sr	Mean	238.58	371.23	225.31	505.08	347.05	489.54	289.52	347.92	394.60
	SD	64.94	144.94		81.13	30.56	122.43	39.17	17.95	150.12
	Range	163.95-282.25	280.39-343.28		442.73-620.46	325.44-368.66	358.87-601.59	261.83-317.22	335.22-360.61	245.08-545.31

Table 5.27. Elemental concentrations of sagittal otoliths in *S. sagax* from the eastern and southern coasts of Australia. Key: BB=Boston Bay, LE=Lakes Entrance, CB=Coffin Bay, AB=Anxious Bay, Mool=Mooloolaba, PL=Port Lincoln, CR=Clarence River, NZ=New Zealand, PPB=Port Phillip Bay, St.F=St Francis Island, Fl.Is=Flinders Island. Trace element composition (ppm) of sagittal otoliths. Ca and Na expressed as ppt.

Element	Site	LE	Coolum	NZ	Coolum	PPB	PPB	CB	Fl.Is	St. Fran
		14.8.97	30.8.97	24.9.97	8.11.97	22.11.97	11.2.98	4.3.98	14.3.98	17.4.98
		n=2	n=2	n=8	n=3	n=11	n=7	n=5	n=2	n=3
Ba	Mean	2.18	6.36	2.99	4.48	0.97	3.97	3.38	1.05	1.20
	SD	3.08	0.53	1.49	2.31	1.80	1.96	2.33	0.21	1.10
	Range	0-4.36	5.98-6.73	1.44-4.71	3.14-7.14	0-5.32	2.30-8.23	0.99-6.72	0.90-1.19	0-2.18
Ca	Mean	446.6	409.96	357.34	382.22	357.39	3.43.65	345.47	357.26	272.62
	SD	135.88	8.83	41.31	2.41	45.19	140.89	53.01	28.5	113.38
	Range	350.87-543.04	403.68-416.25	321.65-416.94	380.38-384.94	262.90-410.77	39.80-485.83	277.89-425.63	337.11-377.41	141.81-342.78
Cu	Mean	30.54	28.72	0.38	1.26	2.37	9.07	3.64	0	2.97
	SD	43.19	5.1	0.86	2.18	3.95	11.86	7.44	0	3.08
	Range	0-61.08	25.11-32.33	0-1.92	0-3.78	0-12.44	0-32.75	0-16.91	-	0-6.16
Fe	Mean	3540.52	54.78	465.97	11.06	33.93	62.18	327.88	3.46	28.78
	SD	4924.83	41.20	518.60	4.02	68.29	60.64	691.19	4.89	27.97
	Range	58.14-7022.90	25.65-83.91	10.38-1306.36	7.24-15.26	0-173.20	0-170.91	0-1562.89	0-6.91	9.01-60.78
K	Mean	712.23	650.63	1190.15	452.96	430.91	414.63	440.98	562.94	386.88
	SD	312	108.41	1662.2	60.05	82.92	41.48	101.49	144.11	146.51
	Range	491.71-932.94	573.97-727.28	332.93-4158.23	396.34-515.93	276.86-576.11	364.14-492.74	297.13-580.20	461.04-664.84	218.62-486.26
Mg	Mean	41.21	52.82	8.47	49.08	32.44	38.35	47.11	57.78	18.66
	SD	44.69	14.74	6.00	8.57	19.11	22.23	18.84	57.62	4.58
	Range	9.61-72.82	42.40-63.23	0-15.38	39.34-55.47	17.36-74.14	24.97-88.08	25.83-67.88	17.03-98.52	13.38-21.58

Mn	Mean	15.53	2.37	4.13	3.29	0.34	31.98	1.01	1.54	0
	SD	20.79	1.32	2.15	3.23	0.75	74.46	1.81	2.18	0
	Range	0.82-30.23	1.43-3.30	1.66-7.54	0.97-6.98	0-1.88	2.85-200.81	0-4.16	2.56-2.84	-
Na	Mean	3.81	3.33	3.13	2.64	3.16	3.32	2.67	2.70	2.02
	SD	1.21	.05	0.29	.17	0.52	.37	.32	0.20	0.83
	Range	2.95-4.66	3.30-3.37	2.70-3.43	2.49-2.83	2.28-3.95	2.97-4.06	2.32-3.07	2.56-2.84	1.05-2.53
P	Mean	100.91	142.79	66.72	112.41	104.02	122.55	122.73	221.99	58.33
	SD	10.69	28.56	23.64	16.20	56.95	85.50	82.06	229	20.77
	Range	93.36-108.47	122.59-162.98	30.76-94.53	96.91-129.22	46.63-236.38	71.57-312.84	39.37-215.27	60.06-383.91	39.08-80.35
S	Mean	633.97	661.8	599.23	644.29	588.64	600.41	541.90	578.48	440.56
	SD	74.23	1075	58.95	18.31	91.08	35.84	67.35	33.26	197.41
	Range	581.48-686.45	654.23-669.43	518.84-680.66	631.22-665.22	429.66-728.33	558.94-662.71	465.22-611.91	554.90-601.94	215.73-585.51
Sr	Mean	371.23	535.54	324.40	374.75	329.75	338.08	309.88	384.43	254.45
	SD	144.94	86.57	34.57	27.74	47.23	34.19	49.15	118.96	107.46
	Range	268.75-473.72	474.32-596.76	288.82-380.83	344.71-399.38	233.61- 383.76	277.13-394.41	228.04-359.65	300.31-468.55	131.26-328.85

Appendix 5.1. Collection data for pilchards. Key: ?= indeterminable; M= male; F= female; "non - spawning"= gonad maturity stages 1-3; "pre spawning"= stage 4; "spawning"= stages 5-7.

COLLECTION SITE	DATE	N	SEX RATIO (F:M	SIZE RANGE (LCF mm)	BREEDING STATUS	Sample Code
Queensland						
Mooloolaba	29.3.97	99	39:28 (?=22)	90.9-143	NS	MO1
Mooloolaba	22.3.97	94	13:03 (?=78)	74.6-137.6	NS	M37
Mooloolaba	8.10.97	63	22:40 (?=1)	125.7-160.7	NS	M97
Coolum	30.8.97	53	29:24 (?=0)	0-161.9	NS-S	CO1
Coolum	8.11.97	83	39:41 (?=3)	0-155.2	NS-S	CO2
New South Wales						
Clarence River	30.4.97	52	12:13 (?=27)	109.1-158.1	NS	CR1
Clarence River	29.5.97	36	16:13 (?=7)	0-148	NS-PS	CR2
Eden	1.8.97	62	20:25 (?=17)	84-123.5	NS	ED1
Eden	1.8.97	77	60:17 (?=0)	164.5-203	NS-S	ED2
Gosford	14.3.98	58	(?=58)	60.4-86	NS	G98
Victoria						
Lakes Entrance	26.4.95	62	(?=62)	120.7-168.6	NS	LE0
Lakes Entrance	15.8.97	12	1:11 (?=0)	153.5-192.3	NS	LE1
Lakes Entrance	16.8.97	47	2:43 (?=2)	109-160.5	NS	LE2
Lakes Entrance	1.8.97	19	13:06 (?=0)	162-206	NS-S	LE3
Lakes Entrance	1.8.97	44	23:17 (?=4)	0-174	NS	LE4
Port Phillip Bay	21.3.97	36	13:22 (?=1)	109.1-160.5	NS-PS	PPB1
Port Phillip Bay	1.8.97	101	45:56 (?=0)	96-115.5	NS	PPB2
Port Phillip Bay	22.11.97	100	36:64 (?=0)	109.1-141.4	NS-S	PPB3
Port Phillip Bay	11.2.98	100	47:53 (?=0)	119.1-146.2	NS-S	PPB8
South Australia						
Boston Bay	14.3.95	43	13:30 (?=0)	122.8-156.2	NS-S	BB95
Coffin Bay	17.5.96	19	6:13 (?=0)	152.3-168.5	NS	CB1
Coffin Bay	5.6.96	32	24:7 (?=1)	126.4-168.1	NS-PS	CB2
Coffin Bay	4.3.98	100	(?=100)	0-126.3	NS	CB4
Anxious Bay	23.4.97	146	(?=146)	126-154.3	NS	AB1
Port Lincoln	27.3.97	33	14:15 (?=4)	107.7-129	NS-S	PL1
Port Lincoln	23.4.97	44	21:23 (?=0)	113.6-131.6	NS-S	PL2
St. Francis Is.	17.3.98	89	20:69 (?=0)	109.3-150.8	NS-S	STF
Flinders Island	14/15.3.98	30	2:28 (?=0)	111.8-153	NS-S	FIS
New Zealand						
New Zealand	24.9.97	112	57:55	139.5-211	NS-S	NZ1

CHAPTER 6. GENETIC STOCK DISCRIMINATION OF AUSTRALIAN ANCHOVY, AND IDENTIFICATION OF USEFUL GENETIC MARKERS IN YELLOWTAIL SCAD AND BLUE MACKEREL.

Troy Coyle, M. Roseline Yardin, Michelle Avramidis, Alan Wilmot, Maria Catalina Bernal and Patricia. I. Dixon

Objective: This chapter investigates the presence of useful genetic markers in the Australian anchovy (*Engraulis australis*), yellowtail scad (*Trachurus novaezealandiae*) and blue mackerel (*Scomber australasicus*). Markers were assessed for their use in discriminating genetic stocks of the three species. Four polymorphic loci identified in the pilot study were used to investigate the genetic stock structure of the *E. australis*. The results of the population study showed that *E. australis* forms several genetic stocks over very small geographic scales.

6.1 Methods

*Stock Discrimination of the Australian Anchovy (*Engraulis australis*).*

Pilot Study

Paterson (1993) assayed 46 enzymes on eleven buffer systems (Appendix 6.1). Four loci were shown to be polymorphic: Peptidase D (leu-pro, PEP*, E.C.3.4.13), Mannose phosphate isomerase (MPI*, E.C. 5.3.1.8), Phosphoglucomutase (PGM*, E.C. 2.7.5.1) and Isocitrate dehydrogenase (IDHP*, E.C. 1.1.1.42).

Population Study

The electrophoretic methods follow those used in the pilot study (Paterson 1993) and Dixon *et al.* (1992). The four polymorphic loci identified by Paterson (1993) were used in the population study. The population study is ongoing and the results reported in this chapter are preliminary only.

Four *E. australis* sample sets were obtained: non-spawning samples caught during winter 1996, spawning samples caught during summer 1997, non-spawning samples caught during winter 1997 and spawning samples caught during summer 1998. This strategy allowed for both a spatial and temporal analysis of samples. An additional sample obtained from Port Phillip Bay was excluded from the spatial analysis but was included in the temporal analyses.

Samples were obtained directly from commercial fishers or bait suppliers. All samples within each set were caught as close as possible to the same date and generally within one month of each other. For statistical purposes each sample contained at least ninety fish and most samples contained more than 100 fish. Spawning stage and sex were determined macroscopically using Blackburn's (1950) key.

Analysis of Genotypic Data

The statistical package BIOSYS-1, release 1.7 (Swofford and Selander 1989) was used to analyse genotypes scored for four polymorphic loci.

Genetic Variability Within Each Site

Genetic variability within each sample was explored by calculating the allele frequencies, average heterozygosity and the percentage of polymorphic loci. The average heterozygosity was calculated in three ways: the proportion of individual samples that were actually heterozygous "direct-count", the usual estimate based on Hardy-Weinberg expectations, and the unbiased estimate based on conditional expectations (Levene 1949; Nei 1978).

Hardy-Weinberg Equilibrium Tests

Each sample was tested for conformity to Hardy-Weinberg equilibrium using a chi-square goodness-of-fit test. The null hypothesis tested was that genotype frequencies do not deviate from Hardy Weinberg equilibrium, and was rejected if $p<0.05$.

Expected frequencies were calculated using Levene's (1949) formula which results in more accurate values for the expected number of genotypes when small sample sizes, i.e.<100 individuals (Speiss 1989) are used (Nei 1987). When more than two alleles were observed at a locus genotypes were pooled into three classes and the tests repeated (Swofford and Selander 1989). This was done because the chi-square test may be unreliable when expected frequencies of some classes are low (Lessios 1992; Roff and Bentzen 1989; Sokal and Rohlf 1969; Swofford and Selander 1989; Zar 1984). The three classes were: 1) homozygotes for the most common allele 2) heterozygotes for the most common allele and one of the other alleles, and 3) all other genotypes.

To avoid the difficulties encountered when using the chi-square distribution for small samples (Haldane 1954; Elston and Forthofer 1977) the exact significance probabilities were also calculated. Exact tests calculate the probability that the observed sample could be drawn

from the population by chance if the null hypothesis held true (Lessios 1992). Therefore, three chi-square tables were produced; one using Levene's (1949) formula on unpooled genotypes, one using Levene's (1949) formula on pooled genotypes and one using the exact probabilities.

In addition, each sample was tested for conformity to Hardy-Weinberg equilibrium using a log-likelihood ratio test (G-test). This test was used to avoid pooling of data and was performed on adjusted values using Levene's (1949) formula. Chi-square tests will be biased when expected frequencies are less than one or when more than twenty percent of expected frequencies are less than five (Cochran 1954; Zar 1984). This problem is often overcome by pooling data, which does not make use of all the available information. The log-likelihood ratio (Sokal and Rohlf 1981) provides a powerful test for distinguishing stocks and uses all the gene frequency data available. Although, the theoretical distribution of the log-likelihood ratio is complex and poorly known (Sokal and Rohlf 1981) many statisticians prefer this method and recommend its routine use (Williams 1976: Zar 1984).

Tests for Temporal and Spatial Genetic Variation

To test for temporal and spatial variation between samples a log-likelihood ratio test was used. The null hypothesis tested was that there was no significant variation in allele frequency distribution between samples, and was rejected if $p<0.05$.

G-statistics testing for differences in allele frequencies between sites were calculated for each individual locus and for all combined loci. If the total (all sites), G-statistics were significant then they were partitioned to test for differences in allele proportions between geographically adjacent sites. This was done in order to determine the number of genetic stocks and their geographic boundaries. Comparisons at each locus were done in order to assess which locus was mainly responsible for the observed population differentiations. Temporal analyses followed the same statistical process with comparisons based on replicate samples.

The significance level of each test was modified to account for the increase in type 1 error when multiple tests are made on the same sample (Cooper 1968). Tests were considered significant if the G-statistic exceeded the value in a chi-square table associated with a probability of 0.05/n, where n is the number of loci used. This resulted in a rejection criteria of $p<0.0125$. The G-statistic values of the comparisons between adjacent sites for each locus

were examined for greatness; a higher G-statistic value indicating a higher contribution to the significant G-statistic across all loci.

Estimation of Genetic Distance

Genetic distance measures were estimated using four distance measures: Nei (1978) unbiased distance, Rogers (1972) genetic distance, modified Rogers distance (Wright 1978) and the Cavalli-Sforza and Edwards (1967) arc distance. A cluster analysis (Sneath and Sokal 1973) using the Cavalli-Sforza and Edwards (1967) arc distance measures was carried out for the two spawning sample sets by the unweighted pair group method with arithmetic averaging.

In order to test for evidence of panmixia, clines or isolation by distance, plots of genetic distance using the genetic distance coefficient of Cavalli-Sforza and Edwards (1967) for the two spawning sample sets were done. The relationship between genetic and geographic distance was tested using linear, logarithmic, quadratic, power and exponential regression. Since the 1997 spawning sample set contained samples whose distribution may have been regulated by different oceanic currents, it was divided into two sets to further investigate the relationship between geographic and genetic distance. The two sets included: Victoria, Tasmania and Western Australia; and New South Wales and Queensland.

*Stock Structure of Yellowtail (*Trachurus novaezelandiae*).*

Forty-four enzymes were assayed in the pilot study (Table 1). Three loci were found to be polymorphic (AAT-1*, ACP-1* and EST-1*). A small stock discrimination study was undertaken using these three loci and fifteen morphometric measures (Wilmot 1995).

*Identification of Useful Genetic Markers in the Blue Mackerel (*Scomber australasicus*).*

This pilot study assayed twenty-four enzymes on four different buffer systems (Table 2) with the aim to identify polymorphic loci suitable for use in genetic stock identification studies. S. australasicus samples used in this study were obtained from: Wollongong, Shelly Bay (near Wollongong) and Cape Moreton (QLD). Nine polymorphic loci were identified; AH*, FBP-1*, IDH*, MPI*, PEP A-2*, PEP B*, PEP S-2*, PGM*, ADA*, ADH-1*.

6.2 Results

*Stock Discrimination of the Australian Anchovy (*Engraulis australis*).*

The *MPI** locus was inactive at some sites and was consistently inactive for samples from Port Phillip Bay. For this reason results for *MPI** are missing for some sites. Allele

frequency tables (Appendices 3-6) show the presence of rare and private alleles. Private alleles are alleles so rare that they occur only in one population (Slatkin, 1985).

Hardy-Weinberg Equilibrium

Hardy-Weinberg equilibrium and heterozygosity tests are presented in Appendices 2-5. The exact significance test, Levene's (1949) formula (with and without pooling of genotypes) and the log-likelihood ratio tests all gave similar significance levels. Therefore only the results obtained using the log-likelihood ratio test on unpooled data will be discussed (and presented), as it is considered the only statistically unbiased method.

Winter 1996 Non-Spawning Samples

Of nineteen tests for conformity to Hardy-Weinberg equilibrium, seven showed significant departures. The Fingal Bay and Byron Bay samples were in Hardy-Weinberg equilibrium at all loci. The Fremantle sample showed significant departures at all loci. The Geelong and Norfolk Bay samples both showed deviations for *PEP**. No samples were out of equilibrium at all loci. The loci which were out of equilibrium differed between sites. Most loci for each site showed slight heterozygote deficits (indicated by negative D values).

Summer 1997 Spawning Samples

Of thirty tests for conformity to Hardy-Weinberg equilibrium, seven showed significant departures. The Tweed Heads, Manly, Botany Bay and Norfolk Bay samples were in Hardy-Weinberg equilibrium at all loci. No samples were out of equilibrium at all loci. The loci which were out of equilibrium differed between sites. Most loci for each site showed slight heterozygote deficits.

Winter 1997 Non-Spawning Samples

Of twenty-two tests for deviations from Hardy-Weinberg equilibrium, fifteen showed significant departures. No samples were in Hardy-Weinberg equilibrium at all loci. The Port Phillip Bay sample was out of equilibrium at all loci. The loci which were out of equilibrium differed between sites. Most loci for each site showed quite large heterozygote deficits.

Summer 1998 Spawning Samples

Of sixteen tests for conformity to Hardy-Weinberg equilibrium, fourteen showed significant departures. The Gosford, Port Phillip Bay and Fremantle samples were out of equilibrium at

all loci. The Kirra and Port Macquarie samples were only in equilibrium at the *IDHP** locus. Most loci for each site showed quite large heterozygote deficits.

Winter 1996 Non-Spawning Samples

The total G-statistic was significant. Partitioning showed significant differences between adjacent sites over all loci combined. Comparisons between adjacent sites for each locus showed that no single locus was responsible for causing the significant differences.

The dendrogram obtained from clustering the Cavalli-Sforza and Edwards (1967) arc distance showed no evidence of group differentiation (Figure 2).

Summer 1997 Spawning Samples

The log-likelihood ratio tests for differentiation between sites were significant. Partitioning showed significant differences between adjacent sites over all loci combined. Comparisons between adjacent sites for each locus showed little variation at the *PGM** locus and that *PEP** contributed the most to the observed significance levels.

The dendrogram obtained from clustering the Cavalli-Sforza and Edwards (1967) arc distance contained one major group (Figure 3). The genetic distance versus geographic distance plot did not display a significant linear, logarithmic, quadratic, power or exponential relationship ($p>0.05$). The plot for VIC, WA and TAS showed a vertical discontinuity and the NSW and QLD plot showed a significant relationship ($p<0.05$) using all regression methods.

Winter 1997 Non-Spawning Samples

The total G-statistic was significant. Partitioning showed significant differences between adjacent sites over all loci combined. However, the Mooloolaba and Kawana sites were marginally different ($p= 0.05$). Comparisons between adjacent sites for each locus showed that samples did not differ at the *MPI** locus.

The dendrogram obtained from clustering the Cavalli-Sforza and Edwards (1967) arc distance contained one major group (Figure 4).

Summer 1998 Spawning Samples

The total G-statistic was significant. Partitioning showed significant differences between adjacent sites over all loci combined. However, the Port Phillip Bay and Fremantle samples

were only significant at the 0.05 level. Comparisons between adjacent sites for each locus showed that the *PGM** locus was the one that contributed the most to the observed significant differences.

The dendrogram obtained from clustering the Cavalli-Sforza and Edwards (1967) arc distance contained two major groups (Figure 5). However, this genetic grouping may not be attributed to geographic proximity of sampling sitesThe genetic distance versus geographic distance plot did not display a significant linear, logarithmic, quadratic, power or exponential relationship ($p>0.05$).

Port Phillip Bay

The total G-statistic was significant. Partitioning showed no difference between the two samples caught less than one month apart or between the two spawning samples. Comparisons at each locus showed that there was no temporal variation at the *PGM** locus.

Geelong

The two Geelong samples were shown to be significantly different.

Norfolk Bay

The total G-statistic was significant. Partitioning showed no difference between the two spawning samples. Comparisons at each locus showed that there was no temporal variation at the *MPI** locus.

Fremantle

The total G-statistic was significant. Partitioning showed significant differences between: consecutive sample sets, spawning samples and non-spawning samples. Comparisons at each locus showed that *IDHP** did not contribute to the observed significant differences.

*Stock Structure of Yellowtail (*Trachurus novaezelandiae*).*

Samples were obtained from: Forster, Wollongong, Wooli and Southport. Replicate samples, caught six months later, were obtained from Forster and Wollongong. The results of the electrophoretic study showed significant spatial and temporal differences between samples. The morphometric study indicated three putative stocks: southern New South Wales, central New South Wales and southern Queensland.

6.3 Discussion

Hardy-Weinberg Equilibrium

Many samples were out of Hardy-Weinberg equilibrium. Deviations from Hardy-Weinberg equilibrium can be attributed to: non-random mating, small population size, differential selection, a Wahlund effect, errors in typing, selection against one or more phenotypes, null-alleles or assortative mating (Richardson *et al.* 1986).

The possibility of typing errors was minimised by re-running samples with poor activity or poor resolution. Substructuring within each site due to differences in allele frequencies between the sexes or age/length classes is the most probable cause of the deviations from Hardy-Weinberg equilibrium. Within sample substructuring is currently being analysed.

Spatial Genetic Variation

The results of this study show that *E. australis* caught in Australian waters do not come from a single panmictic stock. Significant genetic differences were shown between fish caught over very small geographic distances (according to the log-likelihood tests). Genetic stocks are defined as local populations that contain recognisable genetic differentiation by separation of their spawning place or time (Bailey and Smith 1982; Jamieson 1973; Ovendon 1990). By this definition, the results of this study show that *E. australis* is comprised of several genetic stocks.

It is unclear whether these genetic differences are maintained by separation of spawning time or spawning place. Early work by Blackburn (1950) suggested that *E. australis* spawning occurred from October to April in Tasmania and Victoria, and earlier in northern populations. This may lead to a reduction in gene flow between local stocks, however it would not entirely account for the observed genetic differences shown over small geographic scales.

The genetic differences shown between fish (in spawning condition) caught from Manly and Botany Bay and between fish caught from the Geelong arm and Port Melbourne region of Port Phillip Bay suggest that *E. australis* spawns in very discrete areas over small geographic scales. Temporal comparisons showed that the Norfolk Bay and Port Phillip Bay (Port Melbourne region) fish returned to the same area to spawn in consecutive years. However, the genetic structure of fish caught from these sites during non-spawning periods was shown to be different to that which occurred during spawning periods.

Temporal differences within a site between spawning and non-spawning samples may be the result of stocks mixing during non-spawning periods. However, genetic differences shown between fish samples caught in non-spawning condition suggest that spatial genetic differences persist during non-spawning periods. This suggests that *E. australis* stocks in Norfolk Bay and Port Phillip Bay may be moving as cohesive units to new areas during non-spawning periods while returning to the same areas to spawn. One stock may be replacing another for part of the year, leading to heterogeneity in allele frequencies between sample sets collected in the same area at different times.

The results of the genetic distance versus geographic distance plots did not show any discernible patterns, such as those depicted by Richardson *et al.* (1986). There appeared to be a random pattern so the causes of differentiation between sites is unclear. When comparing plots over smaller geographic areas, there appeared be some discontinuity within the southern and western Australia samples. Evidence of isolation by distance in the NSW and QLD samples was detected by the rise in genetic distance with geographical distance (Richardson *et al.* 1986).

The dendrograms clustering genetic distance measures revealed similarities between the northern NSW/southern QLD fish and fish from Fremantle. The reason for these grouping are unclear, however, there are a few possible scenarios to explain how this may have occurred. For example, fish may be under similar selection regimes at similar latitudes or they may have originated from the same population in the past. The dendrograms did not show high levels of differentiation between samples. So, although there is significant genetic differentiation (detected by the log-likelihood tests) between sites, this differentiation does not appear to be great. Certainly, it is not enough to term these groups "subspecies".

Three "subspecies" of *E. australis* have been recognised on the basis of mean vertebra counts (Blackburn 1950). These "subspecies" should be termed phenotypic stocks since no genetic differences were shown. The distribution of these three stocks was: Queensland and New South Wales north of Twofold Bay; southern New South Wales, Victoria, Tasmania and South Australia; and Western Australia. Genetic and phenotypic differences have been shown between *E. australis* samples taken from St Leonards (southern end of Port Phillip Bay), Lakes Entrance and Iluka using electrophoretic and morphometric methods (Paterson 1993). The results of Blackburn's (1950) and Paterson's (1993) studies collaborate the major finding

of this study; that *E. australis* forms several possibly overlapping stocks within Australian waters.

The results of this study have implications for management. Since *E. australis* has been shown to form several genetic stocks within Australian waters it is recommended that *E. australis* should not be managed as a single unit. This will pose problems for management, since *E. australis* is subdivided into genetic stocks with very small geographic boundaries. Therefore, managers should consider each of these units independently when making management decision relating to the *E. australis* fishery.

This study is ongoing. Further statistical analyses of the electrophoretic data are being performed. In addition; morphometric, otolith shape and otolith microchemical studies are being undertaken to determine the phenotypic stock structure of *E. australis*.

Figure 6.1 Sampling sites for *E. Australis* in Australia.

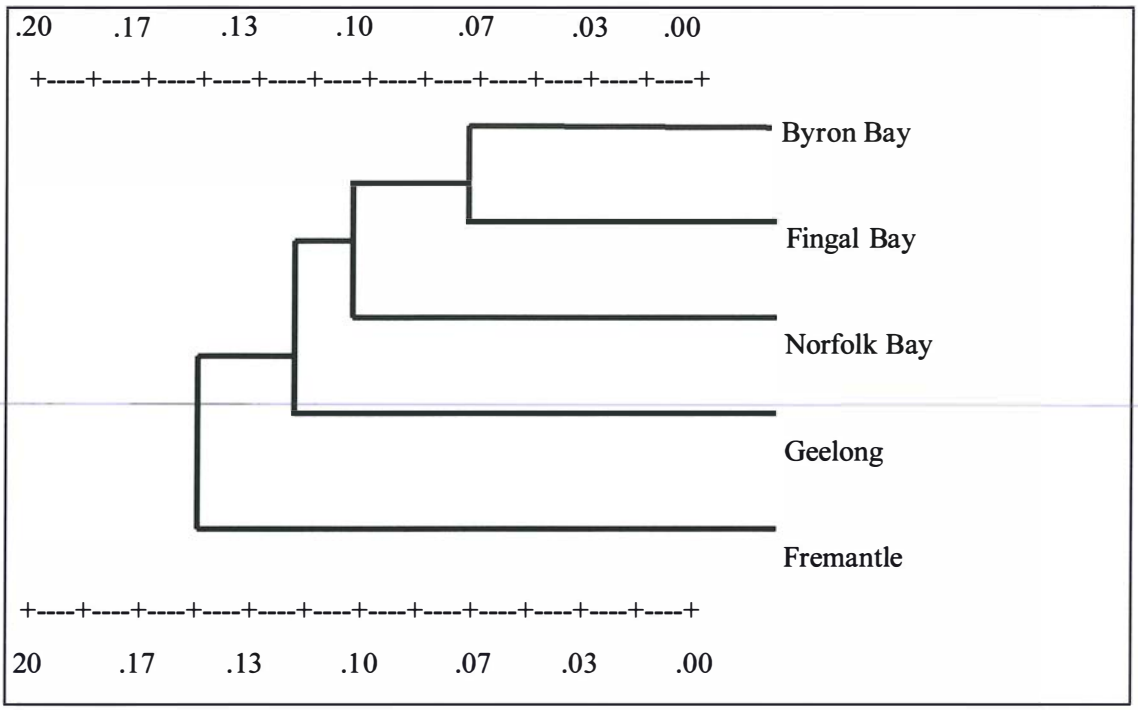


Figure 6.2 Dendrogram using Cavalli-Sforza & Edwards (1967) arc distance - Winter 1996 non-spawners Farris (1972) "f" = 0 .089, Prager and Wilson (1976) "F" = 6.726, % SD (Fitch and Margoliash 1967) = 11.290 Cophenetic correlation = .893

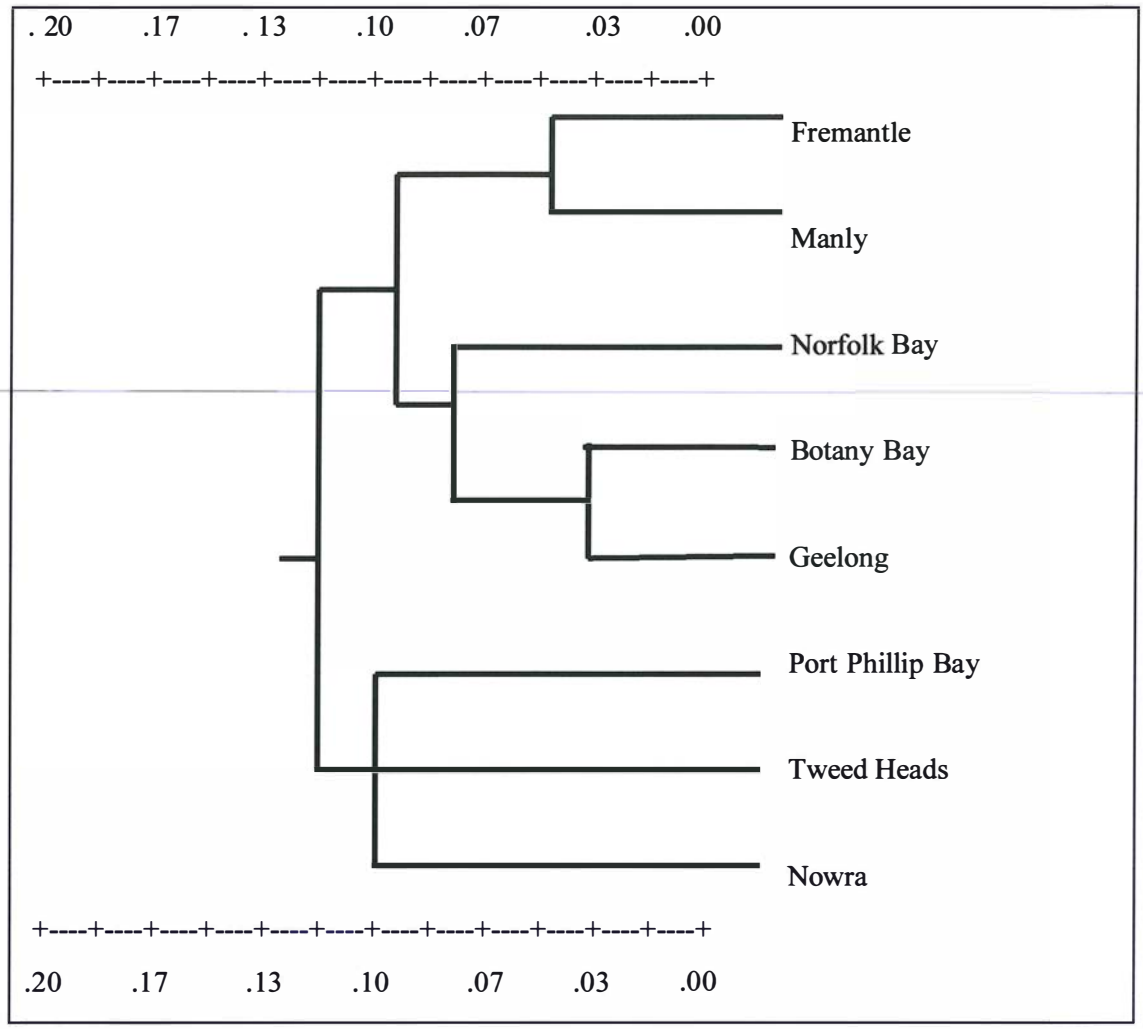


Figure 6.3 Dendrogram using Cavalli-Sforza & Edwards (1967) arc distance - Summer 1997 non-spawners Farris (1972) "f" =.276, Prager and Wilson (1976) "F" = 9.615, %SD (Fitch and Margoliash,1967) = 12.494 Cophenetic correlation = .815

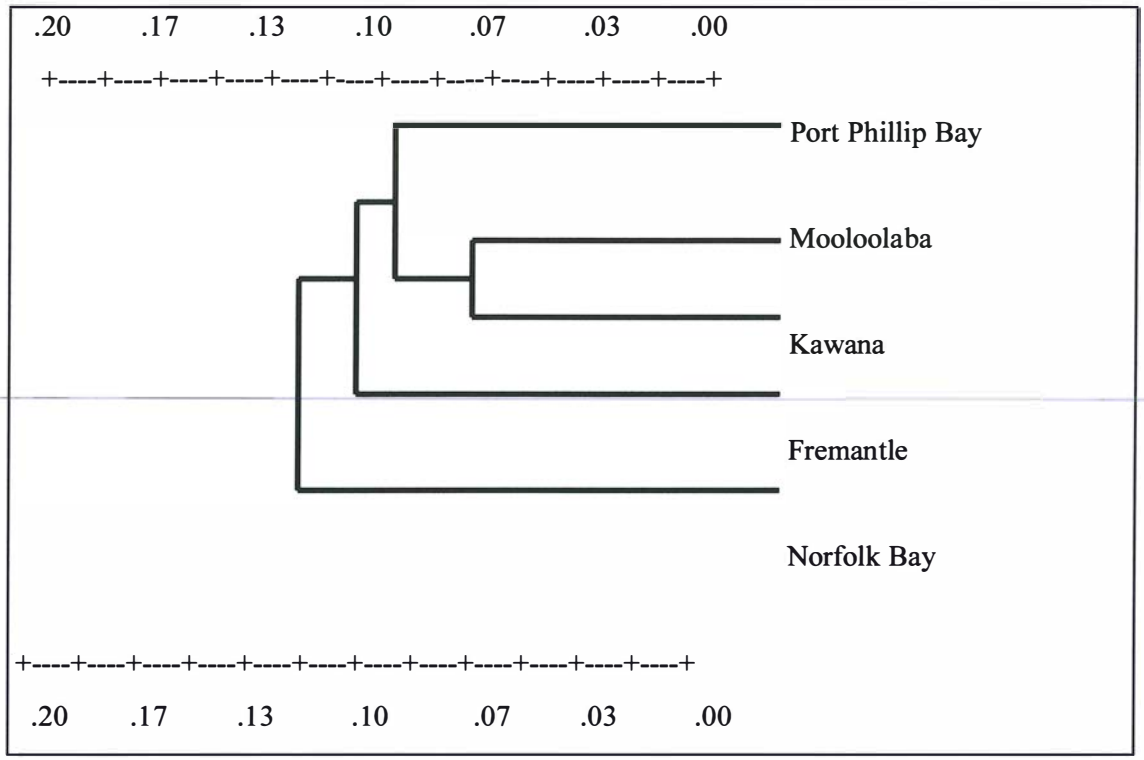


Figure 6.4 Dendrogram using Cavalli-Sforza & Edwards (1967) arc distance - Winter 1997 non-spawners Farris (1972) "f" = .046, Prager and Wilson (1976) "F" = 4.005, %SD (Fitch and Margoliash,1967) = 6.639 Cophenetic correlation = .838

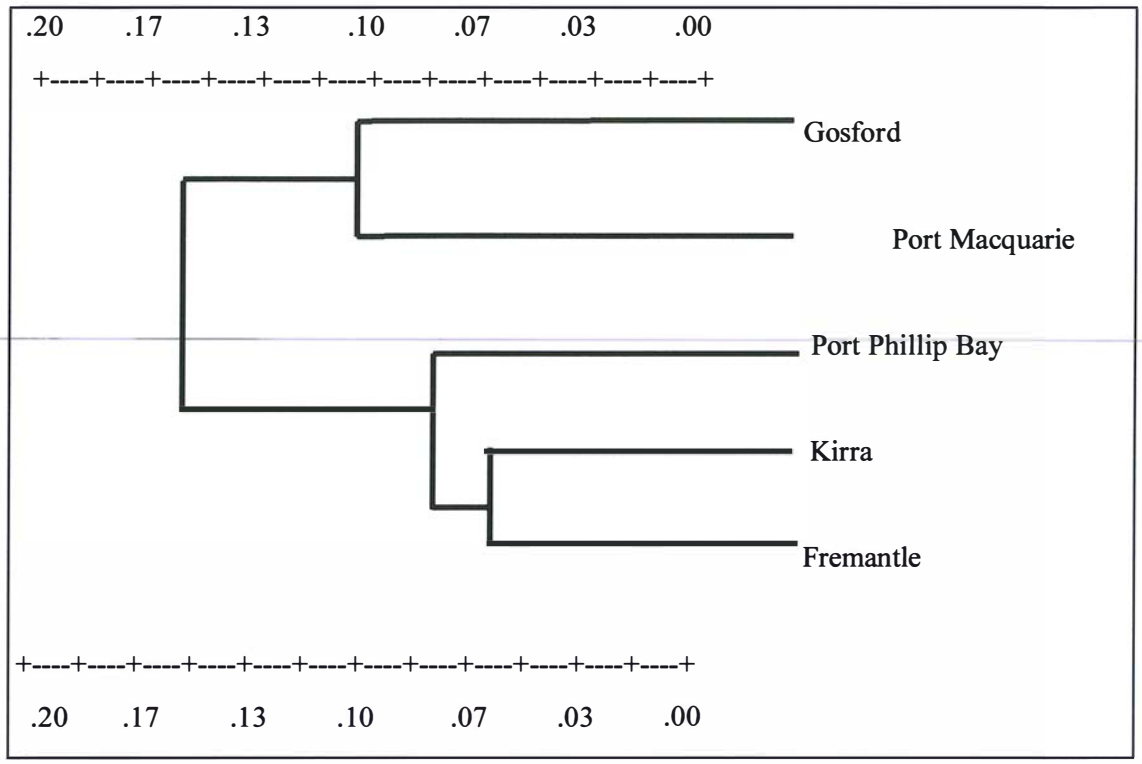


Figure 6.5 Dendrogram using Cavalli-Sforza & Edwards (1967) arc distance - Summer 1998 spawners Farris (1972) "f" = .160, Prager and Wilson (1976) "F" = 12.026, %SD (Fitch and Margoliash,1967) = 18.031 Cophenetic correlation = .817

Table 6.1 Enzymes studied, tissues investigated, running conditions used and polymorphic loci identified in *T. novaezelandiae* pilot study.

Key: L = liver; H = heart; M = muscle; E = eye

1 = Poulik

2 = Tris-EDTA-boric acid pH 9.0

3 = Tris-maleate pH 7.8

4 = Citric acid-aminopropyl-morpholine pH 6.1

5 = Tris-citric acid pH 5.8

* = best tissue/buffer/support matrix for this enzyme

A = anodal

C = cathodal

ST = electrostarch gel

P = polymorphic

Enzyme	Tissue	Buffer(s)	Presumed #loci	Comments
ACP	L	3, 4*	1A	Fair activity, poor resolution
	M	4		No activity
	H	4		No activity
	E	4		No activity
ACP (alt.recipe)	L	1,3,4,*5	1A	fair activity, good resolution, P
AH	L	1,2,3,4,5*	1A	good activity, good resolution
	M	1*,2,3,4,5	1A	poor activity
	H	1*,2,3,4,5	1A	fair activity, poor resolution
	E	4`	1A	poor activity
ADA	L	1,2*,3,4,5	1A	good activity, good resolution
	M	4	1A	fair activity, fair resolution
	H	2,3,4*, 5	1A	fair activity, good resolution
	E	4	1A	fair activity, fair resolution
AK	L	1,2,3,4*,5	2A	good activity, fair resolution
	M	1,2,4	2A	good activity, fair resolution
	H	4	1A	good activity, fair resolution
	E	4	1A	good activity, fair resolution
ADH	L	4	1A, 1C	fair activity, fair resolution

	M	4		no activity
	H	4		no activity
	E	4		no activity
AO	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
ALD	L	4		no activity
	M	4	1C	poor activity
	H	4		no activity
	E	4	1A	fair activity, fair resolution
ALP	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
AAT	L	1,2,3,4*,5	1A 1C	good activity, good resolution
	M	4	1A 1C	good activity, good resolution
	H	4	1A 1C	good activity, good resolution
	E	4	1A 1C	no activity
CAT	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
CK	L	1*, 2, 4	1A	good activity, fair resolution
	M	1,2,4*		good activity, fair resolution
	H	4	1A	fair activity, good resolution
	E	4		no activity
DAMOX	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
DASOX	L	4		no activity
ENOL	L	4		no activity
EST	L	1,2,3,4*,5	2A	good activity, good resolutionP

	M	4		no activity
	H	1,2,3,4,5*	2A	fair activity, fair resolution
	E	4	1A	poor activity, poor resolution
FEBP	L	4	1A	good activity, fair resolution
	M	4	1A	fair activity, fair resolution
	H	4	1A	poor activity, poor resolution
	E	4		no activity
FH	L	2,3,4*	1A	good activity, good resolution
	M	4	1A	good activity, good resolution
	H	4	1A	good activity, good resolution
	E			no activity
GALDH	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
a-GAL	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
ß-GAL	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
GDH	L	4	1A	poor activity, fair resolution
	M	4		no activity
	H	4		no activity
	E	4		no activity
ß-GUS	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
a-GLU	L	4		no activity
	M	4		no activity
	H	4		no activity

	E	4		no activity
GPI	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
GLUDH	L	2,3,4*, 5	1A	fair activity, good resolution
	M	4		no activity
	H	4		no activity
	E	4		no activity
GPT	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
GA3PDH	L	4	1A	fair activity, fair resolution
	M	4	1A	fair activity, fair resolution
	H	4	1A	no activity
	E	4		poor activity, fair resolution
GLYDH	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
GPD	L	4	1A	good activity, fair resolution
	M	1,2,3,4*	2A	good activity, fair resolution
	H	4		no activity
	E	4		no activity
GOX	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
GLO 1	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
GDA	L	4		no activity

	M	4		no activity
	H	4		no activity
	E	4		no activity
HK	L	4	1A	fair activity, fair resolution
	M	4		no activity
	H	4		no activity
	E	4		no activity
HEX	L	4	1A	poor activity, poor resolution
	M	4		no activity
	H	4		no activity
	E	4		no activity
HBDH	L	4		no activity
	M	4		no activity
	H	4		no activity
	E	4		no activity
IDHP	L	4	1A	fair activity, fair resolution
	M	1,2,3,4,5*	1A	poor activity,fair resolution
	H	1,2,3,4*,5	1A	fair activity, fair resolution
	E	4		no activity
LDH	L	4	1A	good activity, good resolution
	M	2,3,4*,5	1A	good activity, fair resolution
	H	4	1A	fair activity, fair resolution
	E	4	2A	poor activity, poor resolution
MADH	L	4	1A	fair activity, fair resolution
	M	4		no activity
	H	4		no activity
	E	4		no activity
ODH	M	2		no activity
	H	2	1A	poor activity
PGM	L	2		no activity
	M	2		no activity
	H	2		no activity
	E	2		no activity
SDH	M	2		no activity

SUCDH	L	2		no activity
	M	2		no activity
	H	2		no activity
	E	2		no activity
XDH	M	2	1A	poor activity

Table 6.2 Enzymes studied, tissues investigated, running conditions used and polymorphic loci identified in *S.australasicus* pilot study.

Enzymes Used

Aspartate Aminotransferase. AAT (GOT). E.C. Number 2.6.1.1.

Adenosine Deaminase. ADA. E.C. Number 3.5.4.4.

Alcohol Dehydrogenase. ADH. E.C. Number 1.1.1.1.

Aconitase. AH (ACON). E.C. Number 4.2.1.3.

Adenalyne Kinase. AK. E.C. Number 2.7.4.3.

Alkaline Phosphatase. ALP (AP; ALKPH). E.C. Number 3.1.3.1.

Aldehyde Oxidase. AO. E.C. Number 1.2.3.1.

D-Aspartate Oxidase. DASOX. E. C. 1.4.3.1.

Esterase. EST. E.C. Number 3.1.1.1.

Fructose 1,6-Diphosphatase. FDP (FBP). E.C. Number 3.1.3.11.

Fumarate Hydratase. FUM. E.C. Number 4.2.1.2.

Glucose Phosphate Isomerase. GPI. E.C. Number 5.3.1.9.

Hexokinase. HK. E.C. Number 2.7.1.1.

Isocitrate Dehydrogenase. IDH. E.C. Number 1.1.1.42.

Malate Dehydrogenase. MDH. E.C. Number 1.1.1.37.

Malic Enzyme. ME (MEP). E.C. Number 1.1.1.40.

Mannose-Phosphate Isomerase. MPI. E.C. Number 5.3.1.8.

Purine Nucleoside Phosphorylase. NP. E.C. Number 2.4.2.1.

Peptidases. PEP-A, -B, -S. E.C. Number 3.4.11 or 13.

6-Phosphogluconate Dehydrogenase. PGD. E.C. Number 1.1.1.44

Phosphoglucomutase. PGM. E.C. Number 2.7.5.1.

Xanthine Oxidase. XO. E.C. Number 1.2.3.2.

Buffers Used

1: Tris-citric acid (TC) pH 5.8.

Running Voltage: 200 V; Running Amperage: 50 mA; Running time: 2h 30 min.

2: Tris-citric acid (TC) pH 7.0.

Running Voltage: 200 V; Running Amperage: 50 mA; Running time: 3h.

3: Tris-maleate (TM) pH 7.8.

Running Voltage: 200 V; Running Amperage: 50 mA; Running time: 3h.

4: Citric acid-aminopropyl-morpholine (CAM) pH 6.1.

Running Voltage: 190 V; Running Amperage: 50 mA.; Running time: 2h.

ENZYME	BUFFER	PRESUMED # LOCI (L)/ ALLELES (A)	ACTIVITY	POLIMORPHISM	COMMENTS
AAT	1 2 3 4	1L, 1A 1L, 1A 3L, 3A 1	GOOD GOOD GOOD GOOD	NO	Clear bands for all the buffers. 1 anodal locus, 2 cathodal loci.
ADA	2 3 4	1L, 3A 1L, 2A 1L, 2A	GOOD GOOD WEAK/ GOOD	YES	
ADH	3	2L, 3A	WEAK	Possibly for anodal locus	1 cathodal locus, weak activity. 1 anodal locus, with possibly 2 alleles; weak activity, weak bands.
AH	4	1L, 2A	WEAK/ GOOD	YES	For samples from Wollongong, Shelly Bay.
AK	2 3 4		NO NO NO		
ALP	3		NO		
AO	1		NO		

DASOX	1		NO		
EST	3	3L, 3A	GOOD	NO	Clear and sharp bands.
FBP	4	2L, 3A	GOOD	YES	For samples from Queensland, Moreton Bay. Clear bands. Both loci are cathodal. FBP-1, 2A FBP-2, 1A
FUM	4		NO		Should be repeated.
GPI	1	1L, 1A	GOOD	NO	
HK	1 3 4	1L, 1A 1L, 1A 1L, 1A	WEAK NO WEAK	NO	3: best buffer.
IDH	1	1L, 4A	GOOD	YES	Strong, sharp bands.
MDH	1		NO		
MEP	1		NO		
MPI	1	2L, 3A	GOOD	YES	Clear/ fuzzy bands.
NP	4	1	WEAK	NO	
PEP A	1 3 4	2L, 4A 2L, 4A 2L, 4A	GOOD GOOD GOOD	YES	PEP A-1, 1A. PEP A-2, 3A.

PEP B	4	1L, 4A	GOOD	YES	Clear bands. Polymorphism for samples from Wollongong and Queensland (Moreton Bay).
PEP S	4	2L, 4A	GOOD	YES	Clear bands. Polymorphism for some fish from Wollongong and Queensland. PEP S-1, 1A PEP S-2, 3A
PGD	3	1L, 1A	GOOD	NO	Sharp bands.
PGM	3	1L, 3A	GOOD	YES	
XO	3	1L, 1A	WEAK	NO	Fuzzy bands.

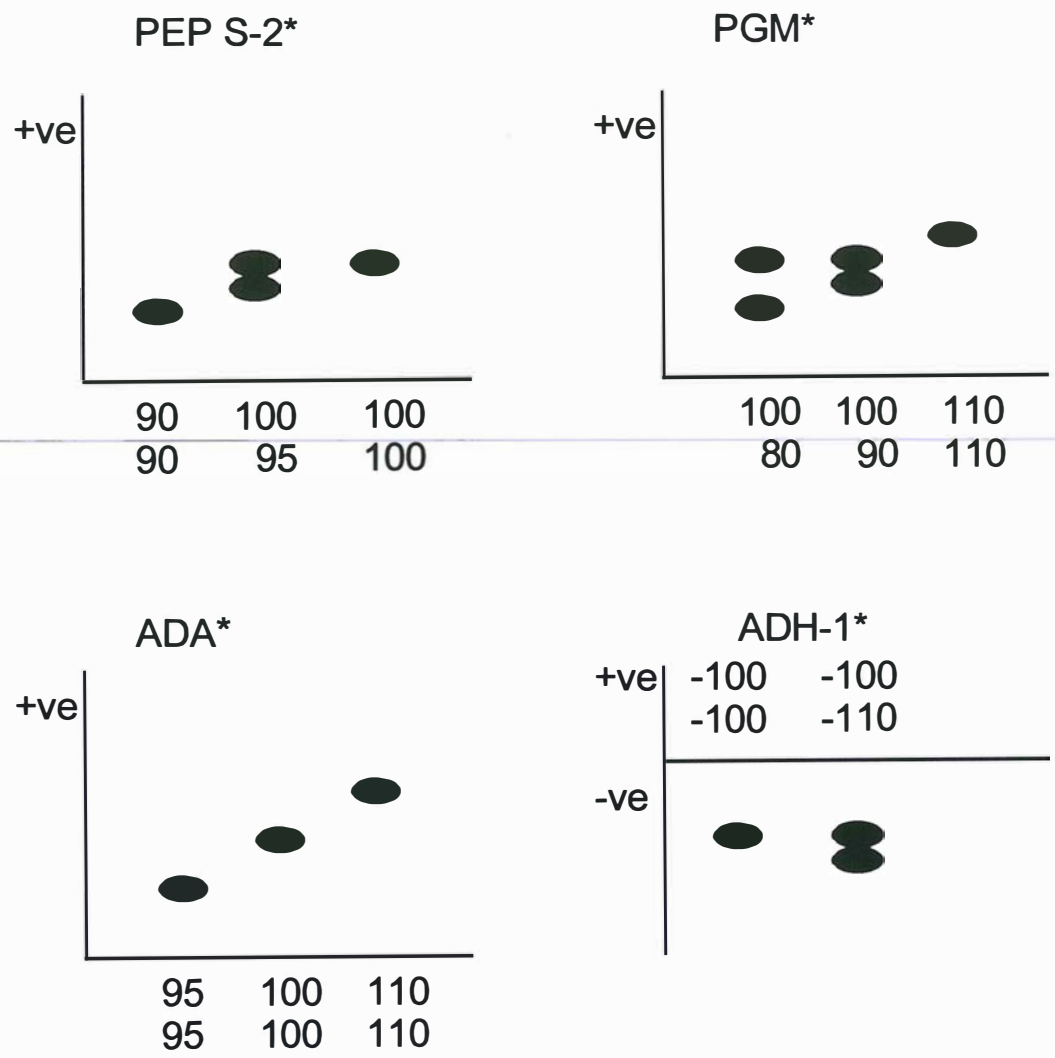


Figure 6.6 Zymograms showing the observed banding patterns and genotype designations for *S. austalasicus* at some loci.

Appendix 6.1 *E.australis* Pilot Study Results

L = Liver, H = Heart, E = Eye, M = Muscle

* = best tissue/buffer/enzyme combination

Key:

1 = Poulik

2 = Citric acid-aminopropyl-morpholine (Cam) pH 6.1

3 = Tris- EDTA-boric acid (TBE) pH 9.0

4 = Tris-citric acid (TC) pH 5.8

5 = Tris-citric acid (TC) pH 6.8

6 = Tris-citric acid (TC) pH 6.9 (Grant, 1985)

7 = Tris-maleate (TM) pH 7.8

8 = LiOHa

9 = LiOHb

10 = LiOHc

11= LiOHd

S = Sigma starch Lot # S4501; E = Electrostarch Lot # 89; P = Polymorphic; M = Monomorphic; A = Anodal; C = Cathodal

Enzyme	**Tissue**	**Buffers**	**Presumed # loci**	**Comments**
sAAT	L, H, E, M	7	1A	Good activity; good resolution; M
sAAT alt	L, H, E, M	5	2A	Good activity; good resultion; M
	L,E, M,	8	1A	Poor migration; M
	L,E,M	4	1A	Good activity: good resolution; M
	L		1C	Fair activity; poor resolution; M
	K, E, M	2	1A	Good activity; Fair resolution; M
	L, M		3C?	Sub-banding; poor resolution
sAH	L	1, *2, 3 4, 5 6, 7, 8, 9		Poor activity; poor resolution
	H, E, M	10, 11, all buffers		No activity
ACP	L	2, 4,	1A	Fair activity; poor resolution,

		5, *6, 7		smearing
	E	6	1A	
	E	2, *4,	1A	Fair act.; poor resolution;
	E	3, 5	1A	Poor activity; fair resolution;
	M	1,2,3,4,5,	1A	No activity
	M	6	1A	No activity
				Fair act.; poor resolution; P?
ADA	L,E,M	5		Sub-banking
	L	*2,3,7	1A	Poor activity; poor resolution;
	E	4,		No activity
	E	7	1A	Poor activity; poor resolution;
	M	4,7	1A	Poor activity, poor resolution;
ADH	L	2,3,4	1A, 1C	Good activity; poor resolution
	L	7,8,9		No activity
	H,E,M	4,7,8,9		No activity
	L,H,E,M	3	1A	Good activity; good resolution; M
ALDH	L	2,3,4	2A?	Poor activity; fair resolution;
	L	5		No activity
	E	3,4,5		No activity
	M	2,3,4,5		No activity
AK	L,H.E,M	3,4,7	2A? or sub-banding	Good activity; fair resolution; sub-banding; M
AO	L,H,E,M,	2,5,7		No activity
ALD	L	1, 4	1A	Good activity; no separation;
	E	3	1A	Good activity, no separation
	M	1,3,4,5	1A	Good activity, no separation
	L,H,E,M,	2		No activity
ALKPH	L	1		Poor activity, poor resolution;
	H,E,M,	1		No activity
	L,H,E,M	4		No activity
CK	L,H,E,M	2,4	2A	Good activity; good resolution; M
DAMOX	L,H,E,M	5		No activity
DASOX	L,H,E,M	4		No activity
DIA	L	7	1A	Good activity; good resolution; M

	L,H,E,M	2		No activity
EST	L,E,M	2,5,7	4A	Good activity; poor resolution;
FDP	L,E	5		No activity
	M	5	2A	Good activity, fair resolution; M
FUM	L,M	5, 7	1A	Good activity; good resolution; M
	E	5,7		No activity
GALDH	L,H,E,M,	7		No activity
GDH	L,H,E,M	1,7		No activity
G6PDH	L	5	?	Good activity; fair resolution; sub-banding; M
	L	1	1A	Poor activity;
	E	2,5	1A	Good activity, good resolution; M
	L,H,E,M	7	1A	Good activity; good resolution; M
	E,M	1		No activity
alph-GLU	L,H,E,M	1		No activity
GPI	L,H,E,,M,	2,4,5,7	1A	Good activity; fair resolution; M
	L,E	8, 9,	1A	Fair activity; fair resolution; slow staining; M
	L,E,M	3,5	3A	Good activity; poor resolution; sub-banding?; M
	L,E,M	11	2A	Good activity; good resolution; M
GLUD	L,E,M	3		No activity
GLYDH	L,E,,M	3		No activity
alph-GPD	M	1	1A	Good activity; fair resolution; M
GOX	L,H,E,M	2,7		No activity
HK	L,H,E,M	2		No activity
IDHP	L	1, *2,3 4,5,7	1A	Good activity; fair separation; P
	E	2	2A	Good activity; poor resolution; P
	E	1,4,5,7		No activity
	M	1,2,4,5,7	1A different loci	Good activity; poor separation; P
LDH	L	1,5,8	2A	Good activity; fair resolution; M

	L,E L,E,M H,E,M E M	10, 12 2,4 3 8, 10, 12	? 1A 2A	Good activity, sub-banding; Good activity; good resolution good activity, fair resolution: M
LAP	L,H,E,M	2,4		No activity
MDH	L,H,E, *M	7	1A	Good activity; good resolution; M
MEP	L,H,E,M	2,4,8,10	1A	Good activity; good resolution; M
MPI	*L,H,*E,M	2 4 3,7,8	1A	Good activity; fair resolution; P No activity Poor activity; poor resolution;
PEP leu Ala	L,E,M	2,4,7	1A	Good activity; fair resolution; sub-banding; M
PEP B leu gly gly	L,H,E,M,	2,7	1A?	Good activity; fair resolution; sub-banding; M
PEP leu try	L,E,M	2,3,4,5, 7,8,10	1-2A?	Good activity; poor resolution; sub-banding; M
PEP D leu pro	*L,H,E M	*2,3, *4, 5,6,7,		Good activity, fair resolution; P
PGM	L,H,M	1,2,3,4,5, *7,8,	2A	Good activity, fair resolution; P
PGDH	L,E,M H	5 5	1A	Good activity, good resolution; M No activity
PNP	L,H,E,M,	7		No activity
SDH	L E,M	7 7	1A	Fair activity; good resolution; M No activity
SUCDH		7		No activity
XDH	L L L	3,4, *5 7 1,8,10		Good activity; fair resolution; poor separation; P? Good activity, good resolution; M? No activity

	H,E,M,	6 1,2,3,4 5,6, 7,.8,10		No activity
XO	L,H,E,M,	2		No activity

CHAPTER 7. AGE AND GROWTH OF PILCHARDS IN SOUTH-EASTERN AUSTRALIA

S. Morison and K. Hall

Objective (4a): To describe and compare the age and growth of South Australian and Victorian pilchards. This objective was achieved by examining the length frequencies, otolith annuli and weights of pilchards sampled from commercial catches in Spencer Gulf and Coffin Bay in South Australia, and from Port Phillip Bay and Lakes Entrance in Victoria. The growth of a cohort in Port Phillip Bay was followed over 11 months from November 1995 to September 1996 (Chapter 4). This provided unequivocal data on growth and showed that several supernumerary zones are formed during the first year of life. Age estimates are unvalidated, but length frequency distributions provide independent estimates of growth. Growth rates vary between years and among areas. Pilchard are difficult to age consistently and best results are obtained by joint use of length frequency, otolith weight and annuli data from monthly samples.

7 .1 Methods

Sample Collection

Age estimates were obtained from a total of 3,447 pairs of sagittal otoliths collected from commercial pilchard catches and submitted for examination to the Central Ageing Facility, at the Marine and Freshwater Resources Institute (Victoria). The locality, month and year of collection and number of otoliths submitted are shown in Table 7 .1.

Otoliths were removed from a sub-sample of fish for which length data had already been obtained. The length data were collected on a monthly basis (when available) from commercial catches (Chapter 4) and from research surveys (Chapter 8).

Preparation and Examination of Otoliths

Each batch of otoliths was registered with a sequential batch number, and otolith pairs were allocated a sequential number within each batch. Data on each batch (e.g. area and date of capture, port of landing, etc.), and the length and sex of individuals within each batch were entered in separate data files. All intact (not broken) otoliths were weighed to the nearest 0.0001 g on an electronic balance prior to ageing.

All counts and measurements were carried out using a stereomicroscope fitted with a polarising filter and a video camera to display the image on a computer monitor. The distal surface of whole otoliths was viewed at 16x magnification under water against a black background using reflected light. Age estimates were made by counting the number of translucent zones (presumed to correspond to slow winter growth) visible along a transect from the primordium to the posterior edge of the otolith. Either the left or right otolith was used for age determination as it is rare to encounter different zone patterns on the two sagittal otoliths from the same fish. Broken and/or deformed otoliths were not aged.

Customised image analysis software (Optimate™) recorded increments counts and enabled on-screen marking and measurements of the distance from the primordia to each zone marked, and to the edge of each otolith (Morison *et al.* 1998). If the otolith was difficult to read on the monitor, increments were identified directly under the stereomicroscope. Measurements were exported to an Excel spreadsheet by dynamic data exchange.

Interpretation of Incremental Structure

Terminology used to describe otoliths follows Kalish *et al.* (1994) and is shown on Figure 7 .1

Examination of the distal side of pilchard sagittae from Victoria and South Australia shows an inner region that is predominantly opaque, but with between one and three faint translucent zones. There is then an abrupt change to a region of diffuse translucent and opaque zones. By examining otoliths from young-of-the-year fish collected in successive months over a period of 11 months (November 1995 to September 1996), it was established that several of these translucent zones may be formed in the first year of life. On larger otoliths (from fish > 1 year old), there is a transition to an outer region of narrower and more clearly defined alternating-opaque and translucent zones, which comprise the annual increments. Age was estimated by counting the translucent zones.

Initially, July 1 was selected as the birthdate for this study, following a preliminary analysis of marginal increments of both the South Australian and Victorian samples which indicated translucent zone formation in winter. Thomas (1983) also suggested that the main annual translucent zone is formed in late winter. Samples caught prior to July 1 were aged as though they were approaching zone formation and any evidence of a zone on the edge was not counted. Samples caught after July 1 were aged as though a zone had formed on the edge, even if it was not fully formed. After ages had been assigned, the birthdate was shifted to January 1 in order to be closer to the known spawning season. Ages were adjusted by adding one year to fish caught prior to July. Ages presented are based on the January 1 birthday unless otherwise indicated.

Validation of Age Estimates

Age estimates provided are unvalidated. However, the length frequency distributions provide independent estimates of growth against which the results from otolith ageing can be compared. Data on otolith weight also allows comparison of the relative age of cohorts. By definition, faster growing fish are younger at a given length than slower growing fish. Also, as otoliths increase in size with age, older pilchards tend to have heavier otoliths than younger fish (Fletcher 1991, 1995). Therefore, for fish of the same length, the otoliths of fish from a faster growing cohort should be lighter on average than fish from a slower growing cohort. Also, differences among cohorts in mean otolith weight for fish of the same length should be consistent with differences in their estimated age.

Precision of Age Estimates

Repeated readings of the same otoliths by the same reader provide a measure of intra-reader variability. This process does not validate the assigned ages but provides an indication of size of the error to be expected with a set of age estimates, due to random variation in a reader's interpretation of an otolith. Twenty five percent or more of the samples were re-read to assess intra-reader variability, or precision.

Repeat readings to assess intra-reader precision were compared using Beamish and Fournier's (1981) index of average percent error (APE):

$$APE = \frac{100}{N}\sum\frac{(|\ X_j - \overline{X}_j|)}{\overline{X}_j}$$

Where N = number of fish aged, X_j = the first age estimate of the jth fish, and $\overline{X}_j$ = the average age of the jth fish.

Age-difference tables were produced to show the frequency and distribution of differences between the primary age and the repeat reading.

Data Analysis

Once age estimates were completed, the ageing data were combined with information on fish length and sex, location and date of capture, and otolith weight, for subsequent analyses.

The von Bertalanffy growth function was fitted to the length and age data for samples using the NLIN procedure in SAS®, a non-linear, least squares procedure. From a grid search over a range of possible values for L_∞ ,K and T_0, the combination with the lowest residual sum of squares was selected as the starting point for iterations. Both the Secant and Marquardt iterative techniques were tried from this point, and the solution with the lowest sum of squares selected. Immature fish were allocated alternatively to either the male or the female samples. Growth functions were fitted to data for each sex separately and for the sexes combined (including samples of males, females and immature fish). This assumes that the growth of immature male and female fish is not significantly different.

Differences in the fitted curves between the sexes were tested using Kimura's (1980) likelihood ratio test:

$$\chi^2=[-N\ln(\sigma_\Omega^2/\sigma_\omega^2)]$$

where σ_ω^2 and σ_Ω^2 are the variances for the hypotheses H_ω, that all parameters are equal, and H_Ω, that all parameters are not equal, respectively.

7.2 Results

Length Frequency Distributions

There was substantial variation between months and years in the length frequency distributions of samples obtained from Coffin Bay and Spencer Gulf (Chapter 4) and little information on growth rates could be obtained from length frequency data.

Discrete size modes in length frequency distributions from monthly samples of the commercial piclards catch in Port Phillip Bay allowed cohorts to be identified and the growth of a single cohort to be followed over an 11 month period from November 1995 to September 1996 (Chapter 4). This mode provides unequivocal data on their growth and provided the basis for interpreting the structure laid down in pilchard otoliths in their first year of life, establishing that several supernumerary zones are formed at this time and giving confidence to the interpretation of the age of the young fish. Although the age estimates provided are unvalidated, the length frequency distributions provide independent estimates of growth against which the results of otolith ageing can be compared.

The length frequency distributions also show how variable growth was between years. In March 1995 the Port Phillip Bay cohort of older fish had a mean length of 12.6 cm and the second cohort which

had entered the fishery had a mean length of 9.8 cm LCF. In March 1996, however, the cohort in Port Phillip Bay had a mean length of 11.7 cm LCF. The length frequency distributions and the plots of mean length against time suggest that the 1995/96 Port Phillip Bay cohort is faster growing than the 1994/95 and 1995 cohorts from Port Phillip Bay, but similar to the 1995 cohort from Lakes Entrance. No difference in the growth between the 1994/95 cohorts from Port Phillip Bay and Lakes Entrance was obvious.

Otolith Weight Distributions

There was no significant difference in mean otolith weight at age between the sexes (Figure 7 .2). Data for females and males were therefore pooled for subsequent analyses.

There were no clear trends in otolith weight distributions by quarter for pilchards from Coffin Bay (Figure 7 .3A). There is a suggestion of a trend for modal progression between January-March and July-September 1996, although the sample sizes were small. Sample sizes were variable for Spencer Gulf although there was a similar trend in the otolith weight distributions (Figure 7 .3B) to the length frequency distributions (Chapter 4). In January-March 1995 the otolith weight distribution covered a wide range of weights including many otoliths of over 2 mg. However, in the latter half of 1996 and in 1997, the range was narrower and few otoliths of over 2 mg were recorded.

There was a modal progression trend in the otolith weight distributions for pilchards from Lakes Entrance between January-March and October-December 1995 (Figure 7 .3C), although the range of weights remained fairly constant from 0.5 mg to 1.5 mg. For the 1995 cohort from Lakes Entrance, the mean otolith weight increased by 0.12 mg between July and August 1995 (a growth rate of 1.44 mg/year). Similarly, there was a modal progression trend in the otolith weight distributions for pilchards from Port Phillip Bay between January-March and October-December 1996 (Figure 7 .3D). This tracks the growth of the cohort first recorded in the length frequency distributions in November 1995 (Chapter 4). For this discrete 1995/96 cohort from Port Phillip Bay, the mean otolith weight increased from 0.3 mg in December 1995 to 0.69 mg by March 1996, and reached 1.07 mg by September 1996, corresponding to an average rate of 0.085 mg/month (or 1.02 mg/year). In January-March 1995 the otolith weight distribution covered a wide range of weights, reflecting the range in size of the fish caught (Figure 7 .3D). This also occurred in the latter half of 1996, including many otoliths of over 2 mg. In October-December 1994 and in January-March 1997, the range of otolith weights was narrower, although this may be have been a result of the smaller sample size.

Substantial variation in the mean otolith weight at age was evident among years and between areas for the South Australian pilchard samples (Figures 7 .4A, 7 .5A). For both Coffin Bay and Spencer Gulf, fish collected in 1995 tended to have heavier otoliths at a given age. However, this may be partly the result of 1995 including a greater proportion of samples collected later in that year, and 1997 including a greater proportion collected earlier in that year. The effect is greater for Coffin Bay, where samples were more heavily biased to the later part of the year. Within years, there was a consistent tendency for heavier otoliths for fish from Coffin Bay, although the difference was not as pronounced in 1996 when samples were distributed through all months of the year in both areas.

Substantial variation in the mean otolith weight at age was evident between areas and between years (Figures 7.4B, 7.5B) for the Victorian samples. As samples were only collected from Lakes Entrance in 1995, only one year could be compared between areas (Figure 7.5B). Fish from Port Phillip Bay and Lakes Entrance had similar otolith weights at age for fish between the ages of 1 and 3.

The distribution of otolith weights at age (Figure 7.6A) and plot of otolith weight against age (Figure 7.7A) for the South Australian samples show a substantial variability within age classes and a considerable overlap of ages for a given otolith weight. A scatter plot of otolith weight against age expressed as a decimal (Figure 7.7A) shows the generally linear nature of the relationship and also the extent of variation within age classes. For any given otolith weight there was approximately a two year range in ages in the samples examined. This range increased with increasing age. Linear regressions fitted to the relationships for each of the areas indicated significant differences in the otolith weight at age between Coffin Bay and Spencer Gulf (Figure 7.8A).

The distributions of otolith weights at age (Figure 7.6B) and plot of otolith weight against age (Figure 7.7B) for the Victorian samples showed substantial variability within age classes and considerable overlap of ages for a given otolith weight. For example, a heavy otolith for an age 0 fish lies within the ranges of weights for 1, 2 or 3 year olds. Linear regressions fitted to the relationships for each of the areas show the variations between Port Phillip Bay and Lakes Entrance (Figure 7.8B), with a trend for heavier otoliths at an older age for Port Phillip Bay. This difference may reflect the narrower size range of fish collected from Lakes Entrance.

Fish Length-otolith Weight Relationships

There were also no differences between the sexes in the relationship between otolith weight and fish length in samples from South Australia or Victoria (Figure 7.9). There were no consistent differences in the relationship between otolith weight and fish length between Coffin Bay and Spencer Gulf

(Figure 7.10A). However, the 1995 Port Phillip Bay samples had consistently heavier otoliths than both the 1995 Lakes Entrance and Port Phillip Bay samples for fish of the same length (Figure 7.10B).

The differences in growth shown in the length frequency distributions from Victoria (Chapter 4) were also examined using the fish length/otolith weight relationships: faster growing fish should have lighter otoliths than slower-growing fish of the same length. Differences in the fish length/otolith weight relationships were examined for Port Phillip Bay between the 1994/95 and 1995/96 cohorts, between Port Phillip Bay and Lakes Entrance for the 1994/95 and 1995 cohorts, and the 1994/99 and 1995 Lakes Entrance and 1995/96 Port Phillip Bay cohorts. Plots of mean otolith weight against fish length (Figure 7.11) show that, for fish of the same length, the 1994/95 and 1995 Port Phillip Bay had consistently heavier otoliths than both the 1995/96 Port Phillip Bay cohort and the 1994/95, 1995 Lakes Entrance cohort. However, the 1994/95, 1995 cohort from Lakes Entrance and the 1995/96 Port Phillip Bay cohort were very similar in this regard.

Precision of Age Estimates

Age determinations for the South Australian samples had a low degree of intra-reader variability. Over one third (35.2%) of the samples from Coffin Bay were re-aged, with an APE of 0.37%. All but four (97.8%) fish were assigned the same age for both estimates and the maximum difference between the estimates was 1 year (Table 7.2A and B). Almost one third (31.5%) of samples from Spencer Gulf were re-aged, with an APE of 0.71%. All but 10 of the 361 re-aged fish (97.2%) were assigned the same age for both estimates and the maximum difference between the estimates was also 1 year (Table 7.2A, B).

Age determinations for the Victorian samples had a reasonable degree of intra-reader variability. Almost one third (31.8%) of the samples from Port Phillip Bay were re-aged, with an APE of 19.06%. The maximum difference between the estimates was 1 year (Table 7.2C and D). From Lakes Entrance, 27.0% of the samples were re-aged, with an APE of 0.33%. Only 2 of the 163 fish re-aged (1.2%) from Lakes Entrance were not assigned the same age (Table 7.2C and D).

The Port Phillip Bay samples had a higher APE than the two South Australian locations or Lakes Entrance. This was due to an error in classification of members of the 1995/96 cohort caught after January 1996. On first reading, these immature fish were aged as 0+ fish (July birthday), despite a translucent zone near the edge. This zone was known to be sub-annual, as the growth of the cohort had been followed on the length frequency distributions. It showed advanced growth, reaching a larger

diameter at age 1 than other cohorts. At the second reading, the date of capture was not referred to, and the zone near the edge was incorrectly counted as the first increment.

Growth

Growth curves fitted to the data for female and male pilchards from South Australia were significantly different using Kimura's likelihood ratio test (χ^2=20.71, P=0.0001). However, the parameters were very similar (Table 7.3A, 4.4A), the two curves were almost congruent, and the predicted lengths at age for the two fitted curves differed by less than 0.4 mm for fish between 1 and 7 years old (Figure 7.12A). The mean length at age for each sex (Figure 7.14A) also showed a small difference between the sexes. Therefore, this difference, although statistically significant, was judged to be biologically unimportant and for subsequent analyses data for both sexes were combined.

Significant differences were also detected between the von Bertalanffy growth functions fitted to samples from Coffin Bay and Spencer Gulf (χ^2=229.4, P<0.00001) (Table 7.4A, Figure 7.13A).

Substantial variation in mean length at age is observed between years, particularly between 1995 and 1997, and also between areas. The plots of mean length at age show that fish of the same age were smaller at Spencer Gulf than they were at Coffin Bay (Figure 7.14A).

The von Bertalanffy growth functions for females and males from Victoria were found to be significantly different using Kimura's likelihood ratio test (χ^2=15.64, P=0.001). This difference persisted when the allocation of immature fish to each of the sexes was reversed. However, as for South Australian samples, the parameters are very similar (Table 7.3B and 7.4B), the two curves are almost congruent, and the predicted lengths at age for the two fitted curves differed at most by 1.5 cm for fish between 1 and 6 years (Figure 7.12B). The mean length at age for each sex (Figure 7.14B) also show the small difference between the sexes. As with pilchards from South Australia, this difference was judged to be biologically unimportant and data for both sexes were combined.

No growth curve was fitted to the data from Lakes Entrance because the samples contained too few large and small fish to adequately define a growth curve. The von Bertalanffy parameters were, however, calculated for pilchards caught in Port Phillip Bay (Table 7.4B; Figure 7.13B).

The variation in mean length at age between years (Figures 7.15B, 7.16), particularly for Port Phillip Bay between 1995 and 1996, is large compared to the differences between areas or sexes. In 1995, the only year when samples were collected from both Lakes Entrance and Port Phillip Bay, samples from

Port Phillip Bay were generally smaller than those from Lakes Entrance, except for age 1 fish, where fish from Lakes Entrance were smaller than those from Port Phillip Bay.

From the slope of the regressions of fish length and date of capture, the growth of the 1995/96 Port Phillip Bay cohort averaged 0.23 mm/day between November 1995 and September 1996, reaching 0.63 mm/day between November 1995 and January 1996. For the 1995 Lakes Entrance cohort, the growth rate between May and October 1995 averaged 0.52 mm/day (Figure 7.17).

Comparison with Western Australia

When sexes and areas are combined, comparisons of mean length at age between Victoria, South Australia and Western Australia (Figure 7.18) showed that for ages greater than 3 Victorian samples reached a greater length at age than both South Australian and Western Australian pilchards, and that South Australian pilchards reached greater lengths at age than Western Australian pilchardss. For ages greater than 3, the Victorian and South Australian pilchards also showed a greater mean otolith weight at age than pilchards from Western Australia (Figure 7.19).

7.3 Discussion

Choice of Method

Most ageing of pilchards in Australia has been conducted using rings on scales (e.g. Kesteven and Proctor 1941; Blackburn 1949, 1950; Joseph 1981). However, scales fail to grow in a regular fashion and can be influenced by the condition of the fish, i.e. spawning or poor condition may result in absorption of the scale edge, obliterating previous rings and resulting in an underestimation of age (Cassleman 1990). Pilchard scales are also extremely deciduous; they can lose nearly all of their scales while still in the net, making it impossible to collect scales from every fish sampled (Joseph 1981; Fletcher and Blight 1996).

Blackburn (1950) used scales to estimate the age of over 1 000 pilchards from Port Phillip Bay and Lakes Entrance and produced a maximum age of 6 years. Six years was also the greatest age recorded in New South Wales pilchards (Blackburn 1949). Blackburn (1950) also estimated the age and growth of pilchard from Tasmania, Coffin Bay and Western Australia. He found that the growth rate of pilchards was higher in New South Wales than Victoria. Of 72 pilchards from Tasmania, the majority were 5 or 6 years old and grew at similar rates to Victorian stocks. Scales were aged from 133 fish from Coffin Bay, producing a maximum age of 4. Scales of 322 pilchards from Western Australia, suggested a maximum age of 5 years and a growth rate similar to New South Wales pilchards. Otoliths were trialled at this time, but were found to be more difficult to read than scales (Blackburn 1949).

This was due to both the lack of clarity of secondary rings and the difficulties in determining the presence or absence of age-rings at the margin.

Using both otoliths and scales from the North American pilchards, a good to moderate agreement was found between the two age estimates for fish of up to three years (Walford and Mosher 1943). The same accessory and spawning rings seen on scales were also observed on otoliths. As the use of otoliths gained popularity, otoliths were examined from pilchards from the Great Australian Bight (Stevens *et al.* 1984). The maximum estimated age for this sample was 6 years, and although the sample size was small (n = 220), no detectable difference in length at age was found between the sexes. Fletcher (1991), using otoliths, assumed that age 6 was the oldest age class in the Western Australia population. However, following cage studies, further analysis of modal progressions and examination of growth zones from 1500 individuals, ages of up to 8 years were assumed (Fletcher 1995). After further otolith examination, the maximum estimated age for Western Australian pilchards was 8 years for males and 9 years for females (Fletcher and Blight 1996). Females were found to grow faster than males, attaining greater lengths in shorter periods. Fletcher (1991, 1995) found the assessment of ages to be subjective, questioned the accuracy of ages assigned and proceeded to use otolith weight for future analysis of the age structure.

While Fletcher and Blight (1996) obtained a poor rate of agreement among different readings of the otoliths, clupeoid studies elsewhere have recorded better rates of agreement, e.g. 94 % for *S. sagax (caerulea)* (Mosher and Eckles 1954), 82% for *S. sagax (ocellata)* (Baird 1970) and 91% for *Sardina pilchardus* (Morales-Nin and Pertierra 1990).

Patterns of Growth

The growth of pilchards in south-eastern Australia is variable and at times quite fast. In South Australia, the mean length of pilchards was over 12 cm at 1 year of age. In Port Phillip Bay the 1995/96 cohort reached a mean size of 14 cm LCF in approximately 12 months. Pilchards from Lakes Entrance showed similar rapid growth, with the 1995 cohort reaching a mean length of 12 cm LCF in 12 months. Pilchards from south-western Australia were reported to take up to two years to reach that size, at which time they are only starting to recruit to the fishery (Fletcher 1995). Pilchards from the Great Australian Bight have also been reported to also take 2 years to reach a mean length of 14.3 cm (Stevens *et al.* 1984). These and other studies of pilchards (e.g. Blackburn 1950) have not had the benefit of a time series of length frequency distributions upon which to assess the age and growth of immature fish, and may have overestimated the age of fish, particularly smaller ones.

Growth was variable between years and between areas both in South Australia and Victoria. The differences between areas in both states, at least partially, reflect the differences in the size range of fish collected in these different areas. Samples from Coffin Bay tended to include larger pilchards than those from Spencer Gulf, whereas samples from Port Phillip Bay often included only small fish. The variability in length-at-age of fish between years was similar to the variation between areas within years. This may be indicative of variation both in spawning times and growth rates, and was particularly evident among samples from Port Phillip Bay. In March 1995 the cohort of older fish had a mean length of 12.6 cm LCF and the second cohort which had entered the fishery had a mean length of 9.8 cm LCF. However, the single cohort present in March 1996 had a mean length of 11.7 cm LCF. The 1994/95 Port Phillip Bay cohort had significantly heavier otoliths than the 1994/95, 1995 cohorts of either Port Phillip Bay or Lakes Entrance, indicating it reached a given length in a longer time. Otoliths of the 1995 Lakes Entrance cohort were of similar weight-at-length to those of the 1995/96 Port Phillip Bay cohort, supporting the suggestion that this was also a relatively fast growing cohort. However, the growth of this cohort was out of phase with the 1995/96 Port Phillip Bay cohort, suggesting a different spawning time. The variability in growth among years in South Australia and the faster growth of the more recent cohorts from both Port Phillip Bay and Lakes Entrance remains unexplained.

Assessment of Methods

The calculated von Bertalanffy growth functions must still be regarded as preliminary because of the often restricted size range of pilchards in samples. Samples from Coffin Bay and Lakes Entrance frequently included only a few size classes, and samples from Spencer Gulf and Port Phillip Bay frequently included no large fish. These differences probably reflect size-related movements of pilchard from juvenile to adult habitats.

Annuli in pilchard otoliths are difficult to interpret consistently. In the samples examined, repeated reading produced results which exhibited both very low variability (APE values < 1% for South Australia and Lakes Entrance) and very high variability (an APE value of 19% for Port Phillip Bay). This difficulty is attributable to the frequent formation of sub-annual translucent zones in the otoliths. Similar difficulty has been reported for pilchards and other clupeid species from other areas of Australia and overseas. Fletcher and Blight (1996) found that younger age groups (<4 years) of pilchards from Western Australia showed evidence of multiple zones being formed annually, but not in all years or individuals, and multiple zones were not evident in older individuals (>3 years). Thomas (1983) found that the main translucent zone in pilchards off south west Africa was formed in late winter, but that a large number of translucent zones may form on the pilchard otolith at other

times of the year. He related formation of translucent zones to sea surface temperature and found prominent zones formed when temperatures were colder than average. More than one translucent zone is also not uncommon in other clupeid stocks (as referenced in Thomas 1983). Pawson (1990), studied the Libyan sardine, *Sardinella aurita* and found that "the major problem in determining the age of these sardines from the structure of their otoliths concern growth during the juvenile stage."

The level of variability in growth observed for pilchard in south-eastern Australia means that caution is required when attempting to age fish using otolith weight. Otolith weight may be able to serve as a proxy for age in Australian pilchards, however, it would be necessary to re-calibrate the relationship every couple of years as significant differences in growth were apparent between years (and also between areas). For example, the rapid growth of Port Phillip Bay pilchards is also reflected in the growth of their otoliths: the otolith weight of the young fish from both Port Phillip Bay and Lakes Entrance (the 1995/96 cohort) increased by over 1 mg/year. This is more rapid than the rate of of 0.22 mg/yr reported from south-western Australia (Fletcher 1995), but comparable with rate of 1 mg per year reported for otoliths of *S. sagax* from the Californian coast (Butler *et al.* 1996).

The benefits of using otolith weights to estimate age include a decreased handling time per otolith, but this needs to be balanced against an increase in the required sample size. Fletcher (1991, 1995) used otolith weight to analyse the age structure of pilchards from Western Australia, and acknowledged that it is "imprecise, with arbitrary cut-off values required to separate age classes". However, there is an additional uncertainty in the unknown and probably very variable level of accuracy that it provides. For fast growing cohorts, ages may be overestimated compared to slower-growing cohorts. Studies using fish of known age (Reznick *et al.* 1989; Secor and Dean 1989) have demostrated that the relationship between otolith weight and fish size is strongly influenced by the growth rate of the fish, and suggest that growth rate is not synchronous with the growth of the fish itself, but has an additional time-dependent rate which results in slow growing individuals having relatively heavy otoliths for their body size.

The subjectivity associated with the assignment of ages from otolith macrostructure is avoided by the otolith weight technique, however, the deterministic alternative does not easily accommodate the observed level of variability in growth. Thus, although the mathematical relationships between otolith size and fish length and age have indicated that age is explained principally in terms of otolith weight and fish length (Boehlert 1985), such techniques have a limited application in ageing fish from wild populations with highly variable growth rate (Pawson 1990).

Table 7.1 Pilchard otoliths submitted to the Central Ageing Facility (Marine and Freswater Research Institute, Victoria) for ageing.

Area	Year	Month	Samples	Area	Year	Month	Samples
Victoria				South Australia			
Lakes Entrance	1995	2	77	Coffin Bay	1995	5	22
		3	30			11	100
		4	80			12	74
		5	100		1996	1	20
		7	79			2	101
		8	84			3	20
		9	80			6	90
		10	75			7	50
		Subtotal	**605**			10	10
Port Phillip Bay	1994	12	60		1997	2	10
	1995	1	54			3	30
		2	100			Subtotal	**527**
		3	74	Spencer Gulf	1995	3	208
		4	74			4	19
		5	77			6	36
		11	76			7	28
		12	76			8	124
	1996	1	80			9	72
		2	28			10	30
		3	83		1996	3	70
		5	78			4	96
		6	75			6	10
		7	105			8	28
		9	74			10	22
		10	102			11	70
		11	101			12	109
		12	100		1997	1	56
	1997	1	77			2	128
		2	76			3	41
Victoria		Total	**1570**	South Australia		Total	**1147**

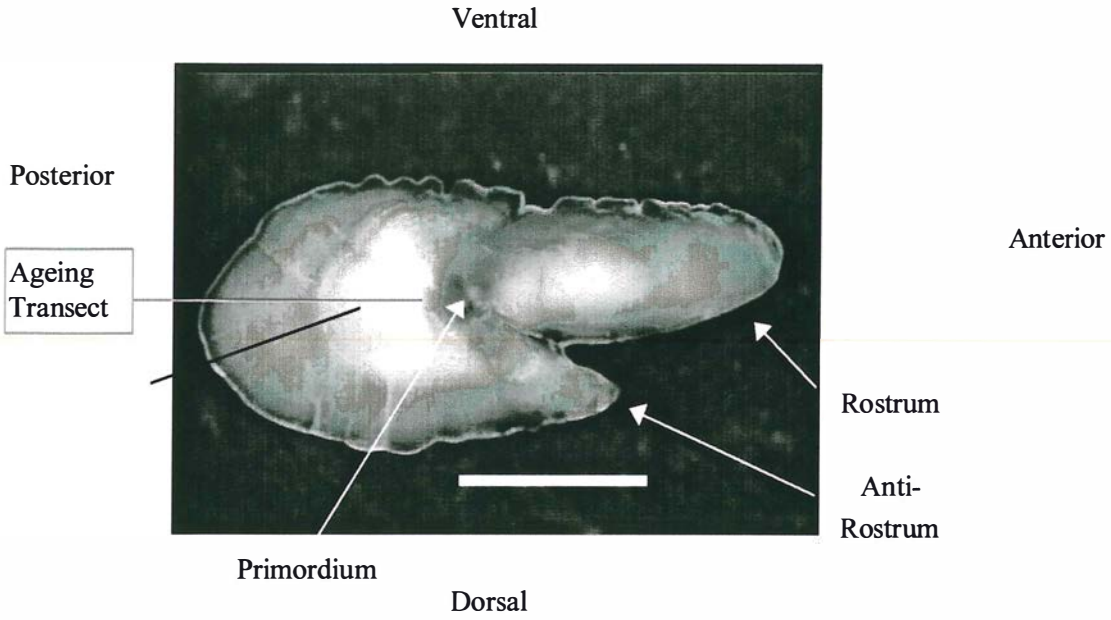


Figure 7.1 Left saggital otolith of a female pilchard (199 cm LCF, 74.1 g) caught off Lakes Entrance in August 1995, viewed with reflected light. The transect line along which measurements were taken and age estimates were made is shown (estimated age of 3 yrs). Scale bar = 1 mm.

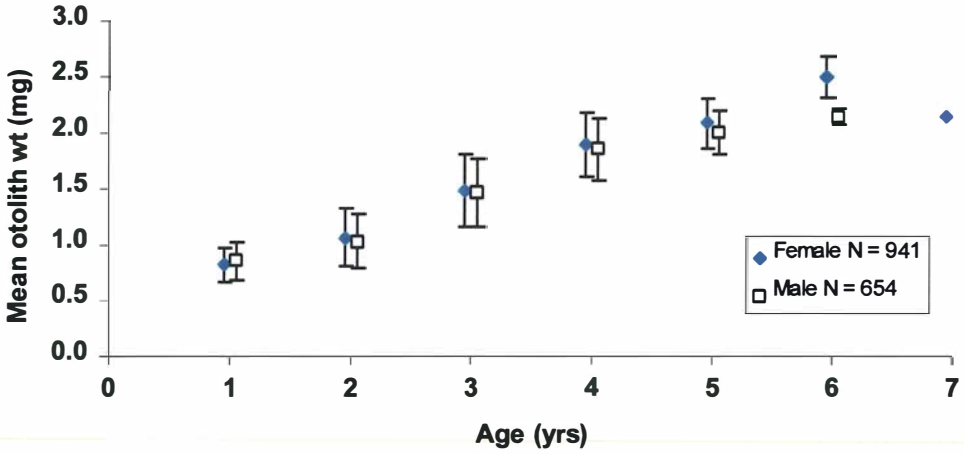


Figure 7.2A. Mean otolith weight (mg) (± 1 SD) at age (years) for female and male pilchards caught in South Australia between March 1995 and March 1997 (years and areas combined).

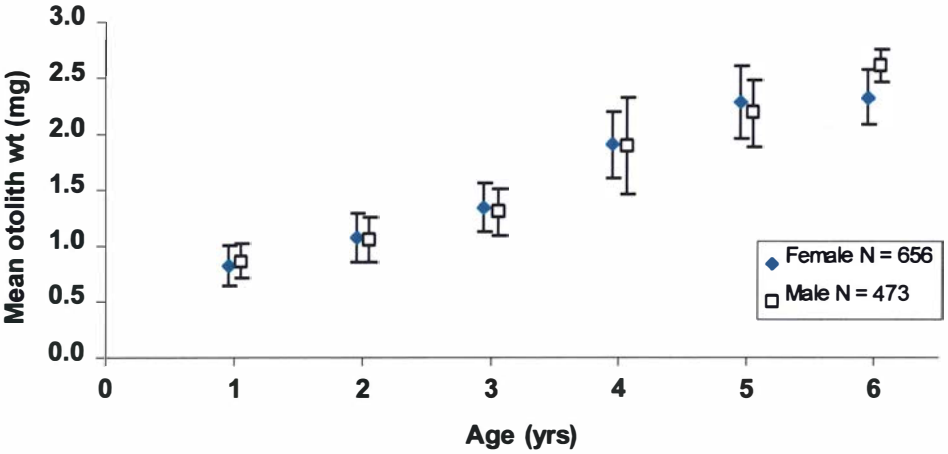


Figure 7.2B Mean otolith weight (mg) (± 1 SD) at age (years) for female and male pilchards caught in Victoria between December 1994 and February 1997 (years and areas combined).

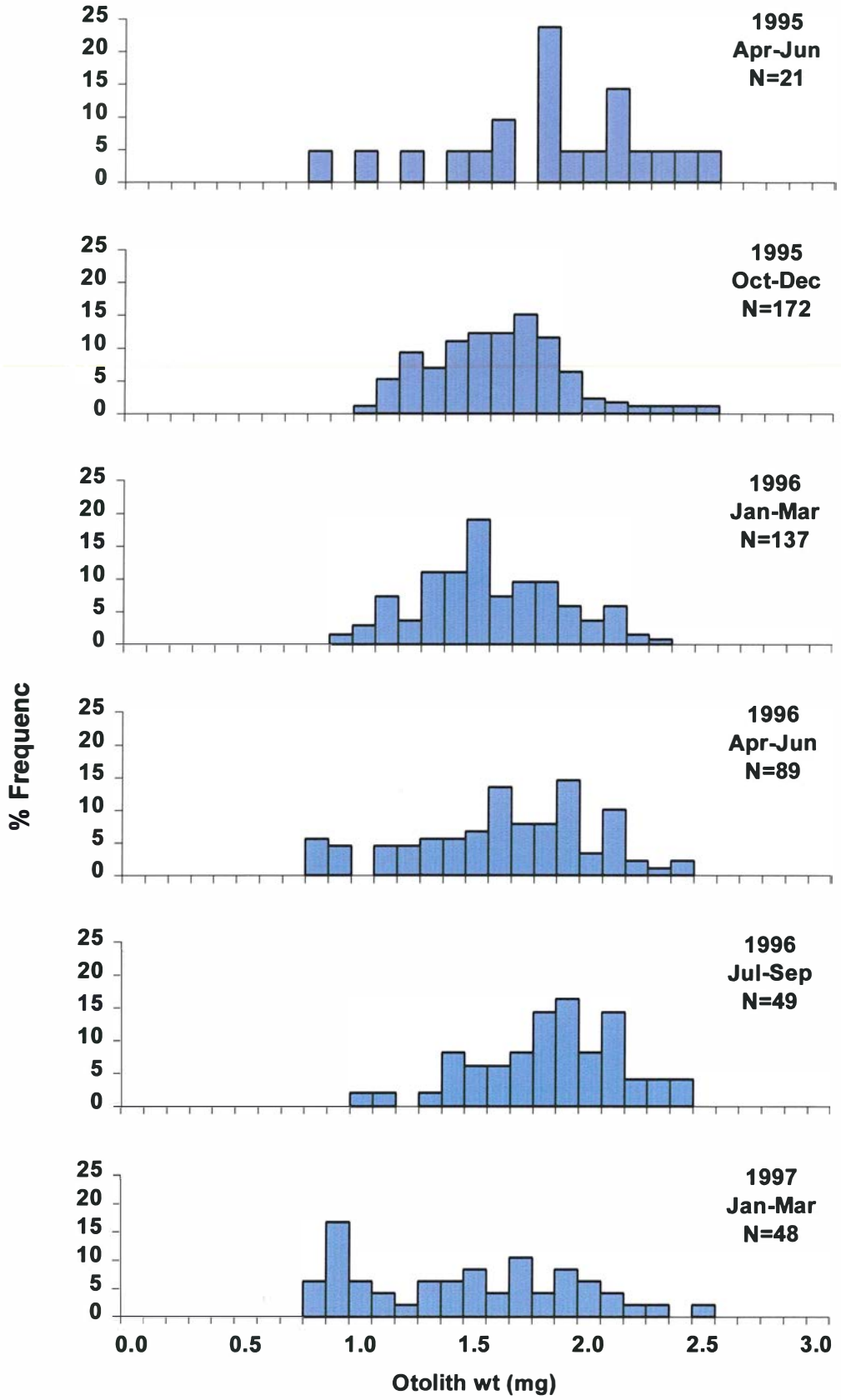


Figure 7.3A Otolith weight-frequency (%) distributions, by quarter, for pilchards caught in Coffin Bay (South Australia) between April 1995 and March 1997.

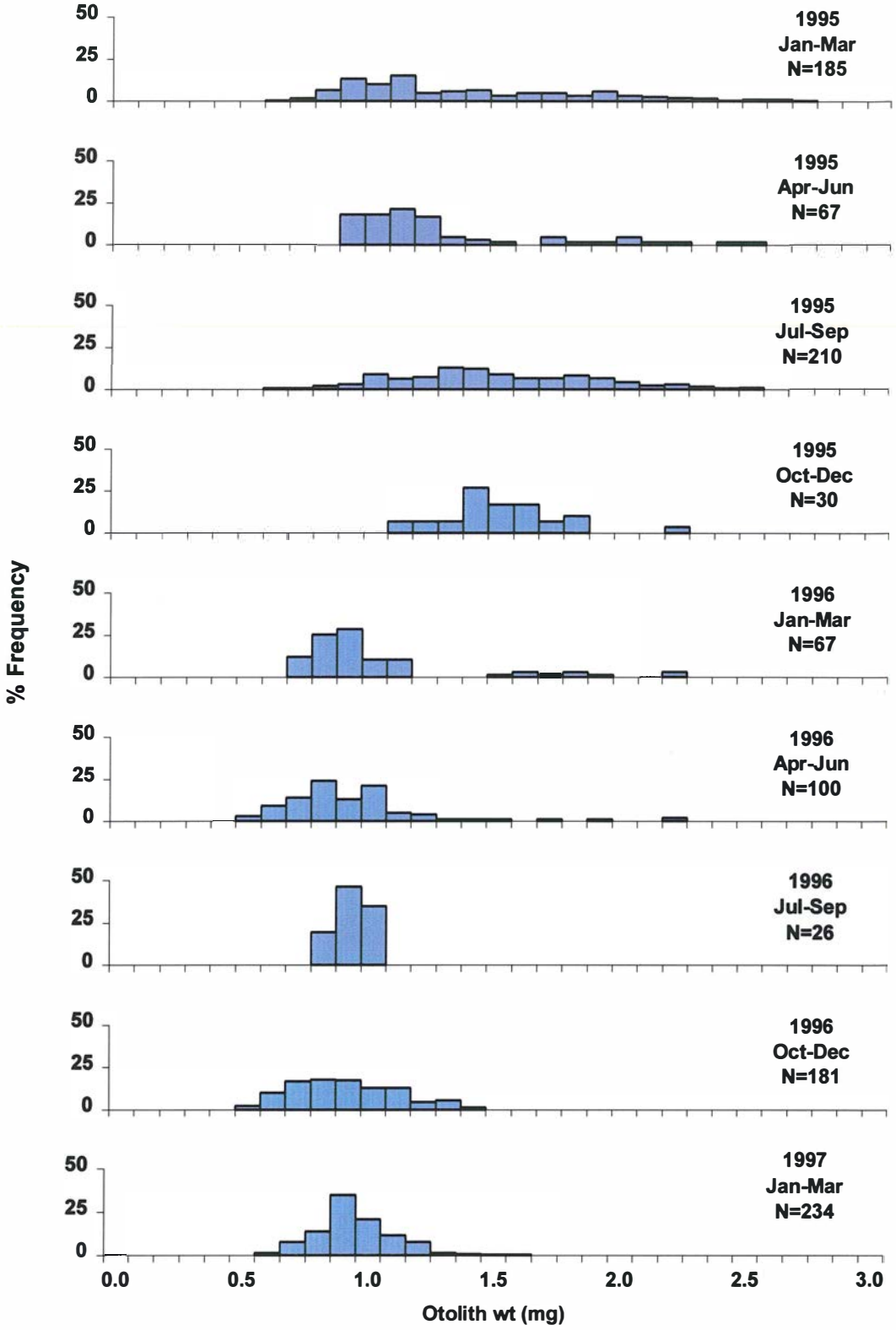


Figure 7.3B Otolith weight-frequency (%) distributions, by quarter, for pilchards caught in Spencer Gulf (South Australia) between January 1995 and March 1997.

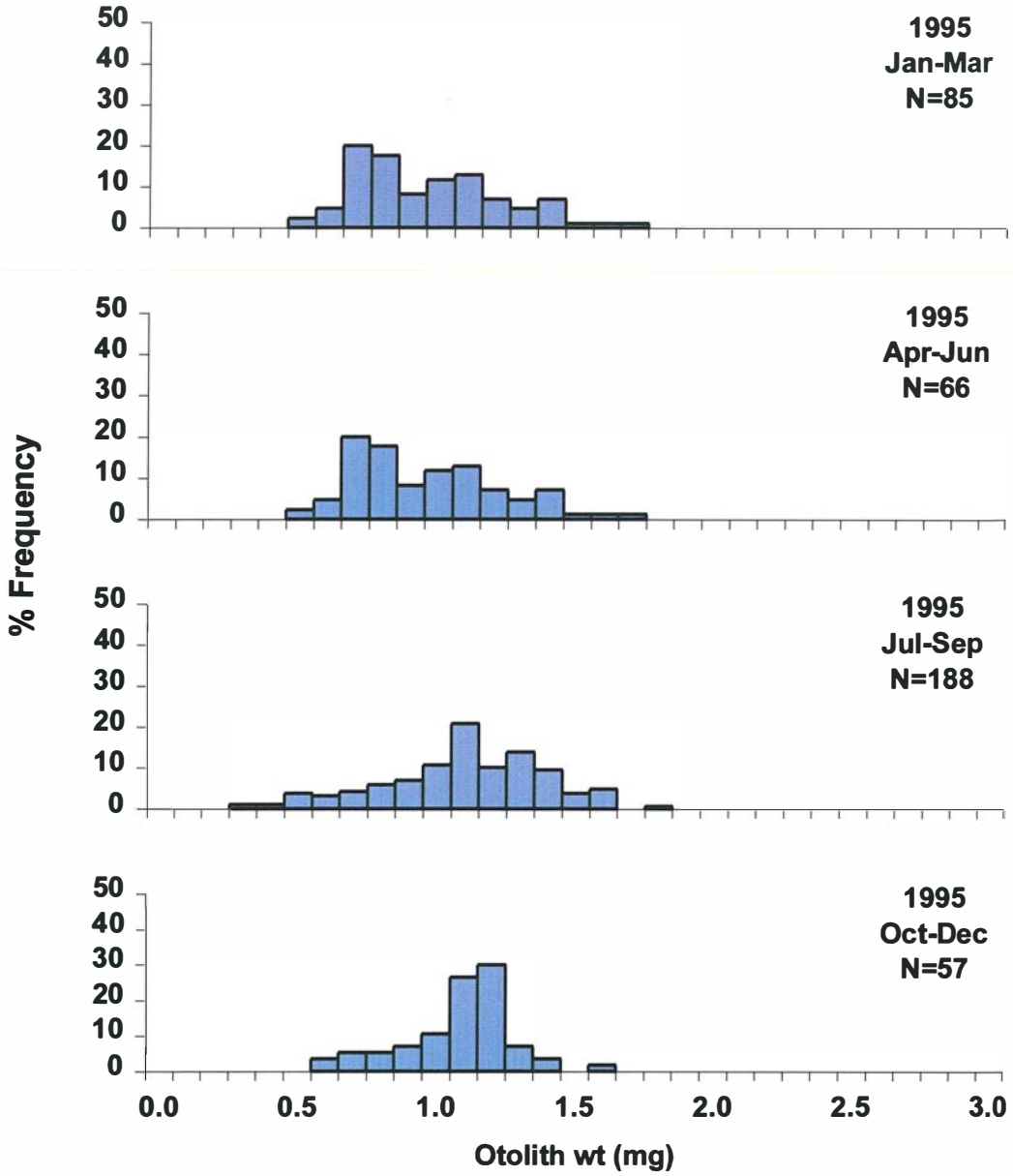


Figure 7.3C Otolith weight-frequency (%) distributions, by quarter, for pilchards caught in Lakes Entrance (Victoria) between January 1995 and December 1995.

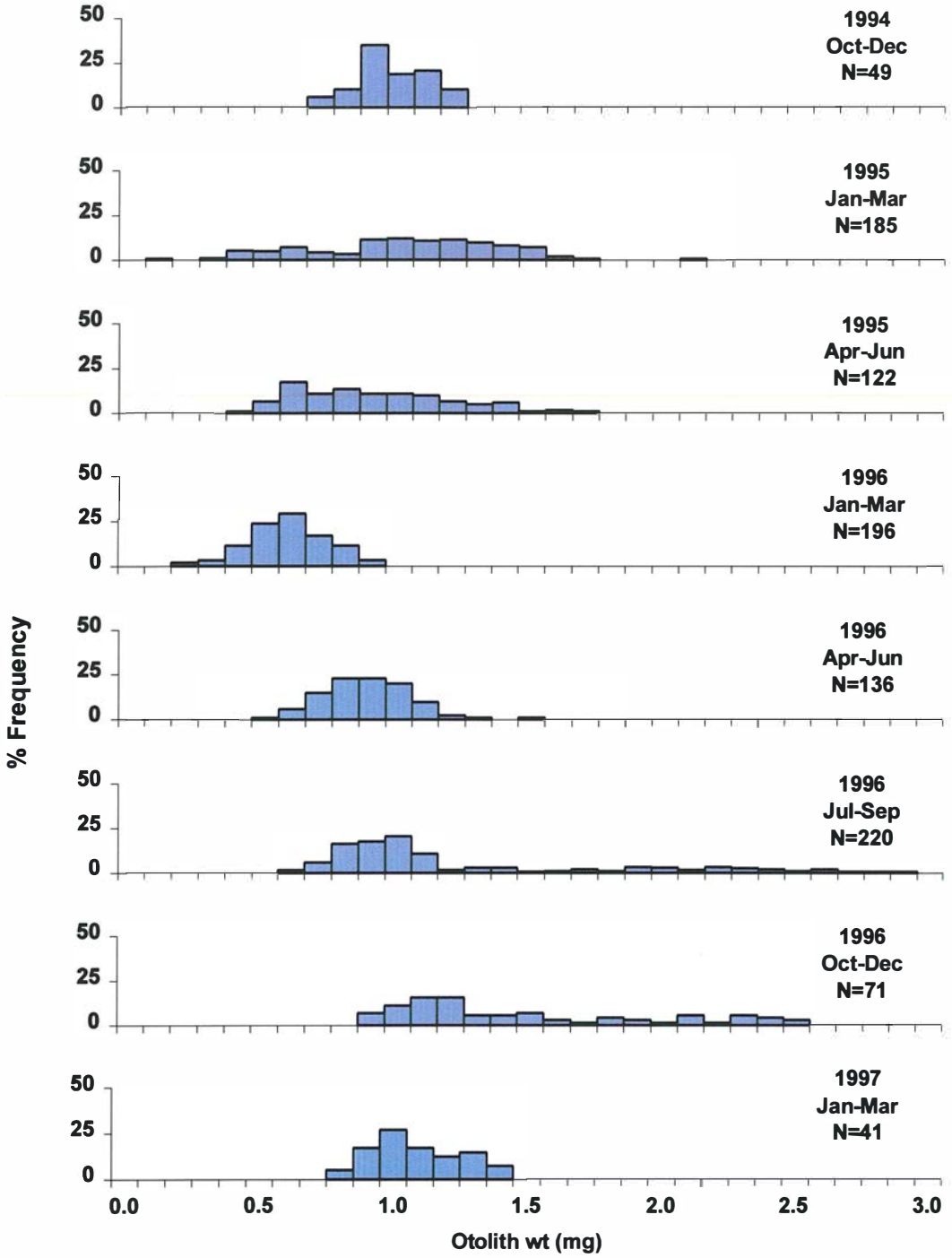


Figure 7.3D Otolith weight-frequency (%) distributions, by quarter, for pilchards caught in Port Phillip Bay (Victoria) between December 1994 and March 1997.

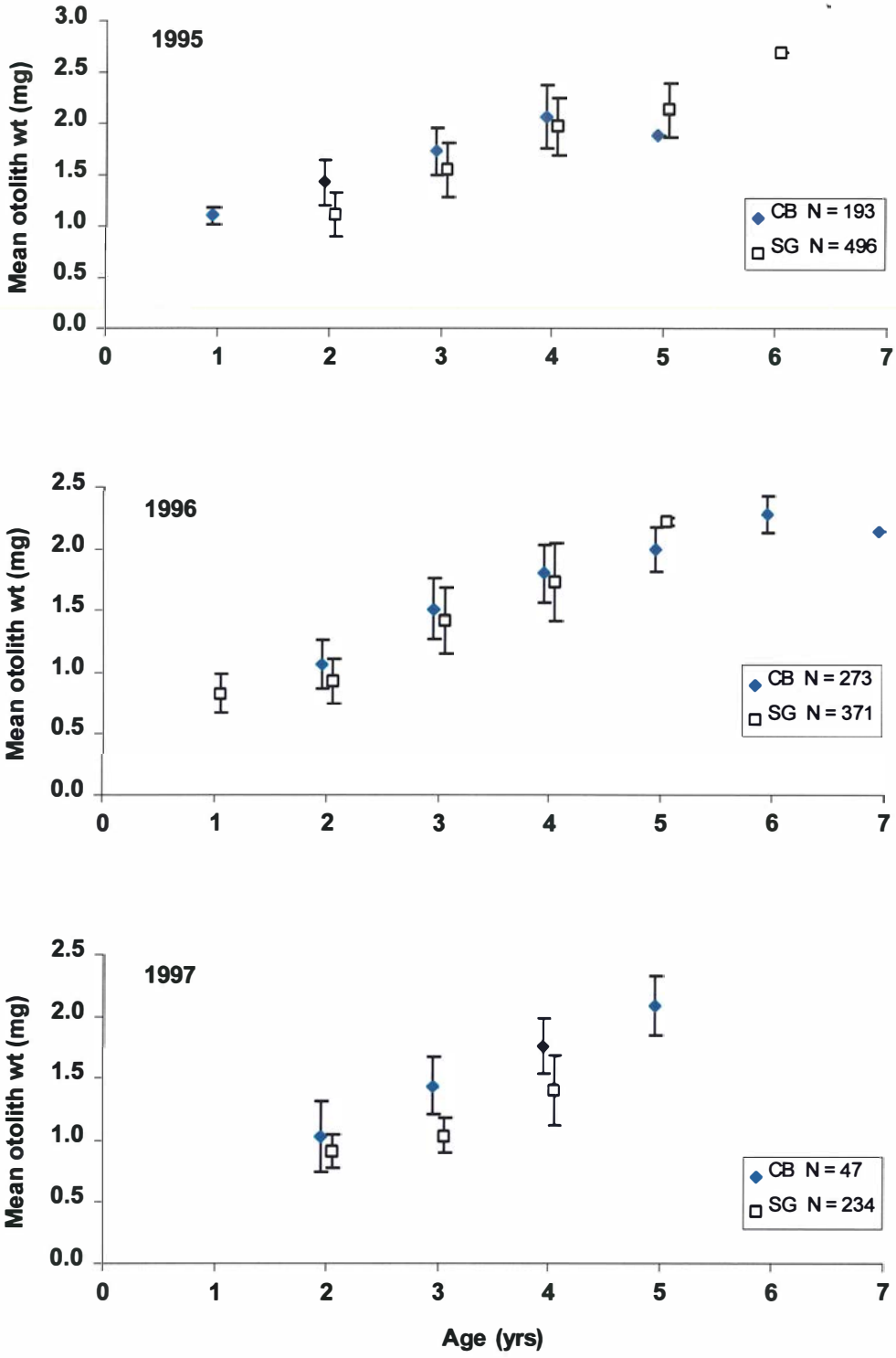


Figure 7.4A Mean otolith weight (mg) (± 1 SD) at age (years), by year, for pilchards caught in Coffin Bay (CB) and Spencer Gulf (SG) (South Australia) between March 1995 and March 1997 (sexes combined).

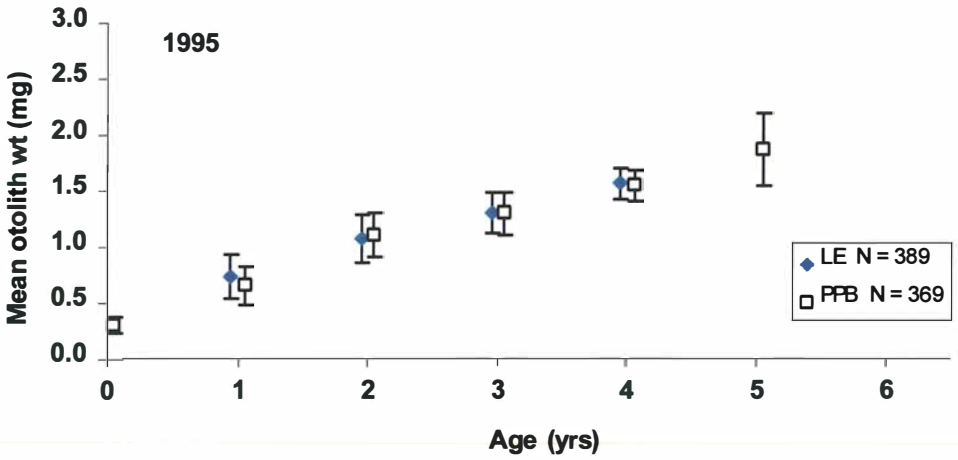


Figure 7.4B Mean otolith weight (mg) (± 1 SD) at age (years) for pilchards caught in Lakes Entrance (LE) and Port Phillip Bay (PPB) (Victoria) in 1995 (sexes combined).

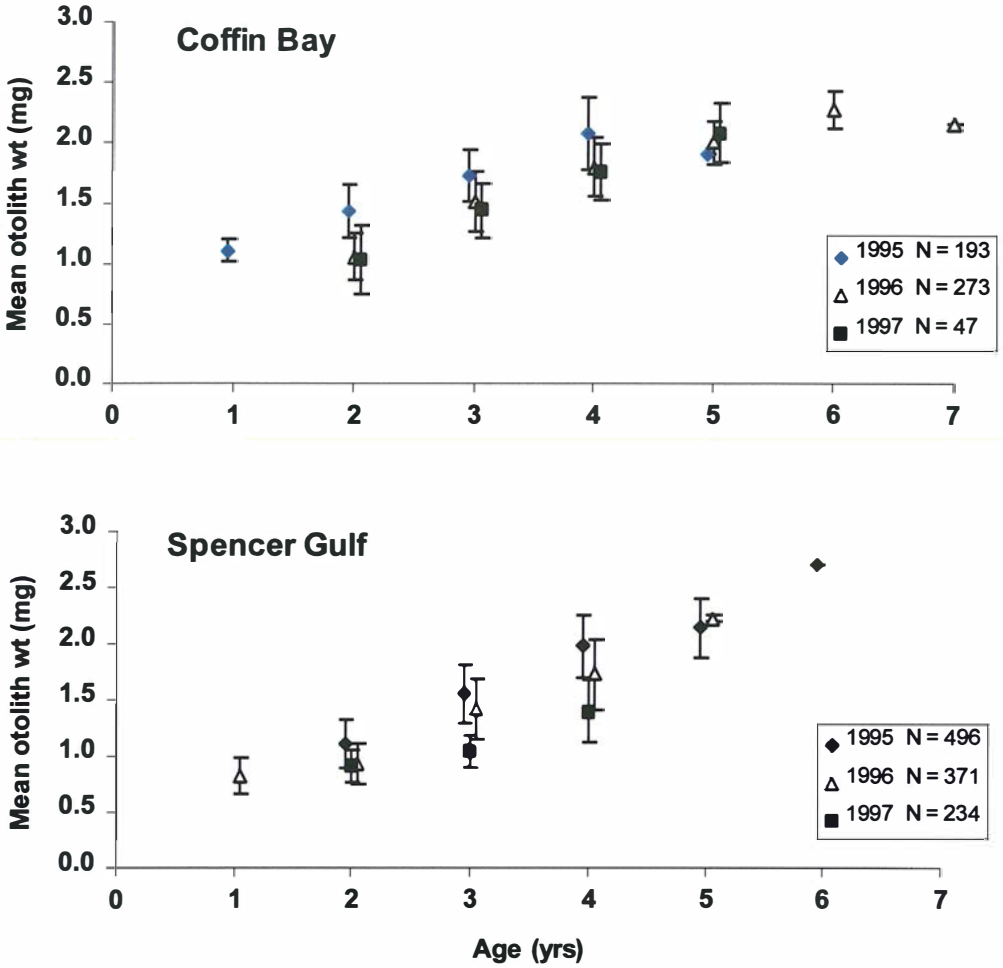


Figure 7.5A Mean otolith weight (mg) (± 1 SD) at age (years), by year, for pilchards caught in Coffin Bay (CB) and Spencer Gulf (SG) (South Australia) between March 1995 and March 1997 (sexes combined).

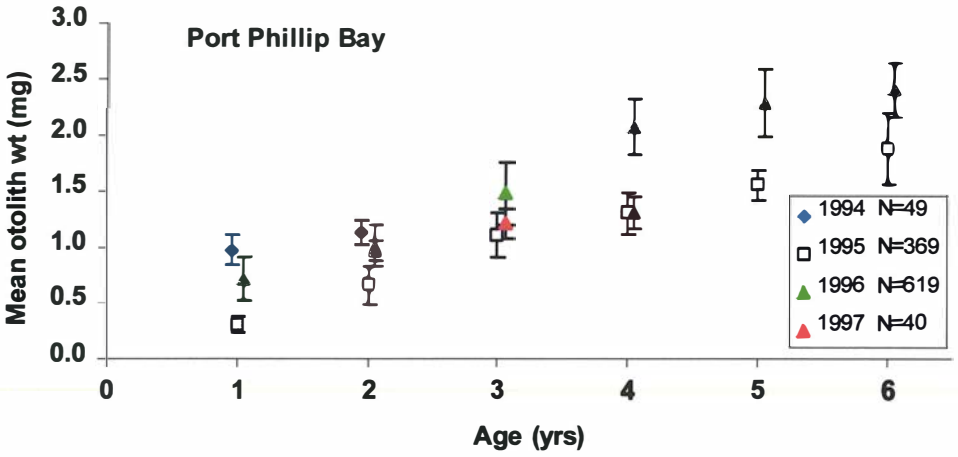


Figure 7.5B Mean otolith weight (mg) (± 1 SD) at age (years), by year, for pilchards caught in Port Phillip Bay (Victoria) between December 1994 and February 1997 (sexes combined).

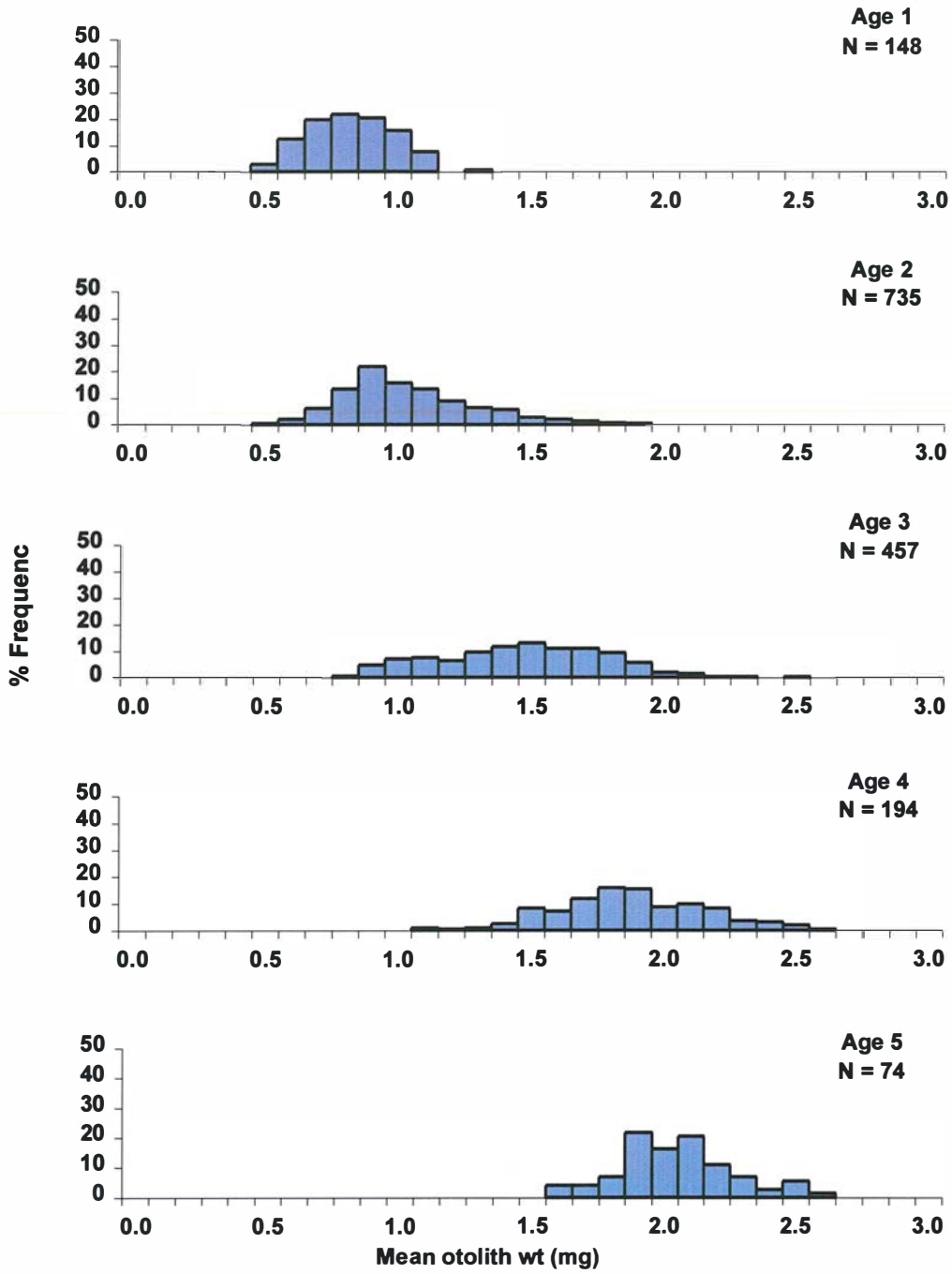


Figure 7.6A Otolith weight-frequency distributions (%), by age class, for pilchards caught in South Australia between March 1995 and March 1997 (areas combined).

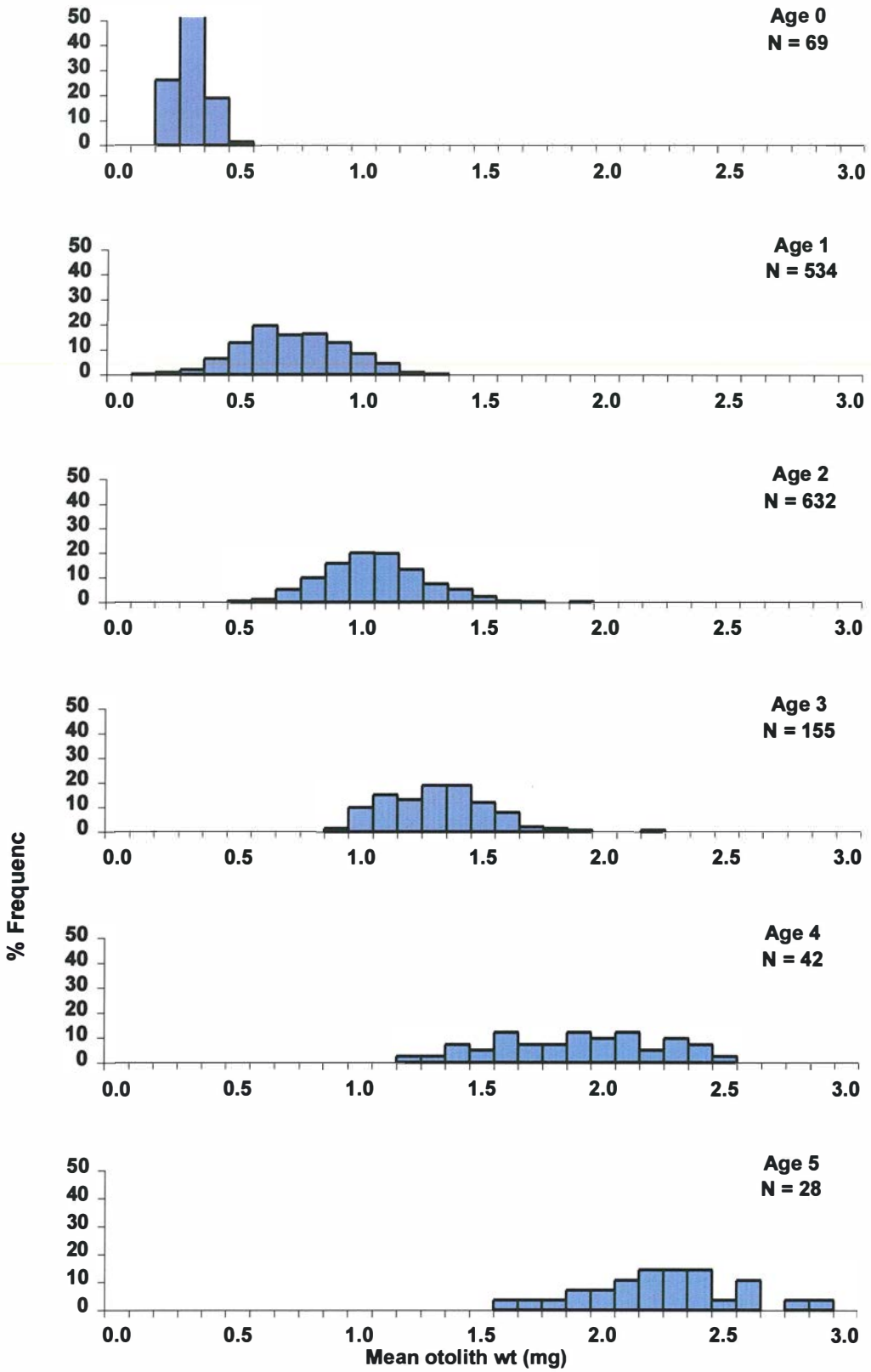


Figure 7.6B Otolith weight-frequency distributions (%), by age class, for pilchards caught in Victoria between December 1994 and February 1997 (areas combined).

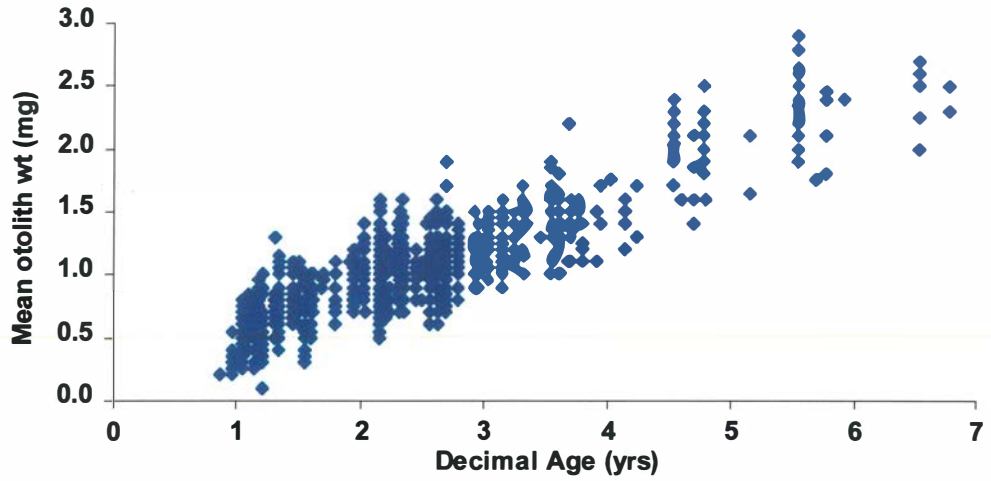


Figure 7.7A Mean otolith weight (mg) at decimal age (years) for pilchards caught in South Australia between March 1995 and March 1997 (years and sexes combined).

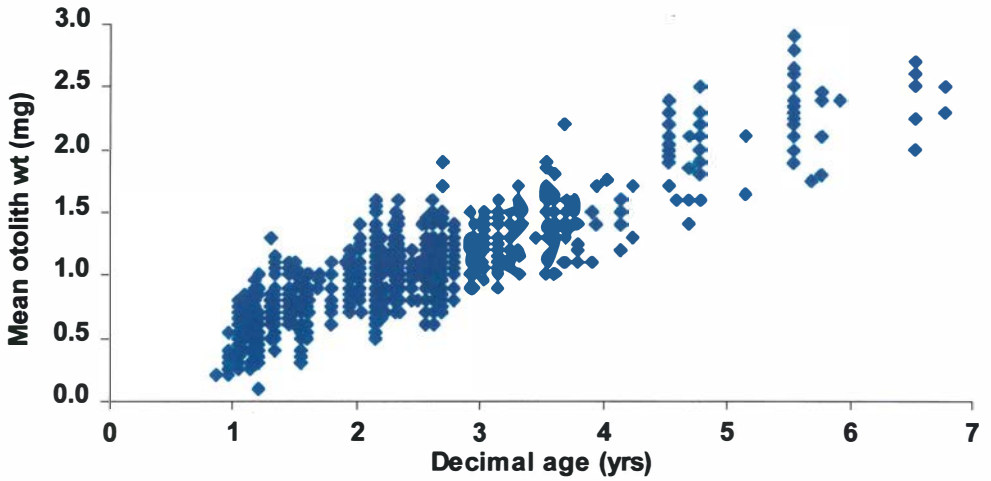


Figure 7.7B Mean otolith weight (mg) at decimal age (years) for pilchards caught in Victoria between December 1994 and February 1997 (years and sexes combined).

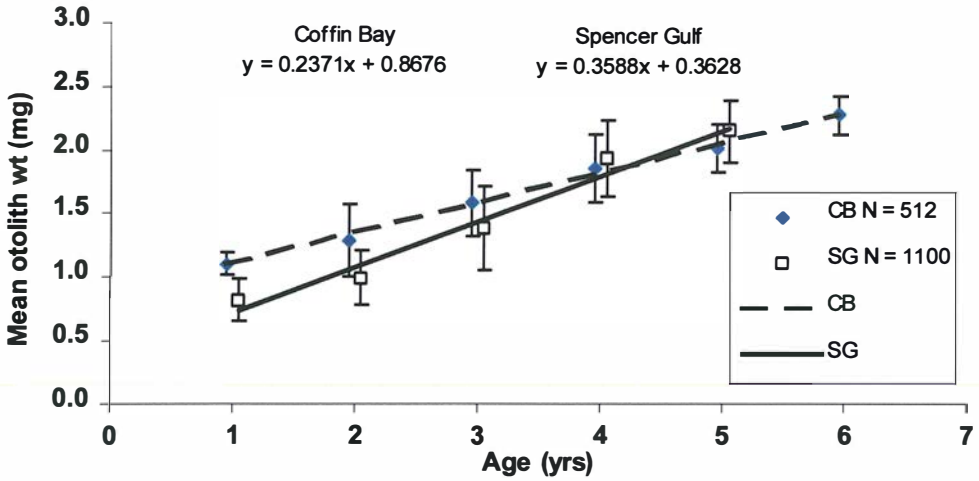


Figure 7.8A Mean otolith weight (mg) (± 1 SE) at age (years) for pilchards caught in Coffin Bay (CB) and Spencer Gulf (SG) (South Australia) between March 1995 and March 1997 (years and sexes combined).

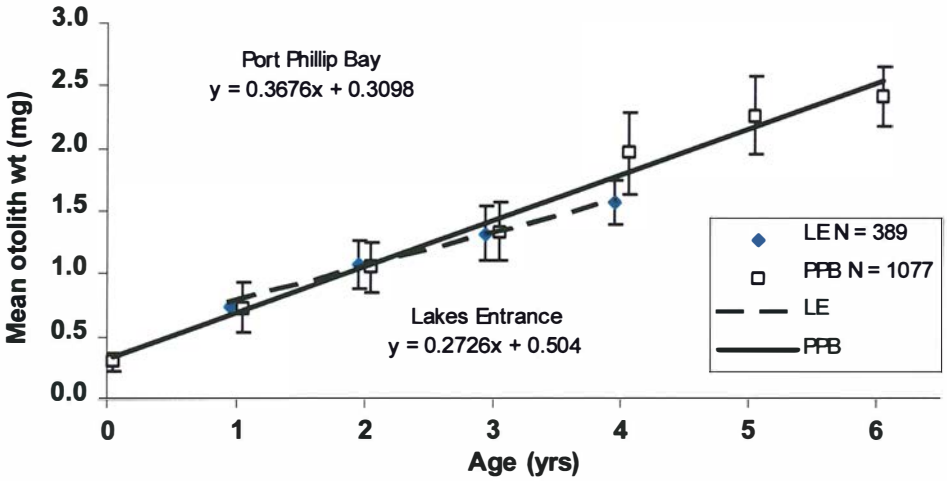


Figure 7.8B Mean otolith weight (mg) (± 1 SE) at age (years) for pilchards caught in Lakes Entrance (LE) and Port Phillip Bay (PPB) (Victoria) between December 1994 and February 1997 (years and sexes combined).

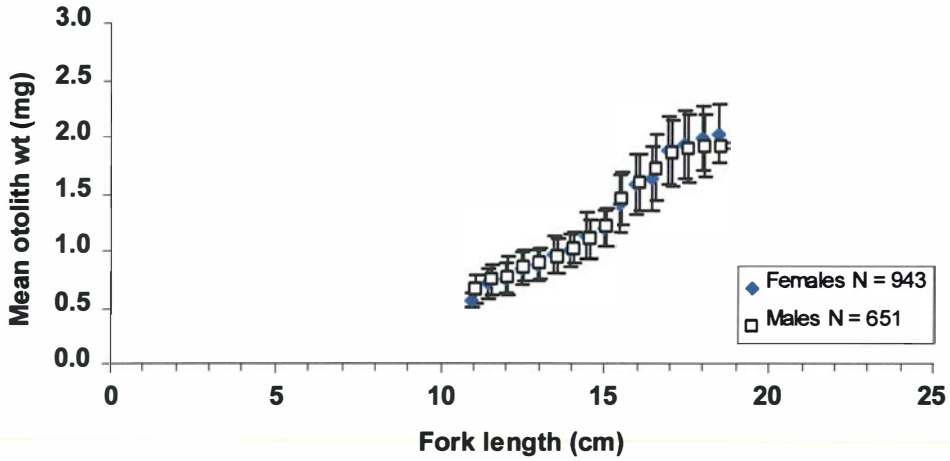


Figure 7.9A Mean otolith weight (mg) (± 1 SD) at length (LCF, cm) for female and male pilchards caught in South Australia between March 1995 and March 1997 (areas and years combined).

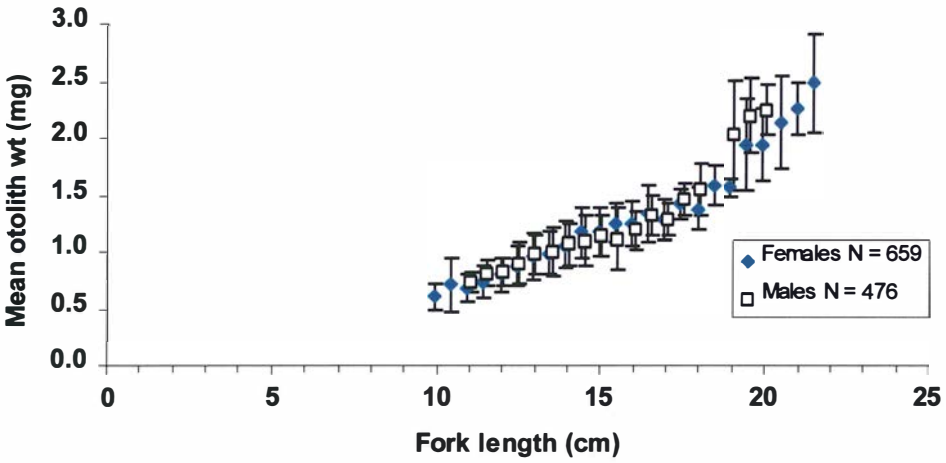


Figure 7.9B Mean otolith weight (mg) (± 1 SD) at length (LCF, cm) for female and male pilchards caught in Victoria between December 1994 and February 1997 (areas and years combined).

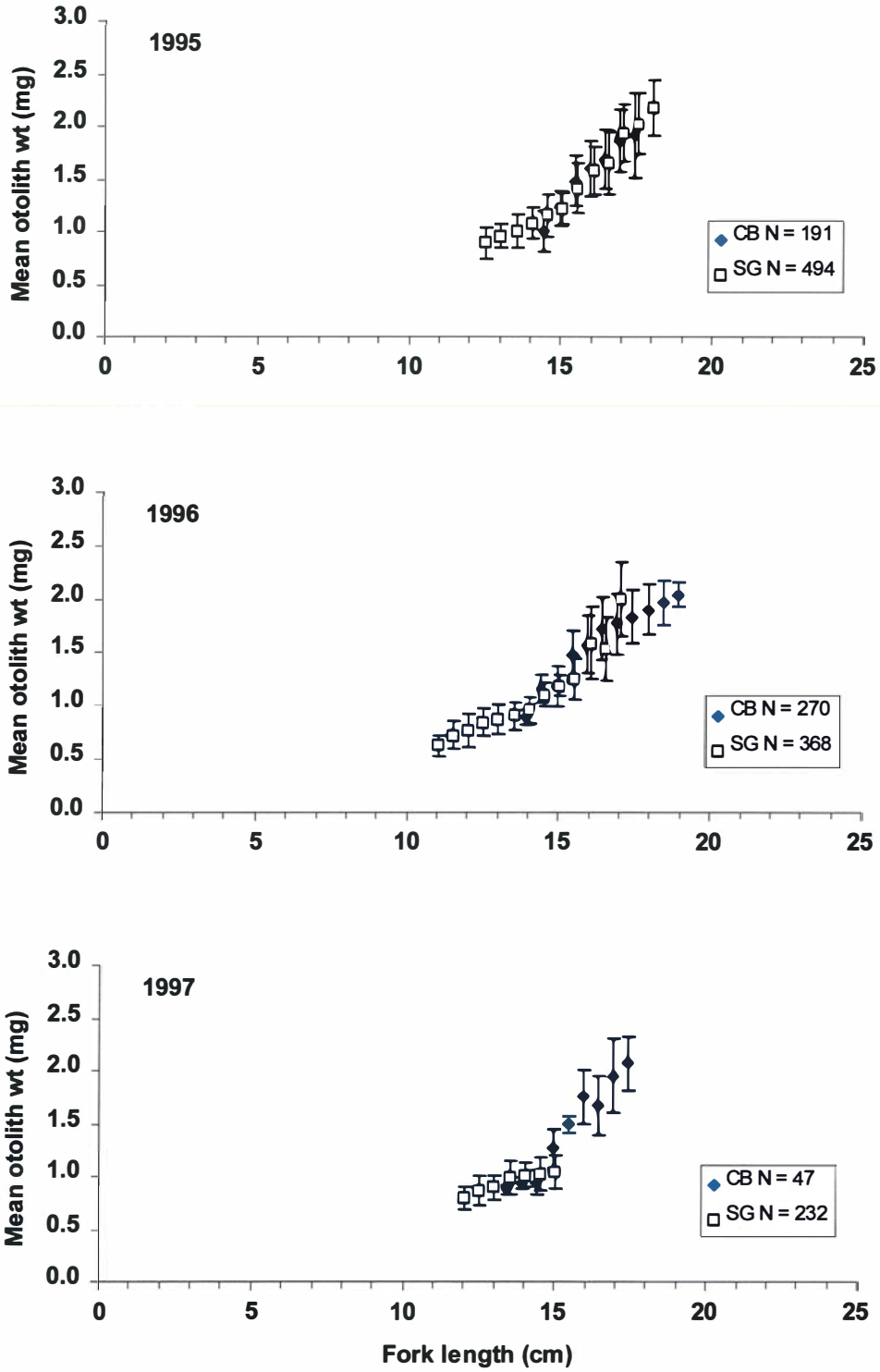


Figure 7.10A Mean otolith weight (mg) (± 1 SD) at length (LCF, cm), by year, for pilchards caught in Coffin Bay (CB) and Spencer Gulf (SG) (South Australia) between March 1995 and March 1997 (sexes combined).

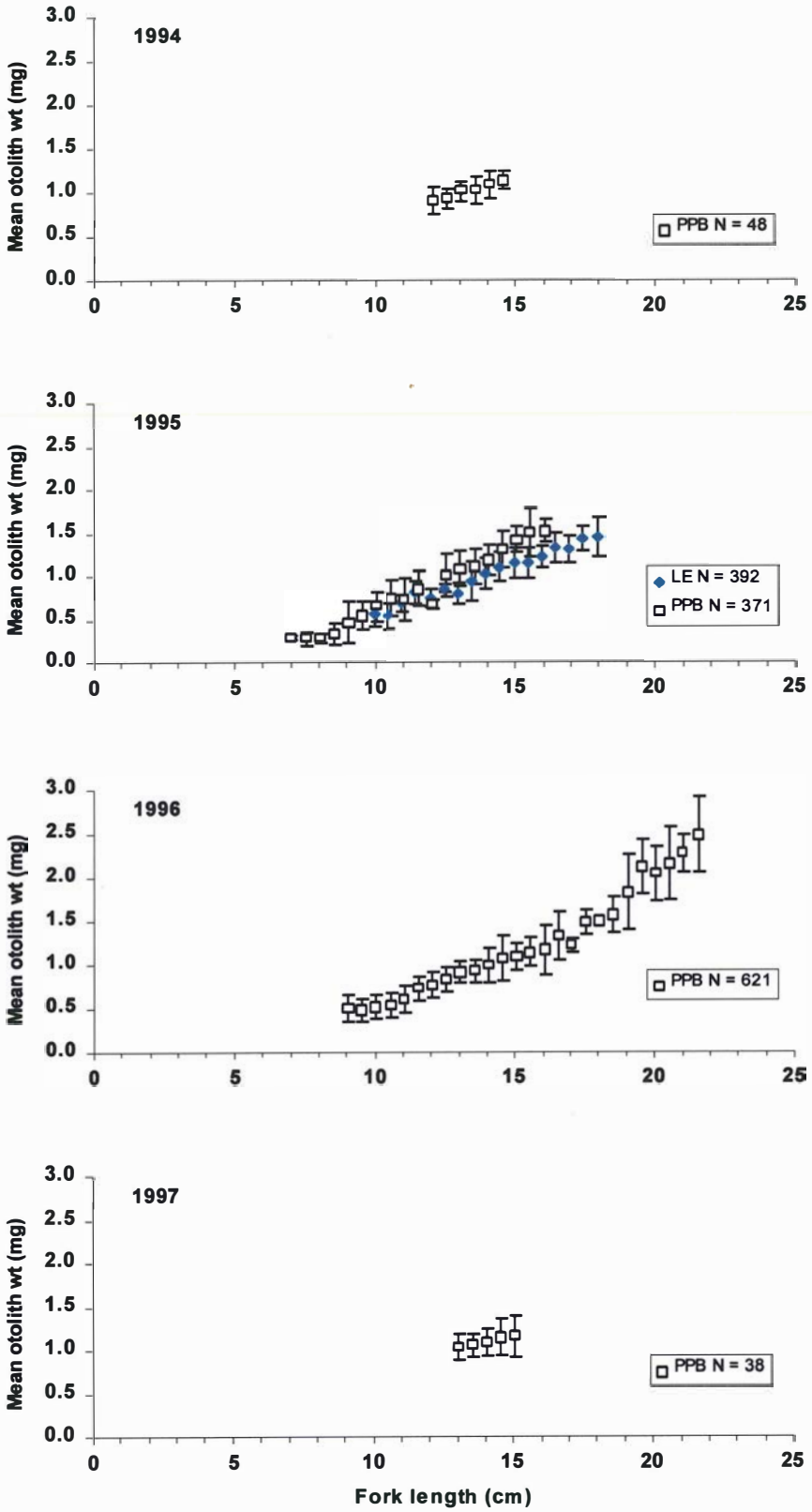


Figure 7.10B Mean otolith weight (mg) (± 1 SD) at length (LCF, cm), by year, for pilchards caught in Lakes Entrance (LE) and Port Phillip Bay (PPB) (Victoria) between December 1994 and February 1997 (sexes combined).

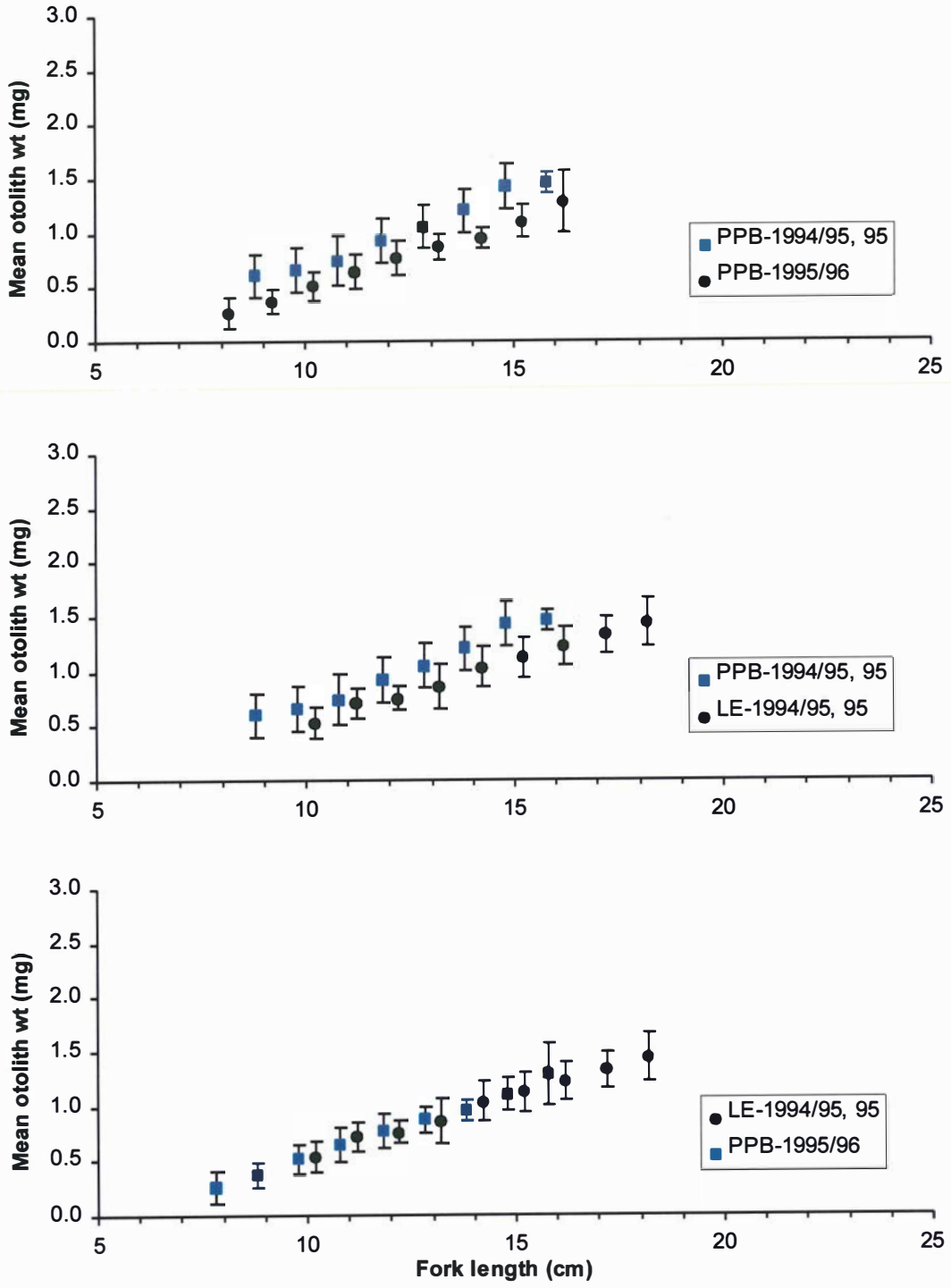


Figure 7.11 Mean otolith weight (mg) (± 1 SD) at length (LCF, cm), for selected cohorts of pilchards caught in Lakes Entrance (LE) and Port Phillip Bay (PPB) (Victoria) between December 1994 and February 1997 (sexes combined).

Table 7.2A Age differences for pilchards caught in Coffin Bay between May 1995 and March 1997.

N	Initial Age estimate (A1)					
Diff (A1-A2)	1	2	3	4	5	Grand Total
-1	0	2	0	0	0	2
0	17	76	51	33	5	182
1	0	0	0	2	0	2
Grand Total	17	78	51	35	5	186

Table 7.2B Age differences for pilchards caught in Spencer Gulf between March 1995 and March 1997.

N	Initial Age estimate (A1)					
Diff (A1-A2)	1	2	3	4	5	Grand Total
-1	3	1	1	0	0	5
0	168	108	52	19	4	351
1	0	2	2	1	0	5
Grand Total	171	111	55	20	4	361

Table 7.2C Age differences for pilchards caught in Port Phillip Bay between December 1994 and February 1997.

N	Initial Age estimate (A1)							
Diff (A1-A2)	0	1	2	3	4	5	6	Grand Total
-1	98	14	0	0	0	1	0	113
0	56	177	139	19	23	9	3	426
1	0	0	0	1	1	0	0	2
Grand Total	154	191	139	20	24	10	3	541

Table 7.2D Age differences for pilchards caught in Lakes Entrance between February 1995 and October 1995.

N	Initial Age estimate (A1)					
Diff (A1-A2)	0	1	2	3	4	Grand Total
-1	0	1	0	0	0	1
0	22	50	65	22	2	161
1	0	0	0	1	0	1
Grand Total	22	51	65	23	2	163

Table 7.3A Parameters of the von Bertalanffy growth function fitted to length and age data for female (F) and male (M) pilchards caught in Spencer Gulf and Coffin Bay (South Australia), between March 1995 and March 1997.

Sex	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
				Lower	Upper
F	L ∞	18.40	0.204	18.00	18.80
	K	0.609	0.047	0.517	0.710
	T zero	-0.043	0.126	-0.290	0.204

Sex	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
				Lower	Upper
M	L ∞	18.81	0.439	17.95	19.67
	K	0.469	0.060	0.352	0.586
	T zero	-0.537	0.227	-0.983	-0.091

Sex	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
				Lower	Upper
F and M	L ∞	18.58	0.198	18.19	18.97
	K	0.546	0.036	0.475	0.617
	T zero	-0.233	0.113	-0.454	-0.011

Table 7.3B Parameters of the von Bertalanffy growth function fitted to pilchard length and age data for female (F), male (M) and immature (I) pilchards caught in Lakes Entrance and Port Phillip Bay (Victoria) between December 1994 and February 1997.

Sex	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
				Lower	Upper
F and ½ I	L ∞	24.12	1.076	22.01	26.23
	K	0.287	0.032	0.225	0.349
	T zero	-0.736	0.113	-0.957	-0.514

Sex	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
				Lower	Upper
M and ½ I	L ∞	21.20	0.901	19.43	22.97
	K	0.379	0.045	0.292	0.467
	T zero	-0.483	0.112	-0.702	-0.263

Sex	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
				Lower	Upper
F, M and I	L ∞	22.96	0.724	21.54	24.38
	K	0.318	0.026	0.267	0.368
	T zero	- 0.637	0.080	-0.794	-0.479

Table 7.4A Parameters of the von Bertalanffy growth function fitted to length and age data for female (F) and male (M) pilchards caught in Coffin Bay and Spencer Gulf (South Australia) between March 1995 and March 1997.

Coffin Bay

Sex	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
				Lower	Upper
F and M	$L\infty$	18.67	0.385	17.92	19.43
	K	0.440	0.081	0.280	0.600
	T zero	-1.380	0.538	-1.436	-0.324

Spencer Gulf

Sex	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
				Lower	Upper
F and M	$L\infty$	19.53	0.561	18.43	20.63
	K	0.372	0.048	0.278	0.466
	T zero	-0.973	0.242	-1.447	-0.499

Table 7.4B Parameters of the von Bertalanffy growth function fitted to length and age data for pilchards caught in Port Phillip Bay (Victoria) between December 1994 and February 1997.

Sex	Parameter	Estimate	Asymptotic SE	Asymptotic 95% CI	
				Lower	Upper
F, M and I	$L\infty$	24.61	1.035	22.58	26.64
	K	0.237	0.024	0.191	0.284
	T zero	-1.189	0.116	-1.416	-0.961

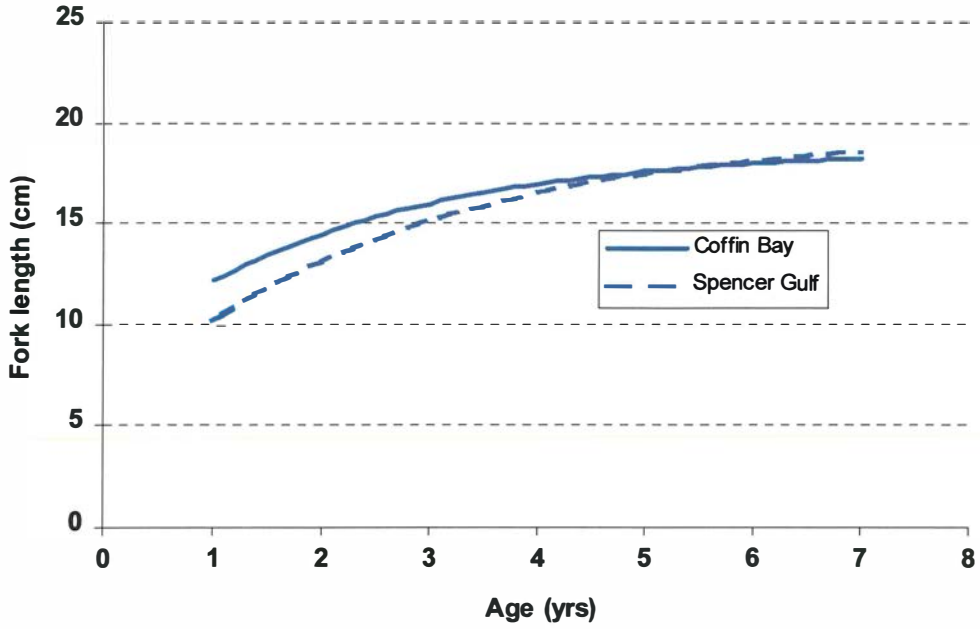


Figure 7.13A Growth curves for pilchards caught in Coffin Bay and Spencer Gulf (South Australia) between March 1995 and March 1997 (sexes combined).

Figure 7.12B Growth curves fitted separately to data for Victorian females and ½ immatures versus males and ½ immatures, showing almost complete congruity of the two curves (areas combined).

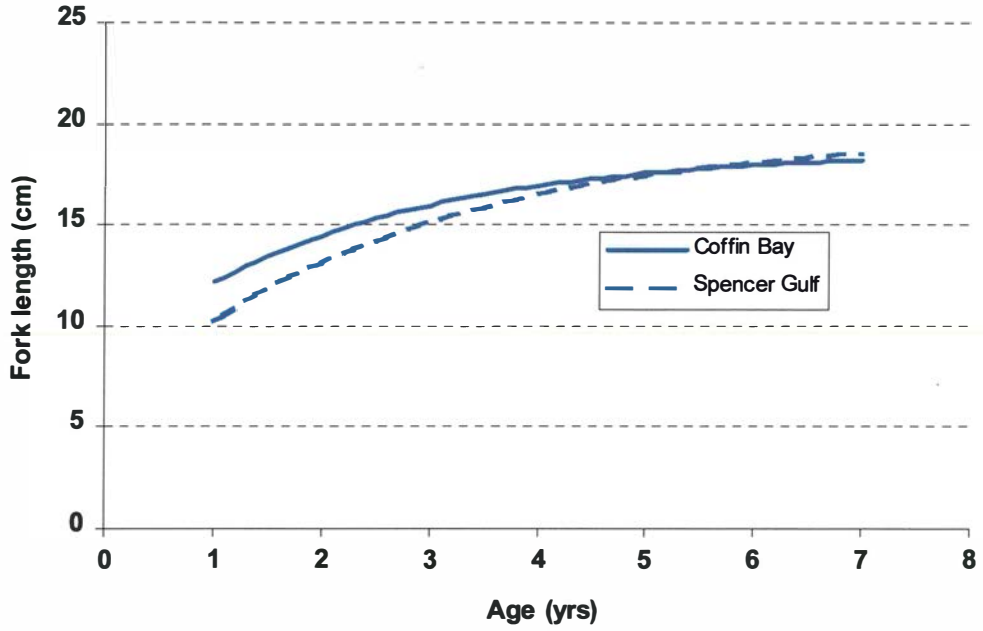


Figure 7.13A Growth curves for pilchards caught in Coffin Bay and Spencer Gulf (South Australia) between March 1995 and March 1997 (sexes combined).

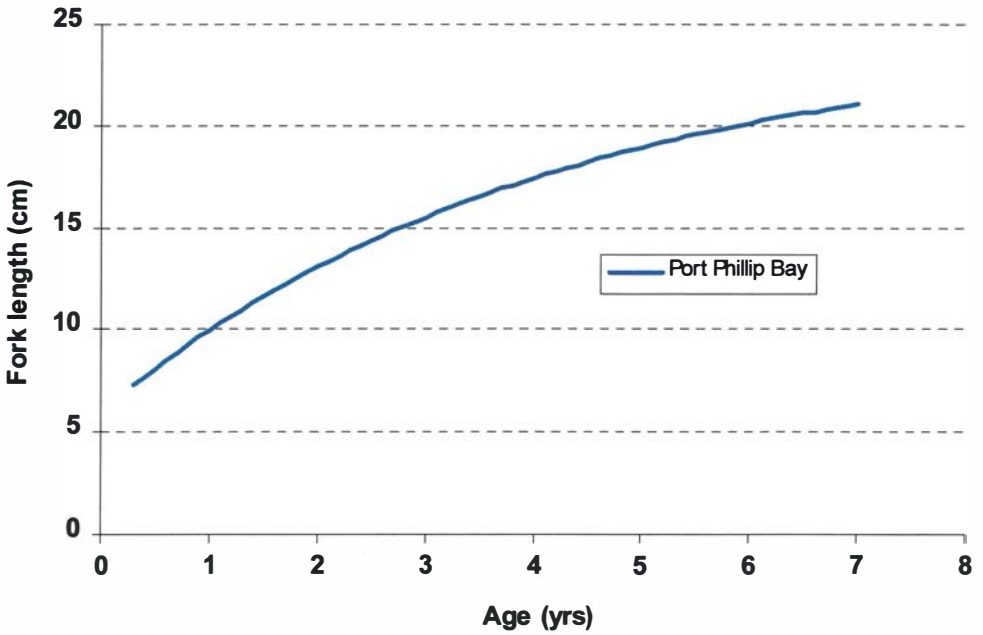


Figure 7.13B Growth curve for pilchards caught in Port Phillip Bay (Victoria) between December 1994 and February 1997 (sexes combined).

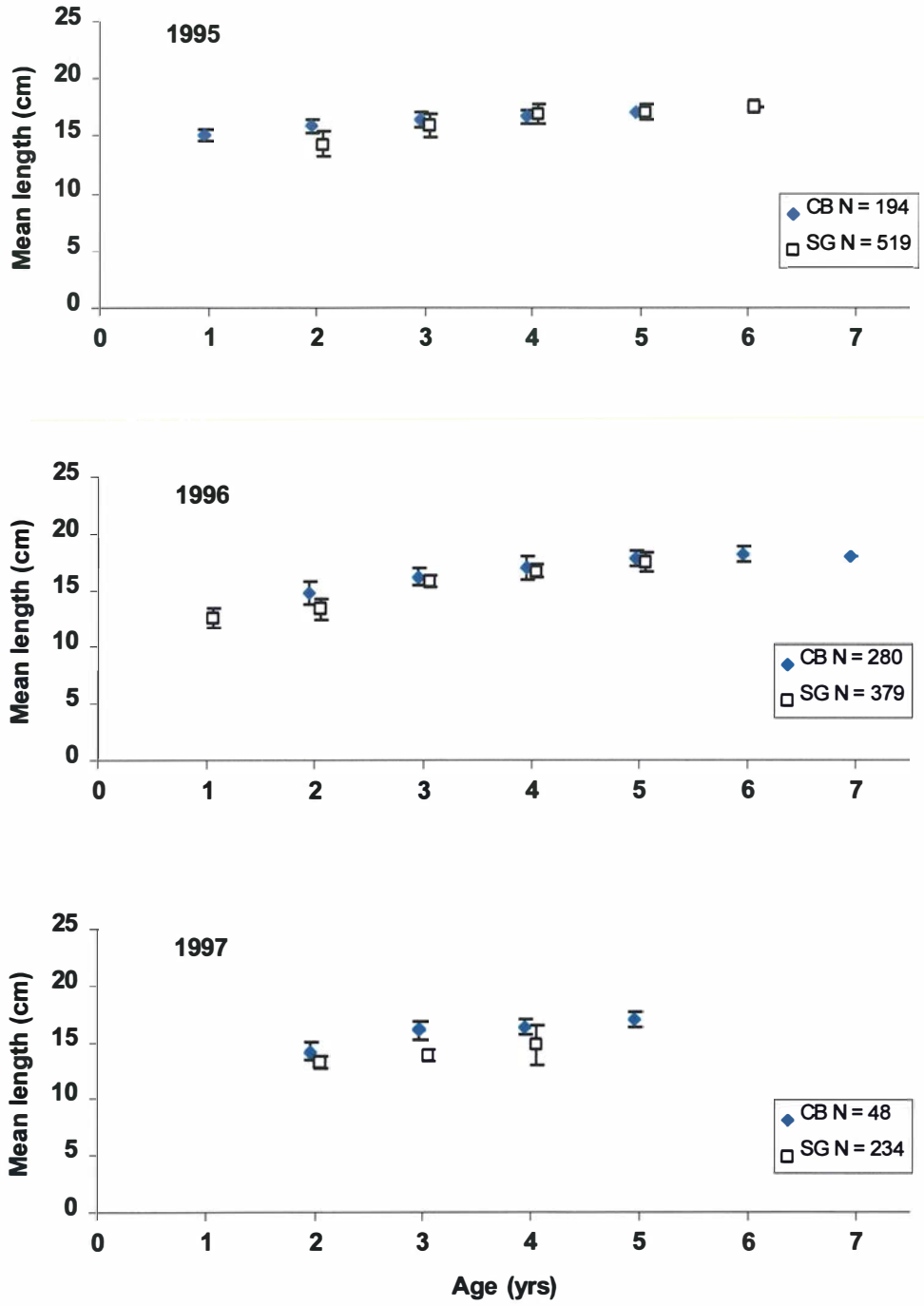


Figure 7.14A Mean length (LCF, cm) (± 1 SD) at age (years), by year, for pilchards caught in Coffin Bay (CB) and Spencer Gulf (SG) (South Australia) between March 1995 and March 1997.

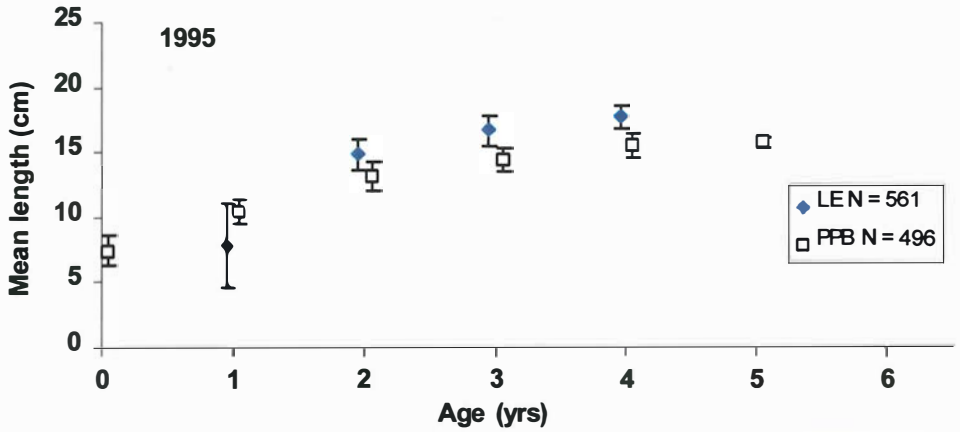


Figure 7.14B Mean length (LCF, cm) (± 1 SD) at age (years), by year, for pilchards caught in Lakes Entrance (LE) and Port Phillip Bay (PPB) (Victoria) in 1995.

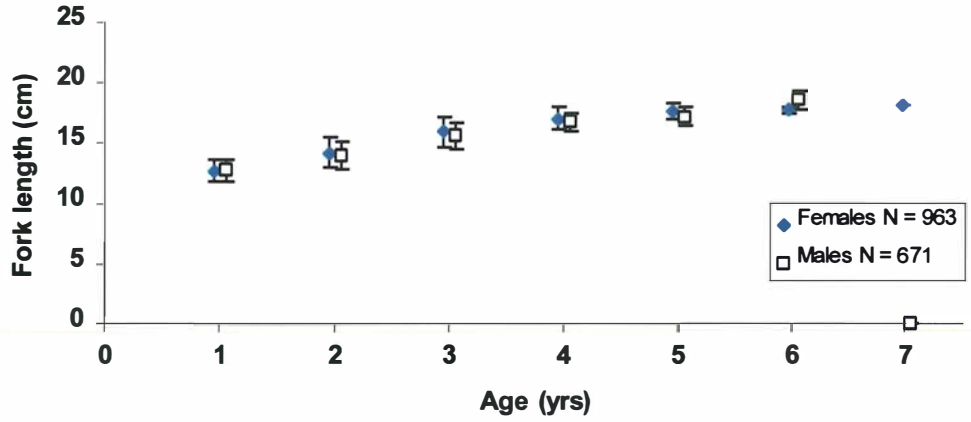


Figure 7.15A Mean length (LCF, cm) (± 1 SD) at age (years) for female and male pilchards caught in South Australia between March 1995 and March 1997 (years and areas combined).

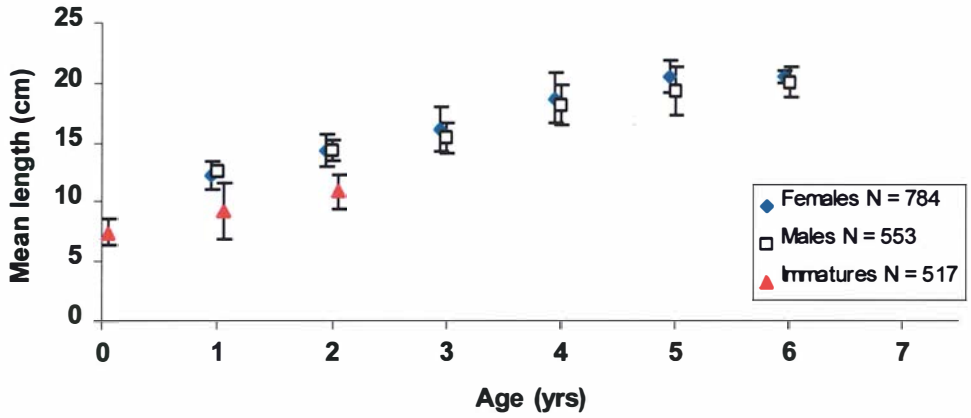


Figure 7.15B Mean length at age (± 1 SD) by sex for Victoria (years and areas combined).

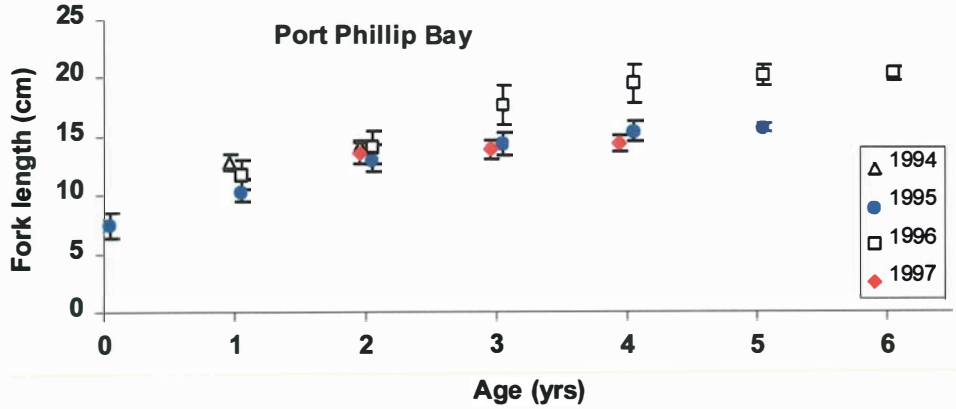


Figure 7.16 Mean length (LCF, cm) (± 1 SD) at age (years), by year, for pilchards caught in Port Phillip Bay (Victoria) between December 1994 and February 1997 (sexes combined).

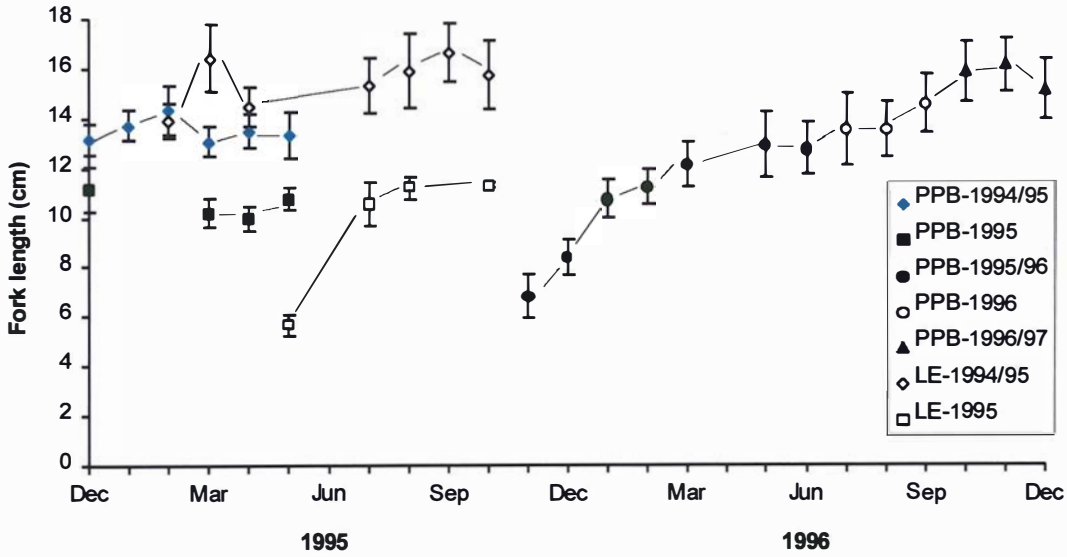


Figure 7.17 Mean length (LCF, cm) (± 1 SD) of identifiable cohorts of pilchards caught in Port Phillip Bay (PPB) and Lakes Entrance (LE) (Victoria) between December 1995 and December 1996.

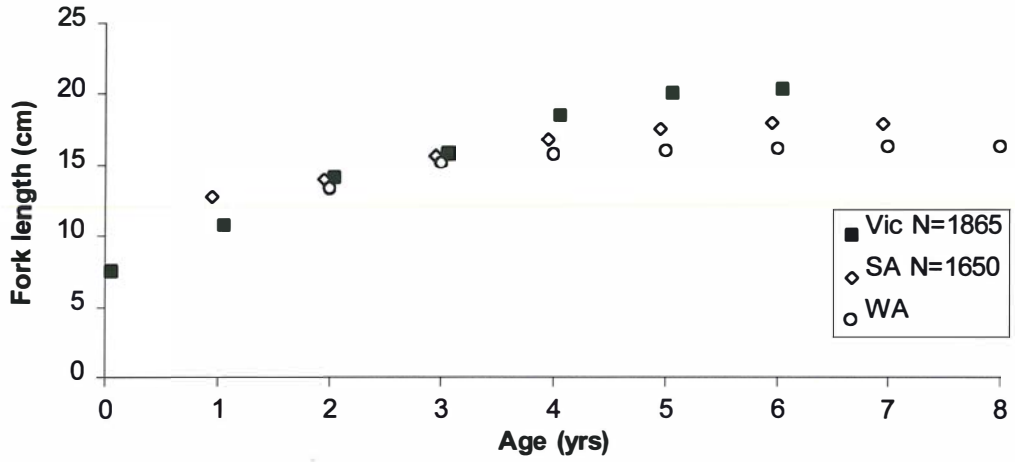


Figure 7.18 Mean length (LCF, cm) at age (years) for pilchards from Victoria, South Australia and Western Australia. Victorian and South Australian ages were determined directly from increment counts in otoliths, whereas West Australian ages were estimated from modes in otolith weight distributions (from Fletcher, 1995).

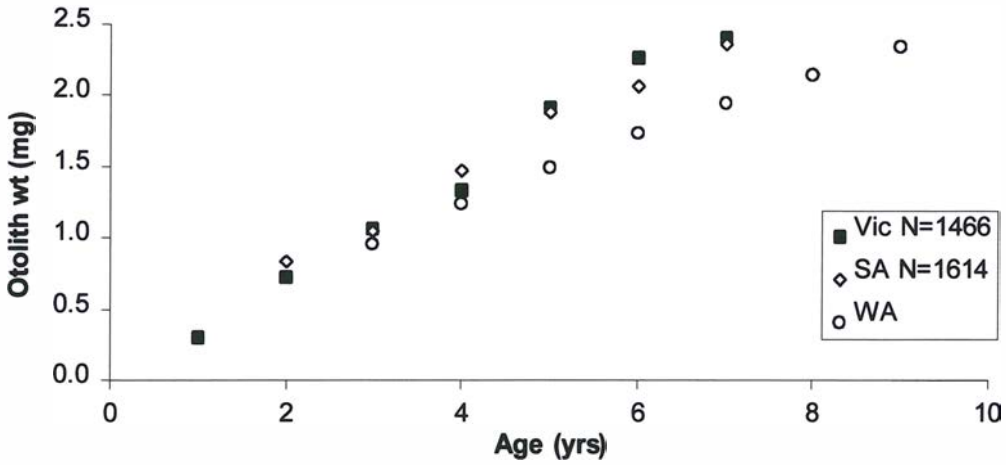


Figure 7.19 Mean otolith weight at age for pilchards from Victoria, South Australia and Western Australia - Western Australian otolith weights at age taken from Fletcher, 1995.

7.4 Appendices

Appendix 7.4A Age-length key: South Australia by area

N	Area	Age_Jan														
	CB							CB Total	PL						PL Total	Grand Total
Floor_FL	1	2	3	4	5	6	7		1	2	3	4	5	6		
8																
9.5									1						1	1
10									1						1	1
10.5									1						1	1
11									4	2					6	6
11.5									10	4					14	14
12									28	22					50	50
12.5									37	54	2				93	93
13		2						2	38	154	8				200	202
13.5		10						10	15	147	39	1			202	212
14		9						9	7	85	29				121	130
14.5	1	7	3					11	2	48	14				64	75
15	1	19	14	2				36	2	44	18	1			65	101
15.5	1	30	40	10				81		41	31	4			76	157
16		34	55	22	3			114		12	48	12	2		74	188
16.5		15	51	22	3			91		16	33	17	4		70	161
17		7	30	22	7			66		2	21	16	12		51	117
17.5			16	19	13	1		49			7	17	2	1	27	76
18			4	14	13	2	1	34			1	9	4		14	48
18.5			1	6	8			15				1	1		2	17
19					2	1		3								3
20.5				1				1								1
21																
Grand Total	3	133	214	118	49	4	1	522	146	631	251	78	25	1	1132	1654

Appendix 7.4B Age-length key: South Australia by area by year

N	Area	Year	Age_Jan																
	CB																		Grand Total
	1995					1995	1996						1996	1997				1997	
Floor_FL	1	2	3	4	5	Total	2	3	4	5	6	7	Total	2	3	4	5	Total	
13							2						2						2
13.5							3						3	7				7	10
14		1				1	3						3	5				5	9
14.5	1	2				3	4	2					6	1	1			2	11
15	1	5	1			7	11	12	1				24	3	1	1		5	36
15.5	1	27	12	1		41	3	25	9				37		3			3	81
16		32	20	6		58	1	34	14	1			50	1	1	2	2	6	114
16.5		15	23	6		44		23	11	2			36		5	5	1	11	91
17		5	15	6	1	27	2	13	16	3			34		2		3	5	66
17.5			7	5		12		9	13	11	1		34			1	2	3	49
18			1			1		3	14	12	2	1	32				1	1	34
18.5								1	6	8			15						15
19										2	1		3						3
19.5																			
20																			
20.5									1				1						1
Grand Tota	3	87	79	24	1	194	29	122	85	39	4	1	280	17	13	9	9	48	522

N	Area	Year	Age_Jan															
	PL																	Grand Total
	1995					1995	1996					1996	1997				1997	
Floor_FL	2	3	4	5	6	Total	1	2	3	4	5	Total	2	3	4		Total	
9.5							1					1						1
10							1					1						1
10.5							1					1						1
11							4	2				6						6
11.5							10	4				14						14
12	1					1	28	14				42	7				7	50
12.5	17	1				18	37	16				53	21	1			22	93
13	25					25	38	76				114	53	8			61	200
13.5	58	4				62	15	42				57	47	35	1		83	202
14	36	8				44	7	25				32	24	21			45	121
14.5	36	7				43	2	8				10	4	7			11	64
15	30	14	1			45	2	14	1			17		3			3	65
15.5	32	25	4			61		9	5			14		1			1	76
16	10	46	9	2		67		2	2	2		6			1		1	74
16.5	16	31	14	4		65			2	3		5						70
17	2	21	15	10		48				1	2	3						51
17.5		7	15	2	1	25				2		2						27
18		1	9	4		14												14
18.5			1			1					1	1						2
Grand Tota	263	165	68	22	1	519	146	212	10	8	3	379	156	76	2		234	1132

Appendix 7.4C Age-length key: South Australia by sex

N	Sex	Age_Jan																	
	F							F Total	M						M Total	U			U Total	Grand Total
Floor_FL	1	2	3	4	5	6	7		1	2	3	4	5	6		2	3	4		
8																				
9.5									1						1					1
10									1						1					1
10.5	1							1												1
11	2	1						3	2	1					3					6
11.5	6	2						8	3	2					5					13
12	19	12						31	9	8					17	2			2	50
12.5	17	23	2					42	20	31					51					93
13	19	78	2					99	19	77	6				102					201
13.5	5	79	21	1				106	10	76	18				104	1			1	211
14	5	55	19					79	2	39	9				50		1		1	130
14.5	2	24	11					37	1	30	6				37	1			1	75
15	1	31	16	2				50	2	29	16	1			48	3			3	101
15.5	1	43	36	10				90		23	35	4			62	5			5	157
16		28	66	20	2			116		17	37	14	3		71	1			1	188
16.5		22	53	25	3			103		9	31	14	4		58					161
17		5	38	29	12			84		4	13	9	7		33					117
17.5			18	25	11	2		56			4	10	4		18		1	1	2	76
18			5	20	15	1	1	42				2	2	1	5					47
18.5			1	5	8			14				2	1		3					17
19					1			1					1	1	2					3
20.5				1				1												1
21																				
Grand Total	78	403	288	138	52	3	1	963	70	346	175	56	22	2	671	13	2	1	16	1650

Appendix 7.4D Mean length-at-age: South Australia by area

		Age_Jan							
Year	Data	1	2	3	4	5	6	7	Grand Total
1995	Mean FL	15.00	14.70	16.06	16.85	17.04	17.50		15.52
	SD	0.50	1.21	0.90	0.74	0.58			1.35
	N	3	350	244	92	23	1		713
1996	Mean FL	12.61	13.55	16.13	16.95	17.79	18.13	18.00	14.64
	SD	0.85	1.04	0.78	0.97	0.67	0.63		2.03
	N	146	241	132	93	42	4	1	659
1997	Mean FL		13.32	14.08	16.05	16.94			13.78
	SD		0.64	0.99	1.04	0.68			1.14
	N		173	89	11	9			282
Total Mean FL		12.66	14.02	15.70	16.85	17.45	18.00	18.00	14.87
Total SD		0.91	1.22	1.19	0.89	0.75	0.61		1.74
Total N		149	764	465	196	74	5	1	1654

Appendix 7.4E Mean length-at-age: South Australia by sex

	Sex	Data								
	F					M				
Age_Jan	Mean FL	SD	Min	Max	N	Mean FL	SD	Min	Max	N
1	12.64	0.90	10.5	15.5	78	12.69	0.92	9.5	15	70
2	14.12	1.26	11	17	403	13.89	1.16	11	17	346
3	15.83	1.19	12.5	18.5	288	15.49	1.14	13	17.5	175
4	16.92	0.93	13.5	20.5	138	16.66	0.78	15	18.5	56
5	17.60	0.69	16	19	52	17.11	0.79	16	19	22
6	17.67	0.29	17.5	18	3	18.50	0.71	18	19	2
7	18.00		18	18	1					
Grand Total	15.12	1.79	10.5	20.5	963	14.53	1.62	9.5	19	671

	Sex	Data								
	U					All				
Age_Jan	Mean FL	SD	Min	Max	N	Mean FL	SD	Min	Max	N
1						12.67	0.90	9.5	15.5	148
2	14.65	1.33	12	16	13	14.03	1.22	11	17	762
3	15.75	2.47	14	17.5	2	15.70	1.19	12.5	18.5	465
4	17.50		17.5	17.5	1	16.85	0.89	13.5	20.5	195
5						17.45	0.75	16	19	74
6						18.00	0.61	17.5	19	5
7						18.00		18	18	1
Grand Total	14.97	1.55	12	17.5	16	14.88	1.74	9.5	20.5	1650

Appendix 7.4F Age-length key: Victoria by area

N	Area	Age_Jan												
	LE				LE Total	PPB							PPB Total	Grand Tota
Floor_FL	1	2	3	4		0	1	2	3	4	5	6		
4														
4.5	4				4	1							1	5
5	21				21	2							2	23
5.5	44				44	7							7	51
6	22				22	14							14	36
6.5						21							21	21
7						17							17	17
7.5						18							18	18
8	1				1	23							23	24
8.5						21	3						24	24
9						14	10	2					26	26
9.5						4	23	1					28	28
10	5				5		58	3					61	66
10.5	7				7		74	3					77	84
11	11				11		70	2					72	83
11.5	7				7		81	8					89	96
12	6				6		61	13					74	80
12.5	3	1			4		72	38	3				113	117
13	3	22			25		51	91	10				152	177
13.5	2	45			47		38	73	8				119	166
14	1	47			48		5	77	15	1			98	146
14.5	1	50	4		55		7	34	21	3			65	120
15	4	47	8		59		2	33	14	3			52	111
15.5		34	9		43		2	15	7	1	1		26	69
16	2	34	10		46			11	3	1	1		16	62
16.5		22	12	1	35			7	7	1			15	50
17		8	21	2	31			6		1			7	38
17.5		7	12	2	21			2	1				3	24
18		3	6	1	10				1				1	11
18.5			2	2	4				3	1			4	8
19			2	1	3			1	8	4	3		16	19
19.5			1		1				3	6	6	1	16	17
20			1		1				2	11	4	2	19	20
20.5								1		4	6	2	13	13
21										4	5	2	11	11
21.5											3		3	3
22											1		1	1
Grand To	144	320	88	9	561	142	557	421	106	41	30	7	1304	1865

Appendix 7.4G Age-length key: Victoria by area by year (Port Phillip Bay)

N	Area	Year	Age_Jan																				
	PPB																						Grand Total
	1994		1994	1995						1995	1996						1996	1997			1997	
Floor_FL	1	2	Total	0	1	2	3	4	5	Total	1	2	3	4	5	6	Total	2	3	4	Total	
4.5				1						1												1
5				2						2												2
5.5				7						7												7
6				14						14												14
6.5				21						21												21
7				17						17												17
7.5				18						18												18
8				23						23												23
8.5				21	1					22	2						2					24
9				14	7	2				23	3						3					26
9.5				4	15	1				20	8						8					28
10					38	3				41	20						20					61
10.5	1		1		29	3				32	44						44					77
11					14	2				16	56						56					72
11.5	1		1		3	2				5	77	5					82	1			1	89
12	5		5		3	4				7	53	8					61	1			1	74
12.5	18		18		4	20	1			25	50	17					67	1	2		3	113
13	14		14		2	52	6			60	35	33					68	6	4		10	152
13.5	9	1	10		2	42	6			50	27	28					55	2	2		4	119
14	2	1	3			29	9			38	3	37					40	10	6	1	17	98
14.5	1	2	3			8	13	1		22	6	22		2			30	2	8		10	65
15						4	8	2		14	2	27	5				34	2	1	1	4	52
15.5						3	6	1	1	11	2	12	1				15					26
16							2	1	1	4		11	1				12					16
16.5							2			2		7	5	1			13					15
17								1		1		6					6					7
17.5												2	1				3					3
18													1				1					1
18.5													3	1			4					4
19												1	8	4	3		16					16
19.5													3	6	6	1	16					16
20													2	11	4	2	19					19
20.5												1		4	6	2	13					13
21														4	5	2	11					11
21.5															3		3					3
22															1		1					1
Grand Tot	51	4	55	142	118	175	53	6	2	496	388	217	30	33	28	7	703	25	23	2	50	1304

Appendix 7.4G Age-length key: Victoria by area by year (Lakes Entrance)

N	Area	Year	Age_Jan		
	LE				Grand Total
	1995				
Floor_FL	1	2	3	4	
4.5	4				4
5	21				21
5.5	44				44
6	22				22
6.5					
7					
7.5					
8	1				1
8.5					
9					
9.5					
10	5				5
10.5	7				7
11	11				11
11.5	7				7
12	6				6
12.5	3	1			4
13	3	22			25
13.5	2	45			47
14	1	47			48
14.5	1	50	4		55
15	4	47	8		59
15.5		34	9		43
16	2	34	10		46
16.5		22	12	1	35
17		8	21	2	31
17.5		7	12	2	21
18		3	6	1	10
18.5			2	2	4
19			2	1	3
19.5			1		1
20			1		1
Grand Total	144	320	88	9	561

ay)

Appendix 7.4H Age-length key: Victoria by sex

N	Sex	Age_Jan																				
	F						F Tota	M						M Tota	I			I Tota	U		U Tota	Grand Tot
Floor_FL	1	2	3	4	5	6		1	2	3	4	5	6		0	1	2		1	2		
4.5															1	4		5				5
5															2	21		23				23
5.5															7	44		51				51
6															14	22		36				36
6.5															21			21				21
7															17			17				17
7.5															18			18				18
8															23	1		24				24
8.5															21	3		24				24
9															14	10	2	26				26
9.5															4	23	1	28				28
10	7						7									56	3	59				66
10.5	14						14	2						2		65	3	68				84
11	23	1					24	5						5		53	1	54				83
11.5	31	2					33	16	4					20		40	1	41	1	1	2	96
12	31	3					34	20	8					28		16	2	18				80
12.5	40	20	1				61	34	18	2				54		1	1	2				117
13	30	64	4				98	24	42	6				72						7	7	177
13.5	22	70	4				96	17	47	4				68		1		1		1	1	166
14	5	76	8				89	1	47	7	1			56						1	1	146
14.5	4	42	15	2			63	4	41	10	1			56			1	1				120
15	4	32	13	2			51	2	48	9	1			60								111
15.5	1	24	12	1			38	1	25	4		1		31								69
16	2	24	9	1	1		37		21	4				25								62
16.5		16	9	1			26		13	10	1			24								50
17		9	14	1			24		5	7	2			14								38
17.5		7	9	1			17		2	4	1			7								24
18		3	4	1			8			3				3								11
18.5			4	3			7			1				1								8
19			8	2			10		1	2	3	3		9								19
19.5			4	1	3		8				5	3	1	9								17
20			3	9		2	14				2	4		6								20
20.5		1		4	5	1	11					1	1	2								13
21				3	5	2	10				1			1								11
21.5					3		3															3
22					1		1															1
Grand Total	214	394	121	32	18	5	784	126	322	73	18	12	2	553	142	360	15	517	1	10	11	1865

Appendix 7.4I Mean length-at-age: Victoria by year

		Age_Jan							
Year	Data	0	1	2	3	4	5	6	Grand Tota
1994	Mean		12.80	14.13					12.90
	SD		0.67	0.48					0.74
	N		51	4					55
1995	Mean	7.42	8.98	14.23	15.81	16.83	15.75		12.26
	SD	1.13	2.82	1.38	1.53	1.40	0.35		3.61
	N	142	262	495	141	15	2		1057
1996	Mean		11.74	14.14	17.68	19.48	20.30	20.36	13.52
	SD		1.17	1.41	1.73	1.55	0.84	0.56	2.87
	N		388	217	30	33	28	7	703
1997	Mean			13.60	13.87	14.50			13.76
	SD			0.85	0.73	0.71			0.80
	N			25	23	2			50
Total Mean		7.42	10.78	14.18	15.87	18.49	20.00	20.36	12.80
Total SD		1.13	2.40	1.38	1.79	2.07	1.41	0.56	3.30
Total N		142	701	741	194	50	30	7	1865

Appendix 7.4J Mean length-at-age: Victoria by sex

	Sex									
	F					M				
Age_Jan	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N
0										
1	12.25	1.17	10	16	214	12.57	0.90	10.5	15.5	126
2	14.26	1.32	11	20.5	394	14.28	1.25	11.5	19	322
3	16.14	1.82	12.5	20	121	15.42	1.64	12.5	19	73
4	18.67	2.09	14.5	21	32	18.17	2.07	14	21	18
5	20.47	1.32	16	22	18	19.29	1.29	15.5	20.5	12
6	20.50	0.50	20	21	5	20.00	0.71	19.5	20.5	2
Grand Total	14.37	2.35	10	22	784	14.29	1.90	10.5	21	553

	Sex									
	U					I				
Age_Jan	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N
0						7.42	1.13	4.5	9.5	142
1	11.50		11.5	11.5	1	9.28	2.34	4.5	13.5	360
2	13.00	0.62	11.5	14	10	10.83	1.47	9	14.5	15
3										
4										
5										
6										
Grand Total	12.86	0.74	11.5	14	11	8.82	2.24	4.5	14.5	517

	Sex				
	All				
Age_Jan	Mean	SD	Min	Max	N
0	7.42	1.13	4.5	9.5	142
1	10.78	2.40	4.5	16	701
2	14.18	1.38	9	20.5	741
3	15.87	1.79	12.5	20	194
4	18.49	2.07	14	21	50
5	20.00	1.41	15.5	22	30
6	20.36	0.56	19.5	21	7
Grand Total	12.80	3.30	4.5	22	1865

CHAPTER 8. REPRODUCTIVE BIOLOGY OF PILCHARDS IN SOUTHERN AND EASTERN AUSTRALIA

M. Kinloch, F.J. Neira, T.M. Ward, F. Hoedt and G. Jackson

Objective (4b): To describe and compare the reproductive biology of pilchards in Victorian and South Australian waters. This objective was achieved by conducting carcass analyses and histological studies of pilchards obtained from commercial and chartered purse-seine vessels in South Australian and Victorian waters. Females were generally more common than males in samples from both states. In South Australia, 50% of male and female pilchards reached sexual maturity at 14.2 cm and 14.8 cm LCF, respectively whereas in Victoria 50% of male and female pilchards reached sexual maturity at 12.9 and 15.1cm LCF, respectively. In South Australia, spawning occurred between January and April, whereas in Victoria the highest GSIs were recorded between September and December. Difficulties in collecting representative samples of spawning fish, restricted the precision of estimates of batch fecundity and spawning fraction in South Australia. Mean estimates of batch fecundity and spawning fraction obtained in 1997 were 13 947 eggs per female and 0.156 females per night respectively. Future studies of the reproductive biology of pilchards will be enhanced by the use of a fishery-independent sampling technique that was developed towards the end of this project.

8.1 Methods

Sex Ratio

Pilchard fisheries in South Australia and Victoria are described in Chapter 4. Between December 1994 and April 1997, monthly samples of between 28 and 1368 fish were collected from commercial catches obtained from Spencer Gulf and Coffin Bay in South Australia (n = 4956) and Port Phillip Bay and Lakes Entrance in Victoria (n = 14 089). Fish from both areas (n = 2227) were measured, weighed (g) and subsequently dissected to determine the sex and stage of maturity.

In Victoria, immature pilchards were not sexed whereas in South Australia immature specimens were sexed according to the method of Hoedt (1995). Monthly sample sizes are shown in Table 8.1 and 8.2. The proportion of females was calculated for each area and month according to the formula:

$$P_f = N_f/(N_m + N_f)$$

where P_f is the proportion of females, N_f is the number of females in a sample and N_m is the number of males in a sample. In South Australia the proportion of females was calculated for both mature and immature fish, whereas in Victoria it was only calculated for mature fish.

Gonadal Stages

Gonadal stages were classified from I to V following the method of Laevastu (1965) for batch or partial spawning fishes, where I is virgin, II is maturing orrecovering spent, III is maturing, IV is running ripe and V is spent.

Gonosomatic Index

Gonads (> Stage II) were removed from the body cavity and weighed to the nearest 0.01 g. The gonadosomatic index (GSI) was calculated using the formula:

$$GSI = (W_g/W_f) * 100$$

where W_g is the weight of the gonad (g) and W_f is the whole weight of the fish (g). GSI values were averaged by month and plotted for each area and sex.

Mean monthly GSIs of males and females were calculated between March 1995 to April 1997 from 639 from Spencer Gulf, 1 700 pilchards from Coffin Bay, 1 172 pilchards from Port Phillip Bay and 589 pilchards from Lakes Entrance. Samples were not available from all months in all areas and sample sizes were sometimes small, so monthly data from each region were pooled across years in order to describe the "mean" annual cycle.

Size and Age at Sexual Maturity

Size at maturity was calculated using fish collected during the spawning season (see Table 8.1, 8.2). The mean length at which 50% of male and females pilchards were sexually mature (L_{50}) was estimated by plotting the proportion of fish with stage II to V gonads present in each length class and fitting a logistic growth function:

$$P_L = 1/[1 + e^{(a+bL)}]$$

where P_L is the proportion of mature fish at fork length class L, and a (intercept) and b (slope) are constants derived from the plot Ln[(1-P)/P] versus fork length (cm). The L_{50} was then derived from the equation $L_{50} = -a/b$.

In South Australia, L_{50} was estimated from 1 466 males and 2 133 females grouped into 0.5 cm length classes whereas in Victoria L_{50} was estimated from 491 males and 682 and females grouped into 1.0 cm length classes. Age-length keys presented in Chapter 5 were used to estimate the age at sexual maturity for males and females from South Australia and Victoria.

Egg Production

Pilchards are batch spawners, and thus release only a proportion of their oocytes during a spawning season. Since eggs that are not spawned during the season are either resorbed or continue developing to be released during the following season, annual fecundity can not be easily determined (Baker 1972; Macewicz *et al.* 1996). It is possible, however, to estimate (i) the proportion of females that spawn on a given day (spawning fraction) and (ii) the number of eggs a female releases during a spawning event (batch fecundity).

Patterns of egg production were examined using pilchards randomly selected from the catches of chartered commercial purse-seine vessels that fished between Coffin Bay to Streaky Bay (Eyre Peninsula) during late January to early March in 1996 and 1997. Two samples obtained by mid-water trawling included no mature fish. Fishes were sexed through a ventral incision. Approximately 15 female pilchards were placed in a 4.5 litre pots containing approximately 4.0 litres of buffered formaldehyde solution. The following information was recorded for each fish: (i) whole fish weight, (ii) gonad-free fish weight, and (iii) gonad weight.

After fixation, one ovary was removed from the formalin solution, cut into approximately $1cm^3$ segments and embedded in paraffin wax. Thin sections were then cut from several segments (7μm) sections and stained with haematoxylin and eosin. Sections were mounted on glass slides and viewed using a bright-field light microscope (Olympus CH).

Batch Fecundity

Batch fecundity was calculated for ovaries with hydrated oocytes according to the gravimetric method described by Le Clus (1977) and Hunter (1985). The presence/absence of hydrated oocytes was determined from histological sections obtained from each ovary (see above). Hydrated oocytes were identified by their large size (~800 μm) and by the presence of protein yolk within the cytoplasm. If hydrated oocytes were identified, the unused ovary was then weighed to the nearest 0.01 g and a sub-sample of tissue was removed from the middle of this ovary and weighed. The number of hydrated oocytes in this sub-sample was counted under a dissecting microscope. The total number of hydrated eggs in the ovary was calculated from the product of the number of mature oocytes per gram of gonad and the total gonad weight.

As relatively few fish with hydrated oocytes were collected during this study, batch fecundity was also estimated using oocytes with migratory nuclei. This was done by cutting serial sections from an entire ovary. Every tenth section was viewed under a light microscope. Oocytes with migrating/migrated nuclei were counted. This method assumes that all oocytes with migratory nuclei will undergo hydration and will be spawned and the number of oocytes with migratory nuclei will be counted accurately.

Spawning Fraction

The presence/absence of post-ovulatory follicles (POFs) was determined from thin sections of the ovary that had been initially stained with haematoxylin and eosin. POFs were staged using criteria developed for *E. mordax* (Hunter and Macewicz 1985). The spawning fraction of each sample was estimated from the proportion of fish with Day-0 and Day-1 POFs.

8.2 Results

Sex Ratio

In South Australia, sex ratio data were more complete for Spencer Gulf than Coffin Bay (Figure 8.1). In samples of mature fish, females were usually more abundant than males and comprised more than 60% of mature fish in 9 of the 22 samples obtained from Spencer Gulf between March 1995 and April 1997 and 8 of the 11 samples obtained from Coffin Bay between May 1995 and April 1997. In contrast, males comprised over 60% of mature fish in only 4 of the 22 samples from Spencer Gulf and 1 of the 11 samples from Coffin Bay. Females were also generally more common than males in samples of immature fish from both Spencer Gulf and Coffin Bay (Figure 8.2).

In Victoria, sex ratio data were more complete for Port Phillip Bay than Lakes Entrance (Figure 8.3). Females comprised more than 60% of mature fish in 7 of the 20 samples obtained from Port Phillip Bay between December 1994 and January 1997 and 3 of the 8 samples obtained from Lakes Entrance between February 1995 and October 1995 (Figure 8.3). Males did not comprise more than 60% of any samples obtained from Victorian waters.

Gonadal Stages

In South Australia, immature fish generally dominated samples from Spencer Gulf whereas mature fish dominated samples from Coffin Bay (Figure 8.4). Pilchards with stage III gonads dominated (>60%) samples obtained from both Spencer Gulf and Coffin Bay in February and March 1996. A total of only 14 females with Stage IV ovaries were collected from three samples obtained from

Coffin Bay in November and December 1995, and April 1997 and two samples obtained from Spencer Gulf in April and November 1995. No males were found in running ripe condition (Stage IV). Only one spent female (Stage V) was found.

Pilchards with stage I and II gonads dominated most samples obtained from Port Phillip Bay, and exclusively comprised samples obtained between March 1995 and June 1996. Pilchards with stage III gonads were present in samples obtained between December 1994 and February 1995 and July 1996 and January 1997, and were highly abundant (>40% of fish) in samples obtained between August 1996-January 1997. No samples from Port Phillip Bay contained fish in running ripe condition (stage IV). Spent fish (Stage V) were only obtained from Port Phillip Bay in January-February 1995 and January 1997. Pilchards with Stage I and Stage II gonads dominated most samples obtained from Lakes Entrance, and exclusively comprised samples obtained in February, April and July 1995. Pilchards with Stage III gonads were obtained only in March 1995. No spawning (Stage IV) or spent (Stage IV) fish were obtained from Lakes Entrance (Figure 8.5).

Gonosomatic Index

Trends in the monthly GSI values for male and female pilchards from Spencer Gulf and Coffin Bay are presented in Figure 8.6. The GSI values of males and females displayed highly similar trends. The maximum mean GSI values for males (5.32) and females (4.48) were both recorded during February 1996 in Spencer Gulf. Annual patterns were similar in the two locations, i.e. GSIs was relatively high (>2) between January and April, and relatively low (<2) between May and November (Figure 8.7).

In Port Phillip Bay, maximum monthly gonadosomatic indices (GSIs) of approximately 2.5 were recorded in October 1996 (Figure 8.2), whereas in Lakes Entrance peaks of 3.3 (females) and 4.0 (males) were recorded in September 1995 (Figure 8.4). Although few data were available for Lakes Entrance, annual patterns appeared to be similar in the two locations, i.e. GSIs were highest in the period between September and December (Figure 8.2).

Size and Age at Sexual Maturity

Male and female pilchards from South Australia and Victoria reached sexual maturity at similar sizes. In South Australia, 50% of males and females were mature at 14.2 and 14.8 cm LCF respectively. The smallest mature fish appeared in the 12.5-12.9 cm LCF length class (Table 8.2). In Victoria, 50% of male and female pilchards were mature at 12.9 and 15.1cm LCF respectively (Figure 8.7). Fish from both states thus appear to mature at approximately 2 years of age (Chapter 5).

Batch Fecundity

At least two populations of follicles were observed in each pilchard ovary. These included a smaller heterogeneous population of primordial follicles and a more numerous advanced clutch. In 1996, only five of the fish collected had hydrated oocytes (Table 8.1; Figure 8.16). A total of 62 fish had tertiary oocytes. In 1997, two fish with hydrated oocytes and 22 fish with oocytes with migrated nuclei were collected (Table 8.1; Figure 8.1b).

Relationships between female body weight and batch fecundity are shown in Figure 8.14. Batch fecundity was not strongly correlated with female weight (see Chapter 7). Power curves had a slightly higher correlation coefficient than linear regressions when fitted to both whole and clean weight. The relationship between the two variables was described by the equation.

$F = 574.5 \times \text{weight}^{0.874}$ $\quad r^2 = 0.066 \quad (n = 62)$

Mean batch fecundity was estimated at 15 366 eggs in 1995 and 16 422 eggs in 1996.

In 1997, the best fit to the data was provided by the power curve :

$F = 549.3 \times \text{weight}^{0.847}$ $\quad r^2 = 0.057 \quad (n = 22)$

This is very similar to the relationship derived for 1996, therefore a new relationship was derived from the pooled data:

$F = 192.02 \times \text{weight}^{1.139}$ $\quad r^2 = 0.167 \quad (n = 84)$

A mean batch fecundity in 1997 of 13 947 eggs was calculated from 450 mature females.

Spawning Fraction

In 1995, post-ovulatory follicles were not identified as inappropriate preservation techniques resulted in the degeneration of tissues. In 1996, samples obtained from commercial vessels included no pilchards with POFs (i.e. post-spawning fish) (Table 8.3). In 1997, 29, 16 and 5 fish with Day-0, Day-1 and Day-2 POFs respectively (Table 8.3; Figure 8.17, 8.18, 8.19, 8.20) were collected from two mid-water trawls and three samples from commercial fishing vessels. Spawning fraction values for each sample ranged declined from 37.5% in March to 4.0% in mid-April and less than 1% at the end of April. The estimate of mean spawning fraction was 0.156.

8.3 Discussion

Fishing effort and catches by commercial fleets fluctuate according to demand and these fluctuations had negative implications on the sampling regime. Samples were not collected from all areas during all months and sample sizes were highly variable. For example, large samples were obtained from South Australia during summer, particularly late summer, whereas fewer and smaller samples were obtained in autumn and winter. Similarly, fishing for pilchards in Lakes Entrance was sporadic and samples were only obtained from eight months in 1995.

Several other factors also limited the utility of samples obtained from commercial vessels. Firstly, sample collection was constrained by logistical difficulties associated with obtaining monthly samples from a fleet of independent vessels operating in relatively remote locations. Secondly, biases in catches obtained by purse seining are poorly understood but may be significant (Dotson and Griffith 1996). Thirdly, the tendency of pilchards to school according to size limits the information that can be obtained using procedures that only sample one school per 'shot'.

Females were generally more abundant than males in samples obtained from all regions. It is not possible to discern, however, whether this difference reflects a real bias in the sex ratio within these populations or occurred as a result of methods used to collect samples. The sampling regime was not sufficiently rigorous to clearly complete do discern any seasonal trends in the relative abundance of males and females.

Immature fish were more abundant in samples from Spencer Gulf and Port Phillip Bay than fishing grounds around Coffin Bay or Lakes Entrance. This finding supports the view that juvenile pilchards are most commonly found in embayments (e.g. Blackburn 1949, 1950; Fletcher 1992). Relatively few data are available on how or when larvae/juveniles move from shelf waters where they are spawned into (Chapter 8) these embayments. The South Australia and Victorian pilchard fisheries both catch large numbers of young fish,and management of these fisheries would be greatly enhanced by the acquisition of additional information on the ecology of juvenile pilchards (e.g. recruitment indices).

Relatively few mature fish (stages III-V) were collected during this study. Data obtained recently obtained a concurrent study in southern Queensland, suggest that spawning pilchards are generally under-represented in samples obtained from purse-seine vessels (Queensland Department of Primary Industries unpubl. data).

In South Australia, 50% of male and female pilchards were found to reach sexual maturity at 14.2 and 14.8 cm LCF respectively. Stevens *et al.* (1984) stated that "sexual maturity in both sexes is assumed to be attained at about 12 cm LCF, based on the size of the smallest fish having stage III (or later) gonads". In Victoria, 50% of male and female pilchards were found to reach sexual maturity at 12.9 and 15.1 cm LCF, respectively. These estimates are considerably higher than those of Blackburn (1950) who reported that Victorian pilchard bgin to mature between lengths of 7.0 and 10.5 cm SL.

Viewed in conjunction with overseas studies, these results support the idea that the sizes at which pilchards attain sexual maturity varies between locations. At least 50% of pilchards in Australia and New Zealand are mature by the time they reach approximately 15 cm (Blackburn 1941, 1950; Joseph 1981; Stevens *et al.* 1984). In contrast, 50% of female pilchards from the Pacific coast of California are sexually mature at approximately 16.0 cm (Macewicz *et al.* 1996) whereas in South Africa, 50% of males and females are sexually mature at 18.5-20.0 and 20.5-22.0 cm, respectively (Davies 1956). This difference in size is directly related to the higher growth rates and larger sizes attained by pilchards in Californian and South African waters, and could be linked to the high nutrient levels.

The timing and duration of spawning by pilchards in Australian waters varies between locations (see Fletcher 1990a; Fletcher and Tregonning 1992). In most regions, there is one spawning season per year but in waters off Albany (Western Australia) there are spawning peaks in both June and December (Fletcher and Tregonning 1992; Fletcher *et al.* 1994).

High monthly gonosomatic indices in January and April in South Australia confirm the findings of earlier workers that spawning occurs during late summer and autumn. Blackburn (1941, 1950) collected pilchard eggs and larvae from South Australian waters during December, February and early March, and obtained females in spent and recovering condition during May. Stevens *et al.* (1984) collected pilchards with running ripe gonads in March, April and May, and caught large numbers of pilchard larvae during April and May. Bongo net samples collected from the Gulf St Vincent and Spencer Gulf (South Australia) from March to May of 1986 and 1987 contained high densities of pilchard larvae that were less than 2 weeks old (Jones *et al.* 1995).

Data for Victorian waters are less conclusive but suggest that spawning occurs in late spring and early summer. Hoedt and Dimmlich (1995) found that pilchards in waters off Phillip Island and Cape Schanck spawn mainly in late spring and summer, i.e. November-January.

Values of GSIs obtained for South Australian waters were generally higher than those obtained for Victoria. Values were particularly low for samples obtained from Port Phillip Bay and data from a concurrent plankton sampling program yielded no eggs or larvae. Although other workers (e.g. Jenkins 1986) have found a few eggs and larvae in Port Phillip Bay, it is hypothesised that Port Phillip Bay is not an important spawning area for pilchards and that adult fish may migrate out of the bay to spawn. This hypothesis fits with evidence from other areas that suggest that pilchards usually spawn in shelf waters and not in estuaries or bays (e.g. Blackburn 1950; Davies 1956; Fletcher and Treggoning 1994). A similar pattern was observed in South Australian waters, where little spawning occurred in Spencer Gulf or Gulf St Vincent, and most spawning occurred in inshore shelf waters. The timing and location of spawning by pilchards in Victorian waters are poorly understood and are currently being investigated as part of larger study of the ichthyoplankton assemblage of Bass Strait. Results are likely to have significant implications for the management of Victorian pilchard stocks, as it been hypothesised that juvenile pilchards that enter Port Phillip Bay may be derived from spawning events that occur in South Australian waters.

Investigation of egg production of pilchards were constrained by difficulties in acquiring large and representative samples of spawning fish. Samples obtained from commercial catches during 1996 yielded no fish with POFs and only five fish with hydrated ovaries. Mid-water trawls conducted in 1997 yielded only two small samples that contained spawning fish, whereas catches of chartered commercial vessels yielded 60 fish with POFs and two fish with hydrated oocytes. In 1998, samples were collected using a multi-mesh gillnet. Samples contained more spawning fishes than those obtained during the three previous years, e.g. 54 fish with ovaries containing hydrated oocytes (South Australia Research and Development Institute, unpublished data). These results emphasise the advantages of using fishery-independent sampling procedures.

As only 7 fish with hydrated ovaries were collected during 1995-7, a procedure which used counts of oocytes with migrated nucleus stage to estimate batch fecundity was tested in 1997. This procedure posed several problems, e.g. it was sometimes difficult to determine when a nucleus had actually begun its migration, and procedures used to convert counts from slides into estimates of abundance for the entire ovary were require further investigation.

Correlations between estimates of batch fecundity (obtained from ovaries with hydrated oocytes) and female body weight obtained in this study were low, presumably because of the small number of samples and small size range of fish collected. Mean fecundities estimated in this study are similar to those obtained by other workers. For example, Joseph (1981) estimated that batch fecundities of pilchards in

southern New South Wales waters ranged from 10 800 (13.0 cm LCF, 25.6 g fish) to 47 100 oocytes (17.7 cm LCF, 83.8 g fish). Mean predicted batch fecundity obtained from 583 mature female pilchards from the Pacific coast off California and Baja California was estimated in 24 282 (Macewicz *et al.* 1996).

Studies of the degeneration of POFs carried out by Fitzhugh and Hettler (1995) showed that duration of POF varies for species that spawn at different temperatures and that increases in temperature decrease the time during which POFs can be detected. These authors also suggested that the ability to accurately identify and stage POFs becomes more important as their duration increases and that knowledge of the age of particular features of POFs are critical for classifying post-spawning ovaries. The numbers of pilchards with Day-0, Day-1 and Day-2 POFs collected in this study, suggested that Day-2 POFs may be significantly undersampled. Two possible reasons for this consistency are that Day-0, Day-1 and Day-2 spawners are not equally susceptible to capture, or that the key used to age POFs is not directly applicable to south Australian pilchards.

The implications of inaccurate and or imprecise estimates of batch fecundity and spawning fraction on estimates of spawning biomass obtained using the DEPM are discussed in Chapter .

Table 8.1 Total number of pilchards measured for length frequency data (in bold), the number examined for reproductive data (in brackets) and the length range (LCF, cm) obtained monthly from commercial catches in Spencer Gulf and Coffin Bay (South Australia) between March 1995 and April 1997. Months during which spawning occurred are shaded.

	Port Lincoln		**Coffin Bay**	
Month-Year	**Total fish measured** (number used for reproductive biology	**Length range (LCF, cm)**	**Total fish measured** (number used for reproductive biology	**Length range (LCF, cm)**
March-95	**882** (370)	12.0 – 18.4	-	
April-95	**110** (67)	12.3 – 18.7	-	
May-95	**68** (39)	3.6 – 15.8	**58** (27)	13.3 – 17.0
June-95	**352** (119)	12.6 – 16.5	-	
July-95	**161** (40)	7.0 – 18.3	-	
August-95	**582** (255)	13.2 – 18.6	-	
September-95	**288** (135)	13.8 – 18.2	-	
October-95	**102** (69)	15.7 – 18.6	-	
November-95	-		**457** (351)	14.0 – 18.4
December-95	-		**523** (495)	14.4 – 19.4
January-96	**23** (23)	15.2 – 18.8	**173** (172)	15.0 – 20.2
February-96	**56** (52)	10.6 – 18.7	**860** (800)	12.5 – 18.9
March-96	**259** (162)	11.7 – 18.8	**444** (423)	12.6 – 19.8
April-96	**471** (366)	11.0 – 18.2	-	-
May-96	**128** (0)	11.4 – 14.6	**780** (42)	12.0 – 20.3
June-96	**40** (7)	12.3 – 17.0	**445** (90)	13.1 – 20.5
July-96	-		**130** (50)	13.8 – 19.4
August-96	**304** (29)	10.8 – 16.4	**65** (0)	11.8 – 13.8
September-96	**150** (0)	11.2 – 15.3	-	
October-96	**150** (28)	10.9 – 19.4	-	
November-96	**366** (89)	10.4 – 17.8	-	
December-96	**548** (207)	9.4 – 16.4	-	
January-97	**441** (120)	11.2 – 16.2	**21** (10)	14.4 – 17.8
February-97	**565** (138)	12.2 – 15.3	**21** (10)	15.3 – 17.9
March-97	**298** (51)	12.8 – 15.2	**125** (30)	11.7 – 18.1
April-97	**273** (40)	13.2 – 15.8	**292** (50)	12.5 – 19.2
May-97	**210** (0)	11.4 – 16.1	**42** (0)	14.1 – 17.5
Total	**6827** (2406)	7.0 – 19.4	**4436** (2550)	7.0 – 19.4

Table 8.2 Total number of pilchards measured for length frequency data (in bold), the number examined for reproductive data (in brackets) and the length range (LCF, cm) obtained monthly from commercial catches in Port Phillip Bay and Lakes Entrance (Victoria) between December 1994 and January 1997.

	Port Phillip Bay		**Lakes Entrance**	
Month-Year	**Total fish measured** (number used for reproductive biology)	**Length range (LCF, cm)**	**Total fish measured** (number used for reproductive biology	**Length range (LCF, cm)**
December-94	**241** (60)	11.2 – 15.2	-	-
January-95	**162** (54)	12.1 – 15.4	-	-
February-95	**271** (100)	12.0 – 16.1	**98** (77)	12.8 – 16.1
March-95	**1368** (75)	8.1 – 14.5	**93** (30)	11.8 – 17.6
April-95	**851** (74)	6.2 – 15.0	**80** (80)	12.7 – 16.2
May-95	**875** (77)	8.1 – 15.3	**267** (100)	4.1 – 10.2
July-95	-	-	**152** (79)	8.1 – 18.3
August-95	-	-	**84** (84)	10.1 – 19.1
September-95	-	-	**80** (80)	14.3 – 20.0
October-95	-	-	**321** (75)	11.2 – 18.8
November-95	**521** (75)	4.4 – 09.5	-	-
December-95	**227** (76)	6.1 – 10.2	-	-
January-96	**657** (80)	6.1 – 12.4	-	-
February-96	**826** (80)	7.0 – 13.8	-	-
March-96	**672** (83)	6.8 – 16.2	-	-
May-96	**1240** (78)	8.9 – 17.0	-	-
June-96	**430** (75)	8.5 – 16.8	**272**	9.0 – 18.9
July-96	**952** (105)	10.9 – 21.8	-	-
August-96	**352** (76)	9.6 – 16.6	-	-
September-96	**432** (74)	10.1 – 22.3	-	-
October-96	**453** (102)	11.7 – 21.2	-	-
November-96	**532** (101)	12.4 – 21.7	-	-
December-96	**419** (100)	12.4 – 19.9	-	-
January-97	**1161** (77)	11.9 – 17.9	-	-
Total	**12 642** (1622)	6.2 – 22.3	**1447** (605)	11.7 – 20.5

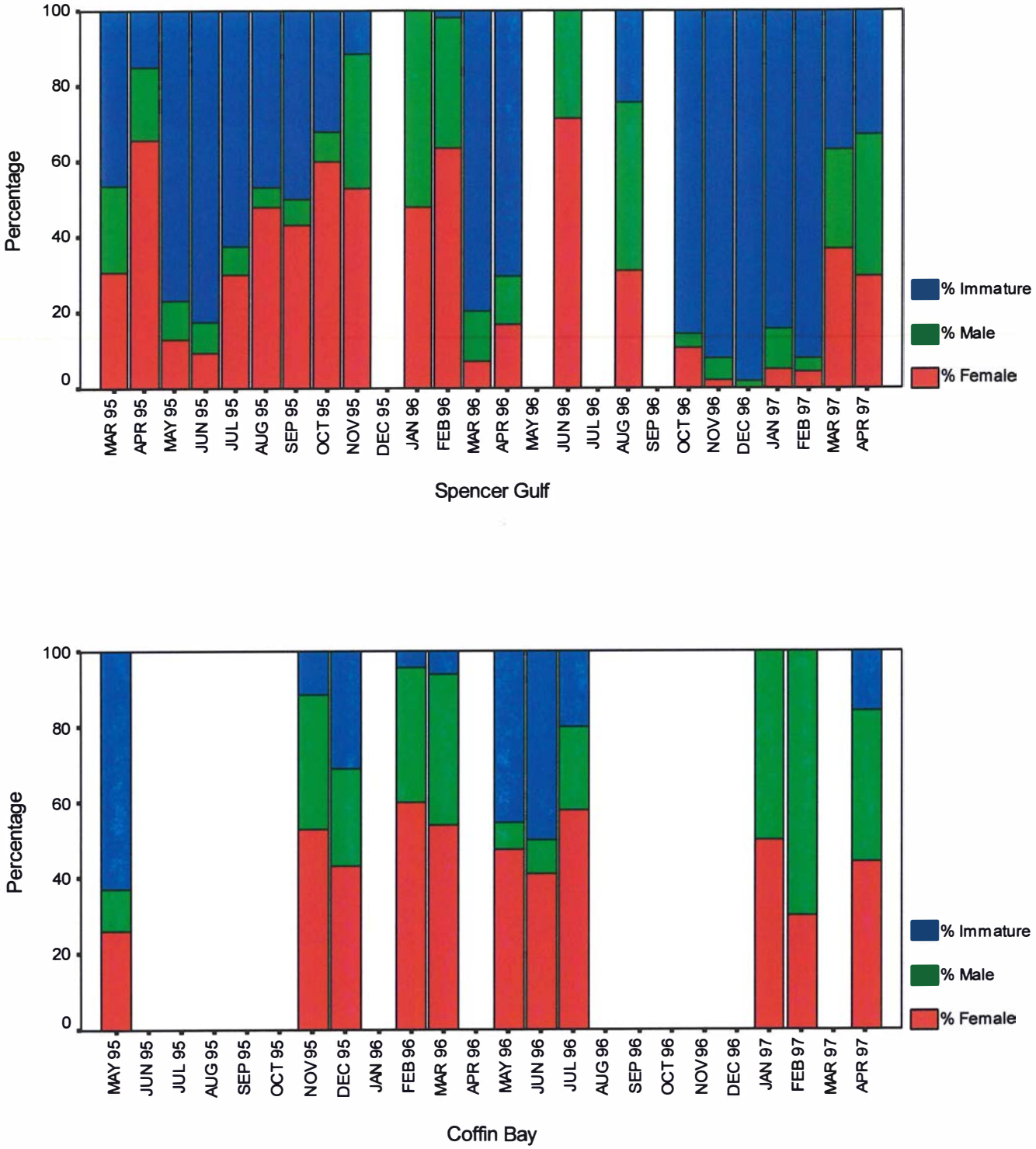


Figure 8.1 Proportion of males, females and immature pilchard obtained from commercial catches in Spencer Gulf and Coffin Bay (South Australia) between May 1995 and April 1997.

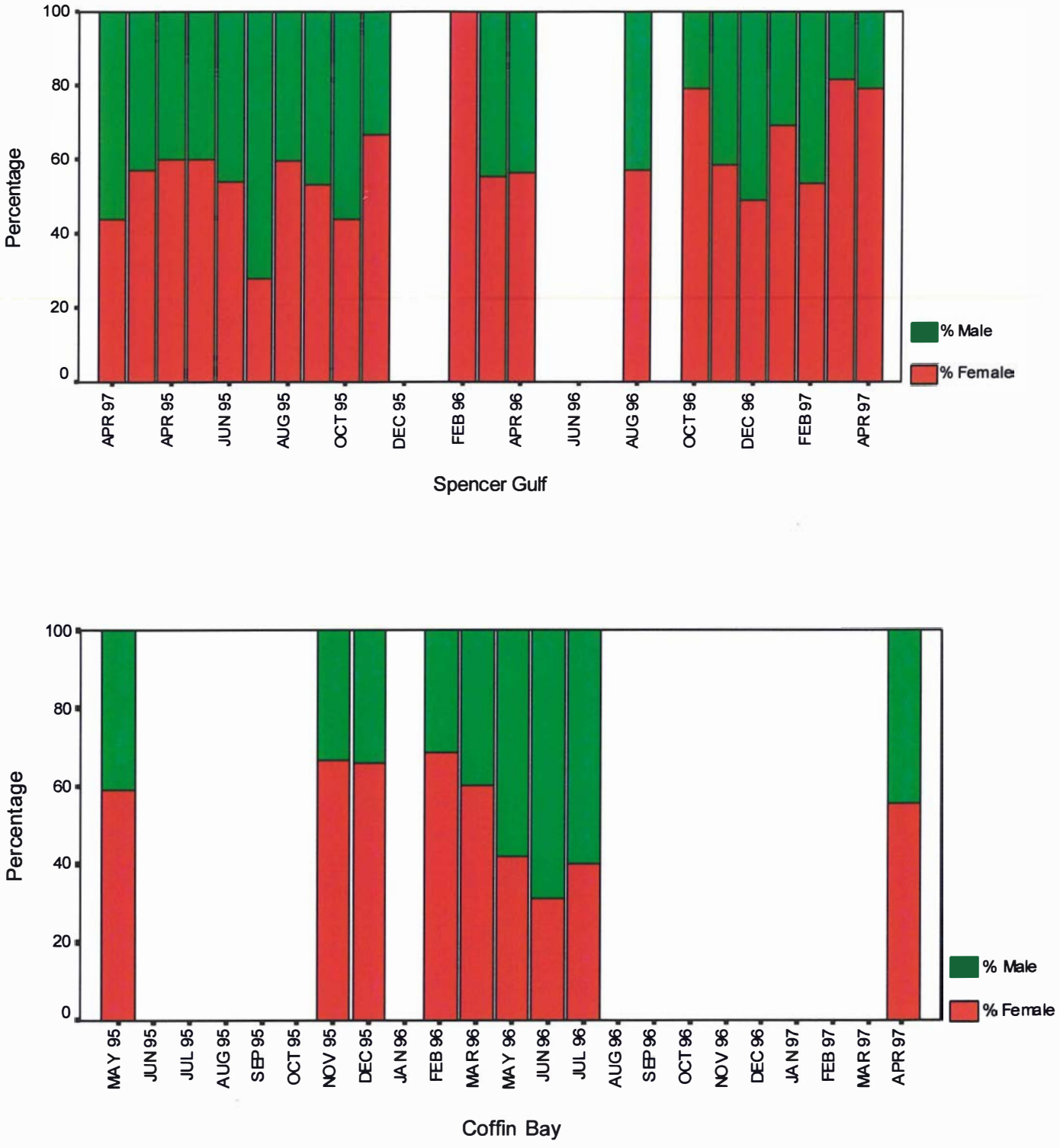
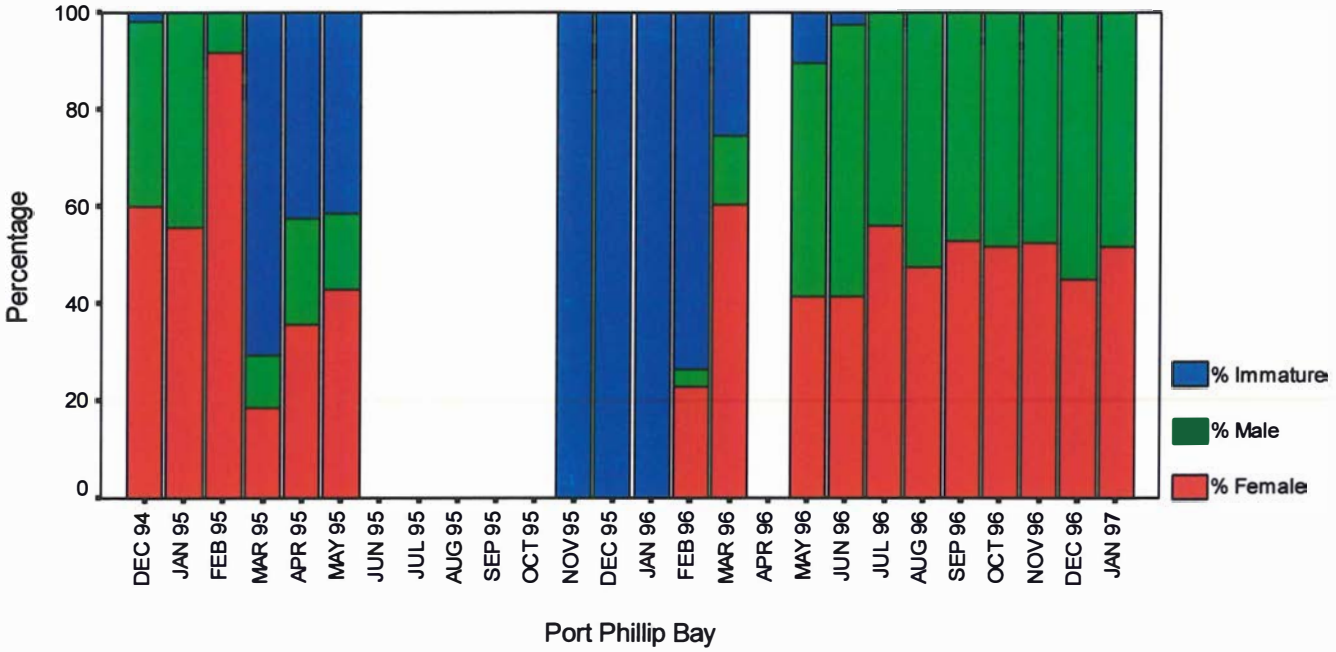


Figure 8.2 Proportion of immature male and female pilchards obtained from commercial catches in Spencer Gulf and Coffin Bay (South Australia) between May 1995 and April 1997.



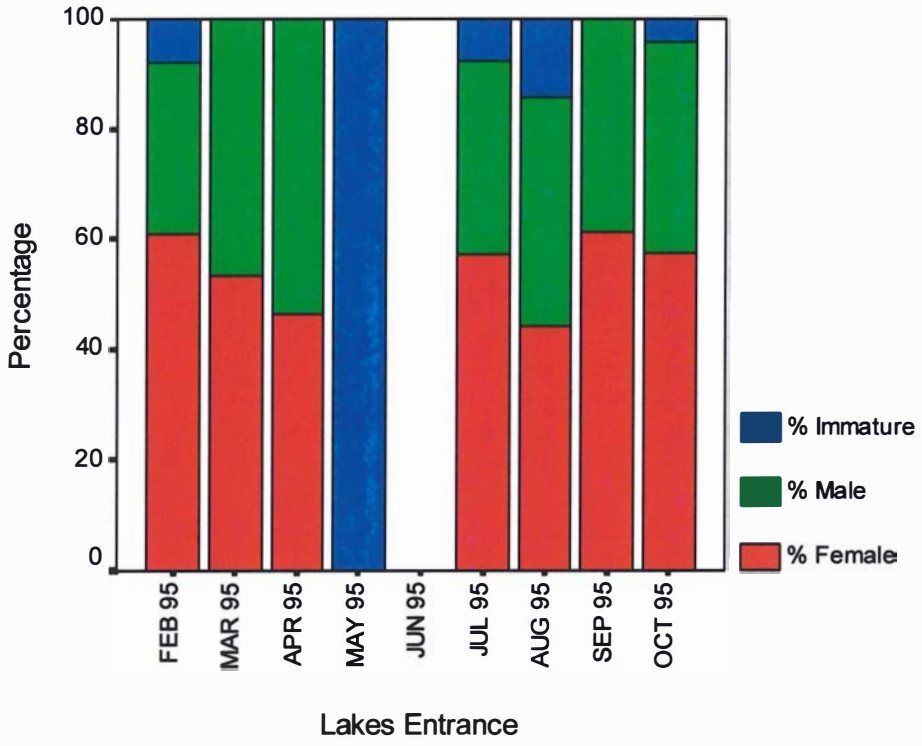


Figure 8.3 Proportion of males, females and immature pilchard obtained from commercial catches in Port Phillip Bay and Lakes Entrance (Victoria) between December 1994 and January 1997.

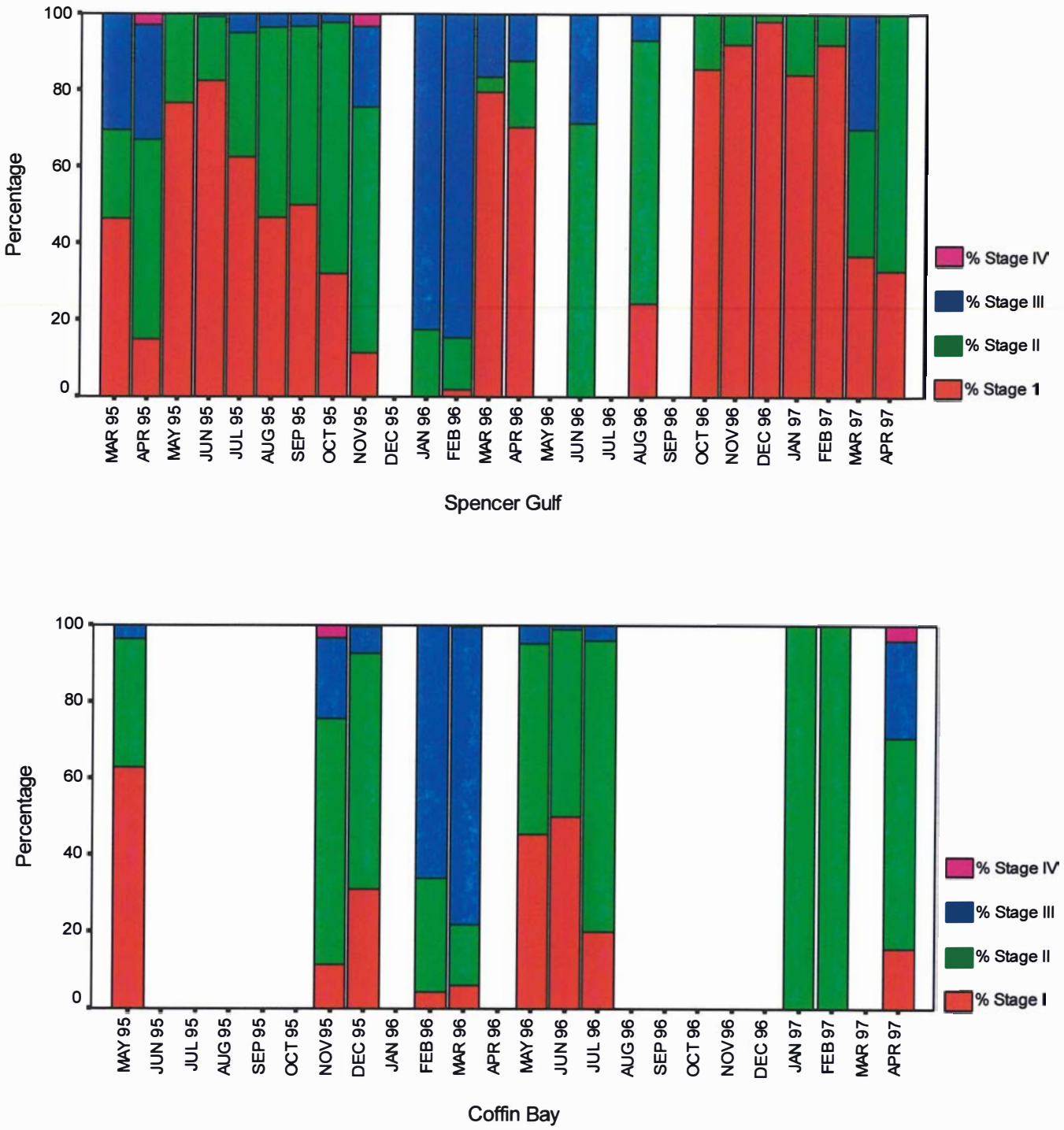
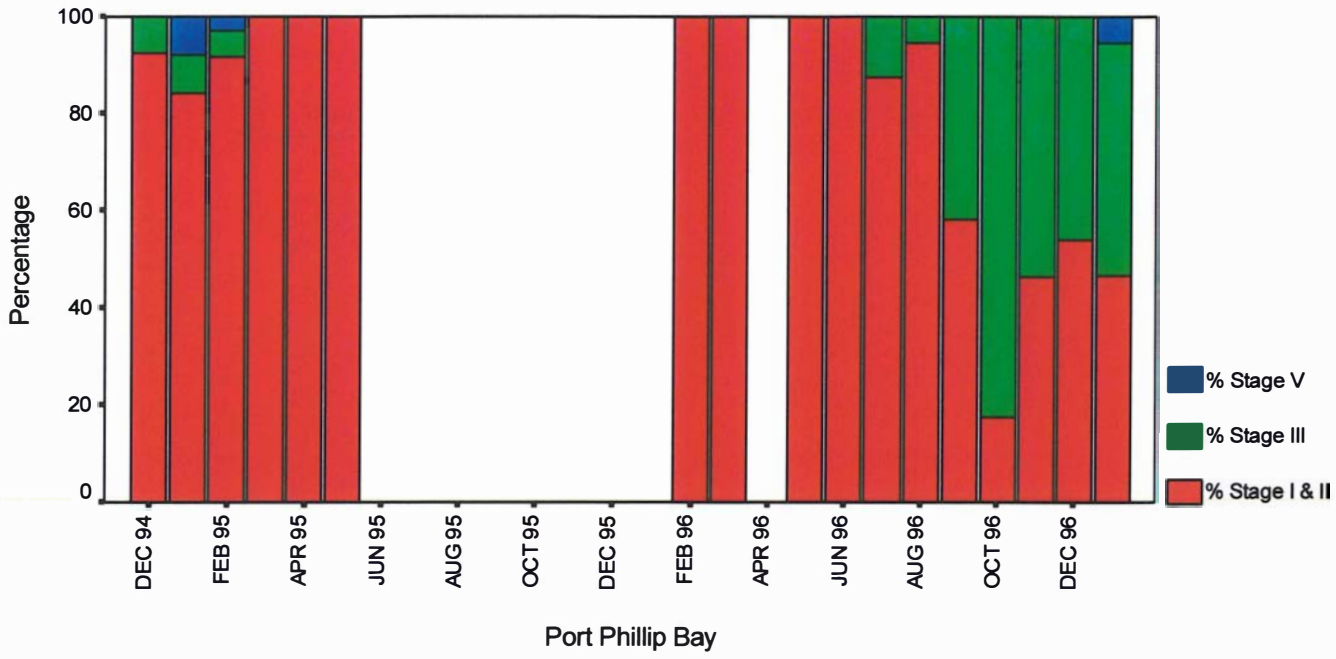


Figure 8.4 Proportion of the different gonadal stages of pilchards (males and females combined) obtained from commercial catches in Spencer Gulf and Coffin Bay (South Australia)between May 1995 and April 1997. (Gonadal stages described in the text).



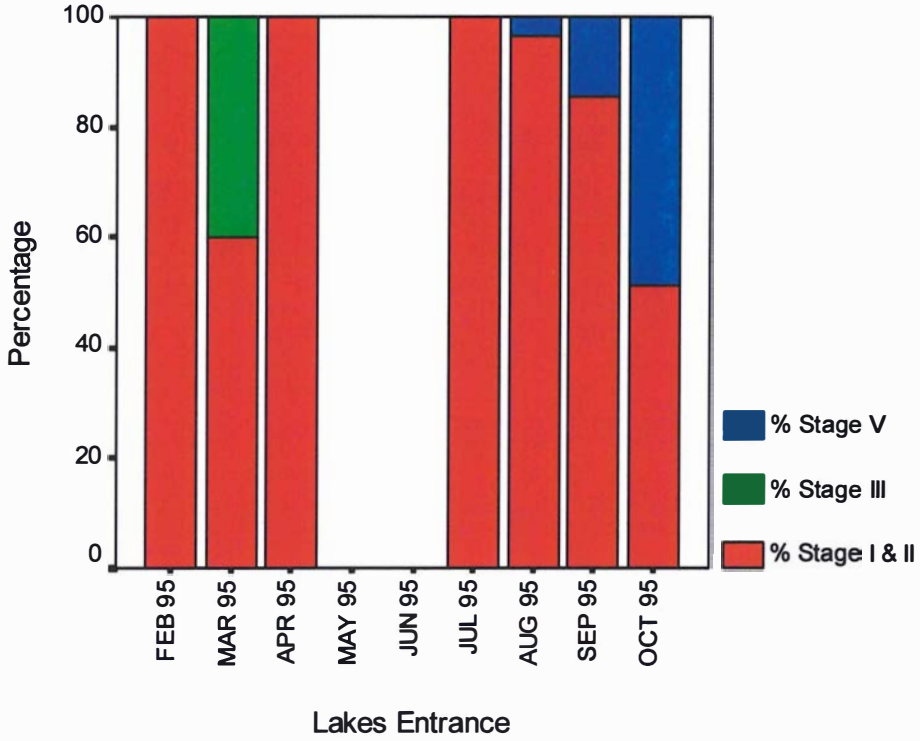


Figure 8.5 Proportion of the different gonadal stages of pilchards obtained from commercial catches from Port Phillip Bay and Lakes Entrance between December 1994 and january 1997. (NB Stages I and II are combined.)

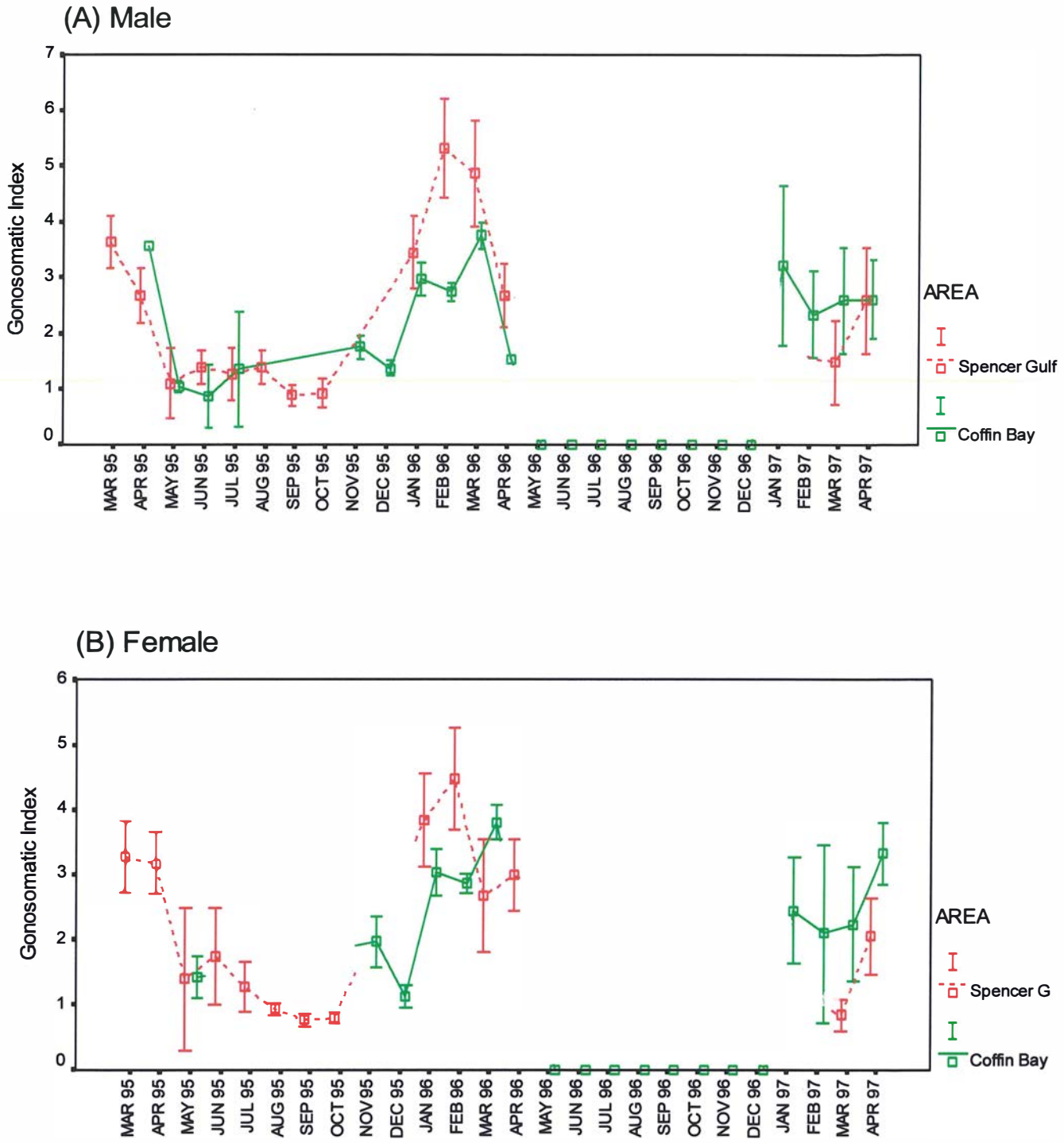


Figure 8.6 Mean monthly gonosomatic indices (± 2 SE) for (A) male and (B) female pilchards from Spencer Gulf and Coffin Bay (South Australia) between March 1995 and April 1997.

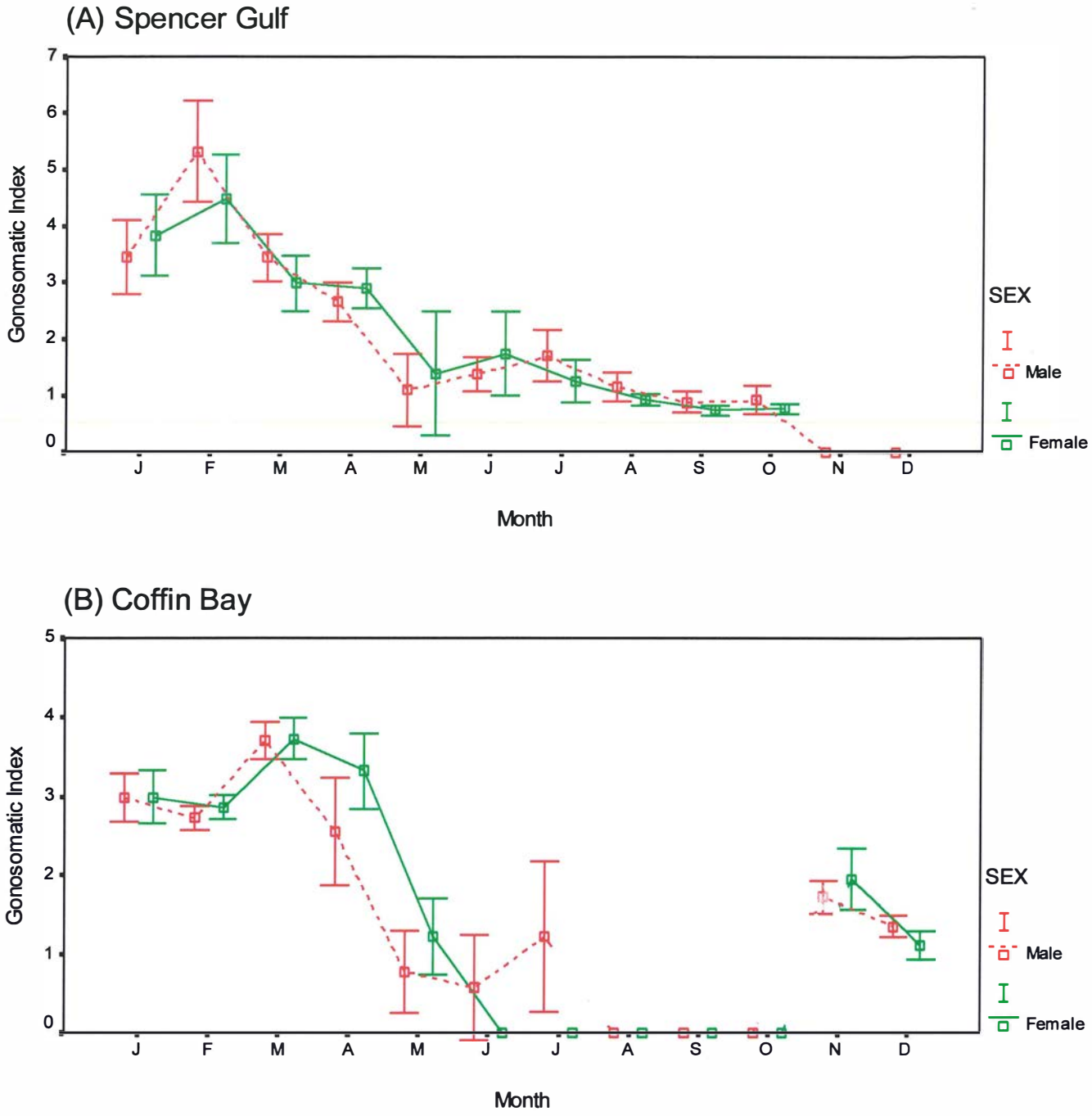


Figure 8.7 Mean monthly gonosomatic indices (± 2 SE) (pooled over years) for males and females in (A) Spencer Gulf and (B) Coffin Bay (South Australia) between 1995 and 1997.

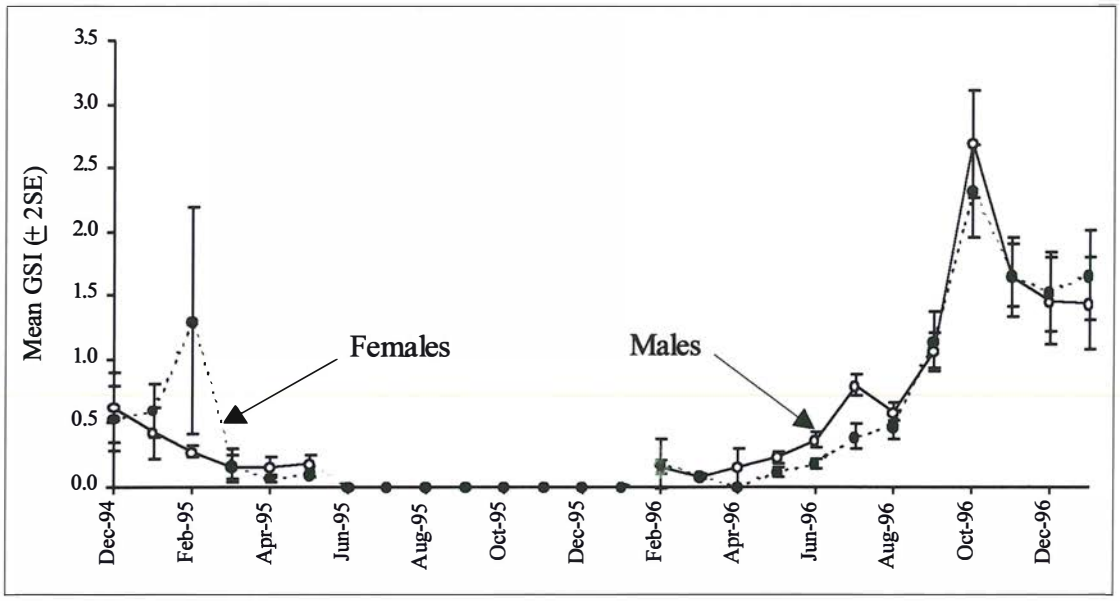


Figure 8.8 Mean gonosomatic indices (GSIs ± 2 SE) in male and female pilchards sampled from commercial catches in Port Phillip Bay between December 1994 and January 1997 (see Table 8.2 for number of fish examined each month). No samples were obtained between June and October 1995 and all fish examined between November 1995 and January 1996 were juveniles in which sex could not be determined.

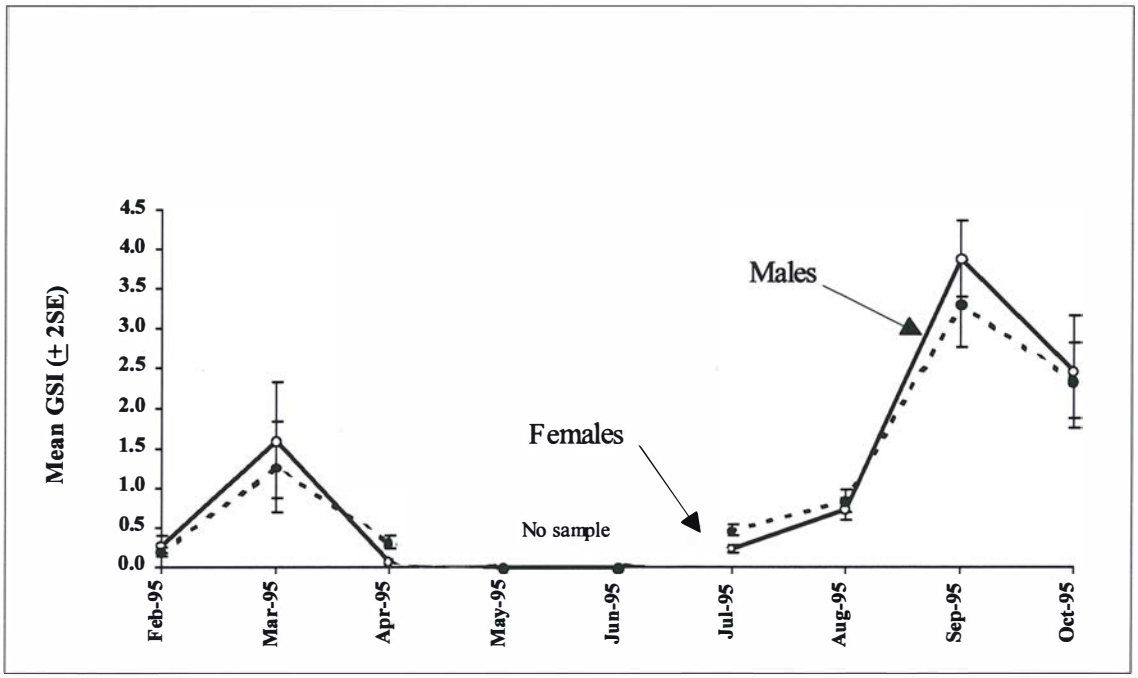


Figure 8.9 Mean gonosomatic indices (GSIs ± 2 SE) in male and female pilchards sampled from commercial catches in Lakes Entrance between February and October 1995 (see Table 8.2 for number of fish examined each month). No samples were obtained in June 1995.

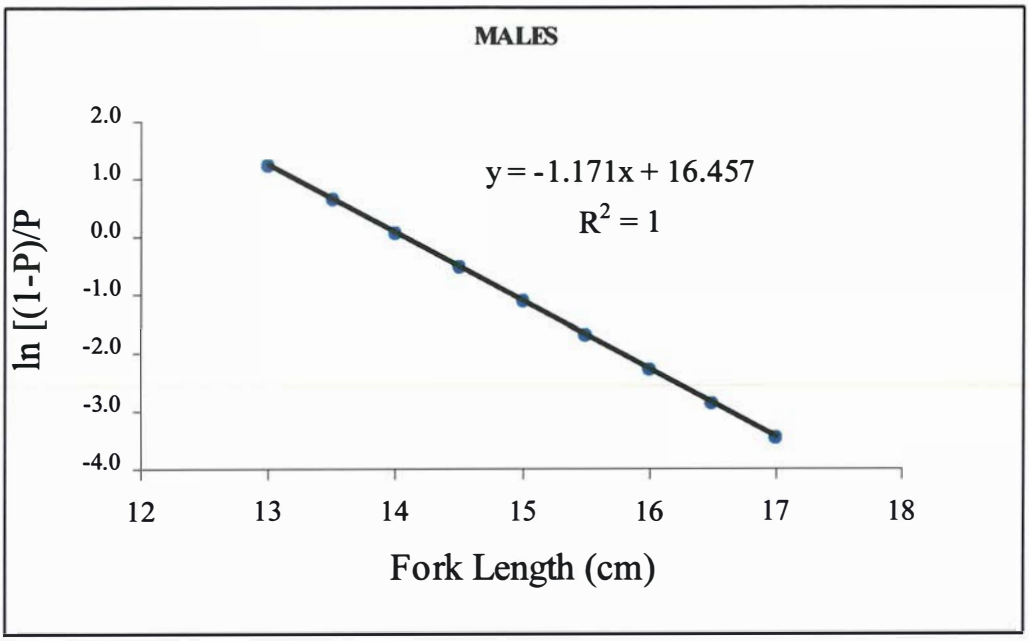


Figure 8.10 Relationship between fraction of male pilchard from South Australian waters that were sexually mature (stages II-V) and fork length (cm). Approximately 50% of males were sexually mature at 14.2 cm LCF (i..e. when ln [(1-P)/P] = 1)

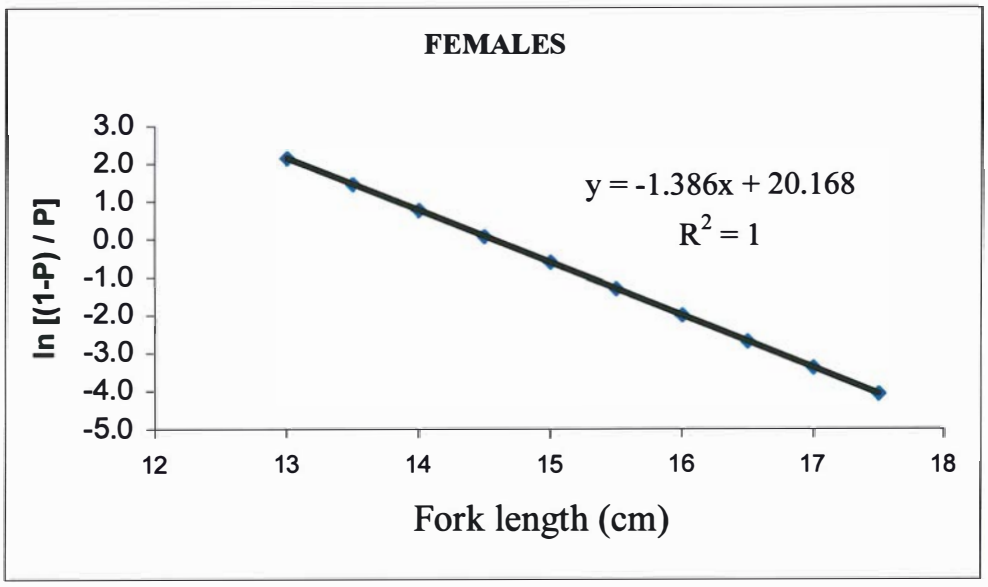


Figure 8.11 Relationship between fraction of female pilchard from South Australian waters that were sexually mature (stages II-V) and fork length (cm). Approximately 50% of the females were sexually mature at 14.8 cm LCF (i..e. when ln [(1-P)/P] = 1).

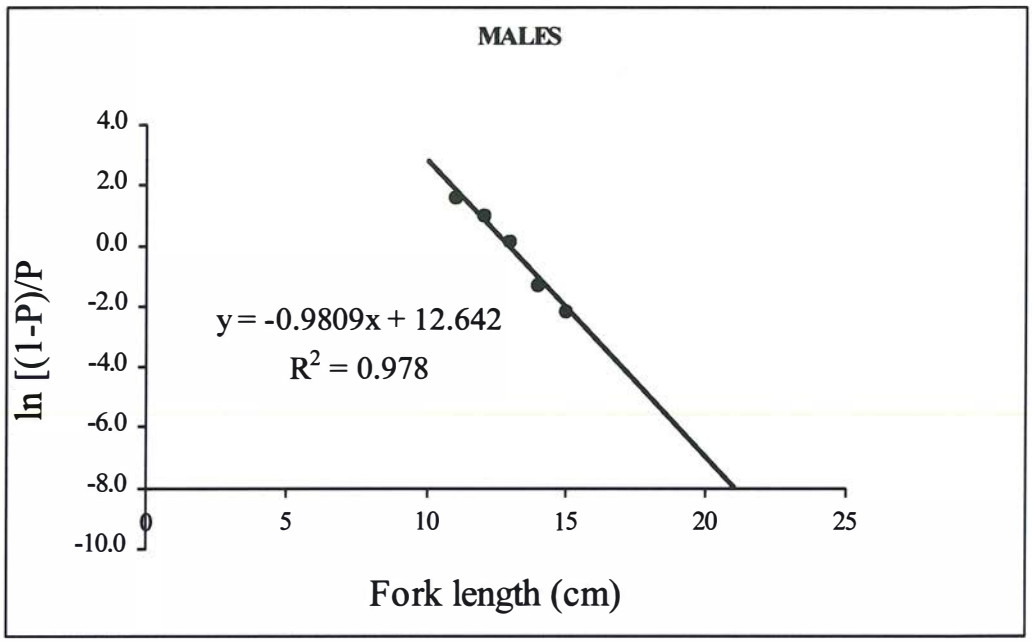


Figure 8.12 Relationship between fraction of pilchard males from Port Phillip Bay that were sexually mature mature (stages II-V) and fork length (cm). Approximately 50% of the males were sexually mature at 12.9 cm LCF (i..e. when ln [(1-P)/P] = 1).

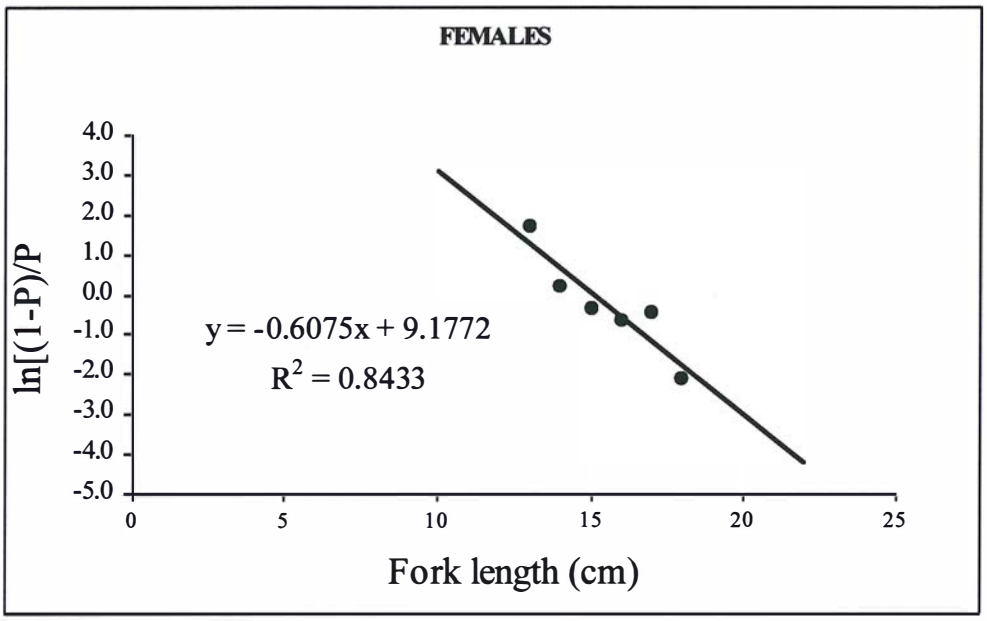


Figure 8.13 Relationship between fraction of pilchard females from Port Phillip Bay that were sexually mature (stages III-V) and fork length (cm). Approximately 50% of the females were sexually mature at 15.1 cm LCF (i..e. when ln [(1-P)/P] = 1).

Table 8.3. Samples used to obtain estimates of egg production in pilchards from South Australia in 1996 and 1997.

Year	Sample	Number Females	No. Hydrated	No. Migrated Nuclei	No. Day-0 POFs	No. Day-1 POFs	No. Day-2 POFs
Sub-Total (1996)	20	600	5	0	0	0	0
1997		22	2 (09.1)	0	4 (18.2)	3 (13.6)	2 (09.1)
		12	0 (00.0)	0	7 (58.3)	3 (25.0)	0 (00.0)
		59	0 (00.0)	2	12 (20.3)	2 (03.4)	1 (01.7)
		59	0 (00.0)	4	2 (03.4)	3 (05.1)	0 (00.0)
		57	0 (00.0)	15	2 (03.5)	3 (05.3)	0 (00.0)
		45	0 (00.0)	1	1 (02.3)	0 (00.0)	0 (00.0)
		134	0 (00.0)	0	0 (00.0)	2 (01.5)	2 (01.5)
Sub-Total (1997)	7	388	2	22	29 (15.14)	16 (7.7)	5 (1.8)
TOTAL	**27**	**988**	**7**	**22**	**29**	**16**	**5**

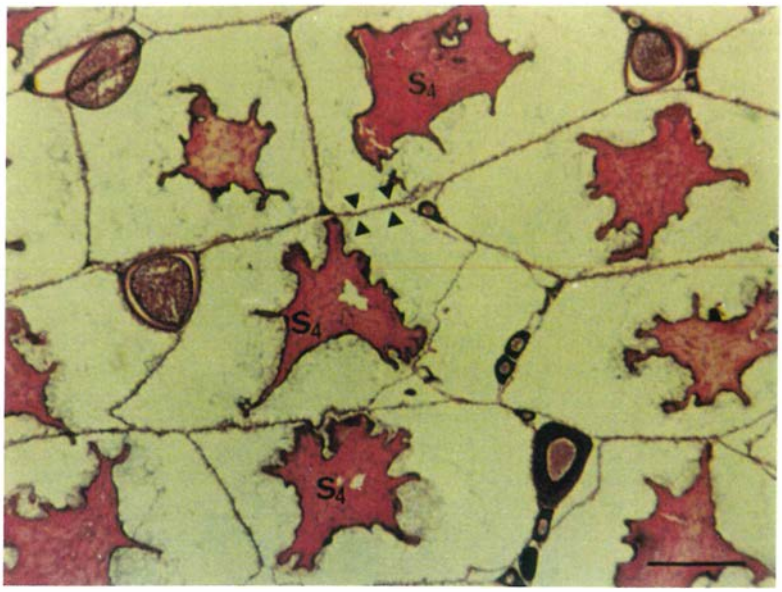


Figure 8.14 A light micrograph of a Stage IV ("ripe") pilchard ovary. Due to a processing artefact, Stage IV oocytes (S_4) shrink leaving an obvious empty spaces between the oocyte and the follicular layer (arrowheads) (Bar = 60 μm).

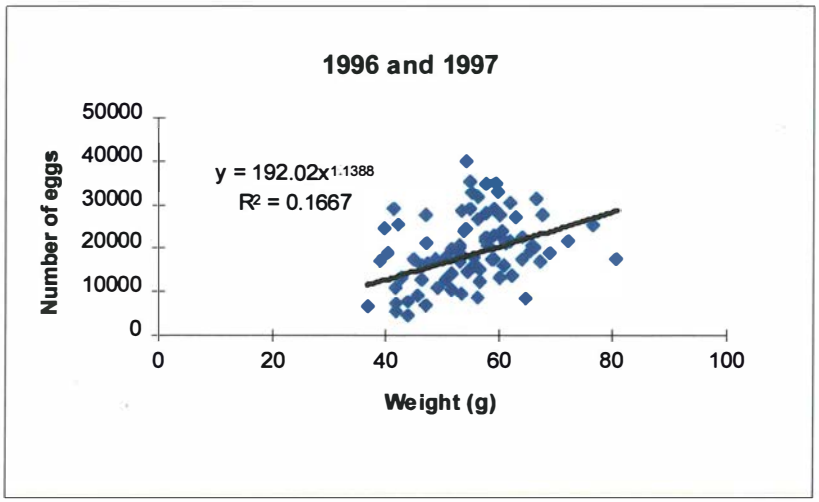


Figure 8.15 Number of eggs produced per gram of female body in South Australia (data for 1996 and 1997 combined).

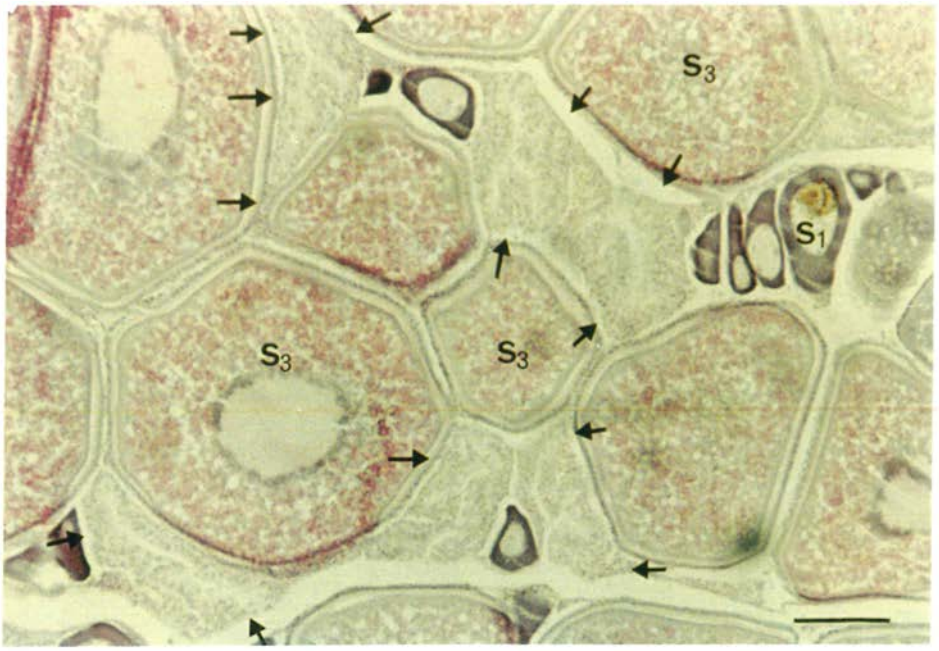


Figure 8.16 Light micrograph of a post-spawned pilchard ovary that was fixed in formalin. Post-ovulatory follicles (POFs) are bounded by arrows and interspersed with Stage I, II and III follicles (S_1, S_2, and S_3). (Bar = 10 mm).

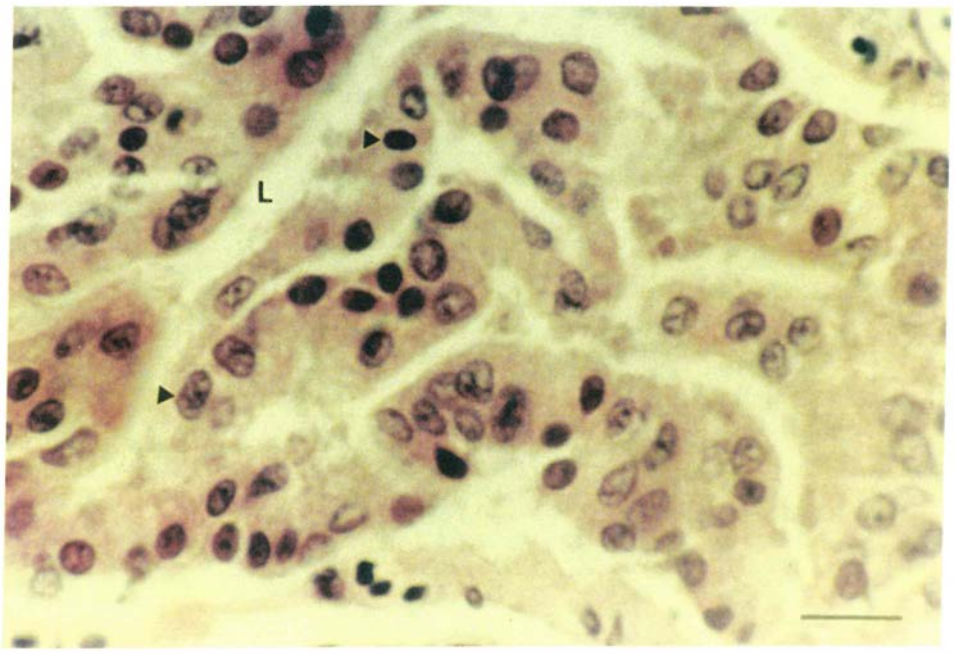


Figure 8.17 A light micrograph illustrating the morphological and histological appearance of a Day-0 post-ovulatory follicle (POF). Note the obvious irregularly-shaped lumen (L) and the granulosa cell layer (arrowheads). (Bars = 1.5μm).

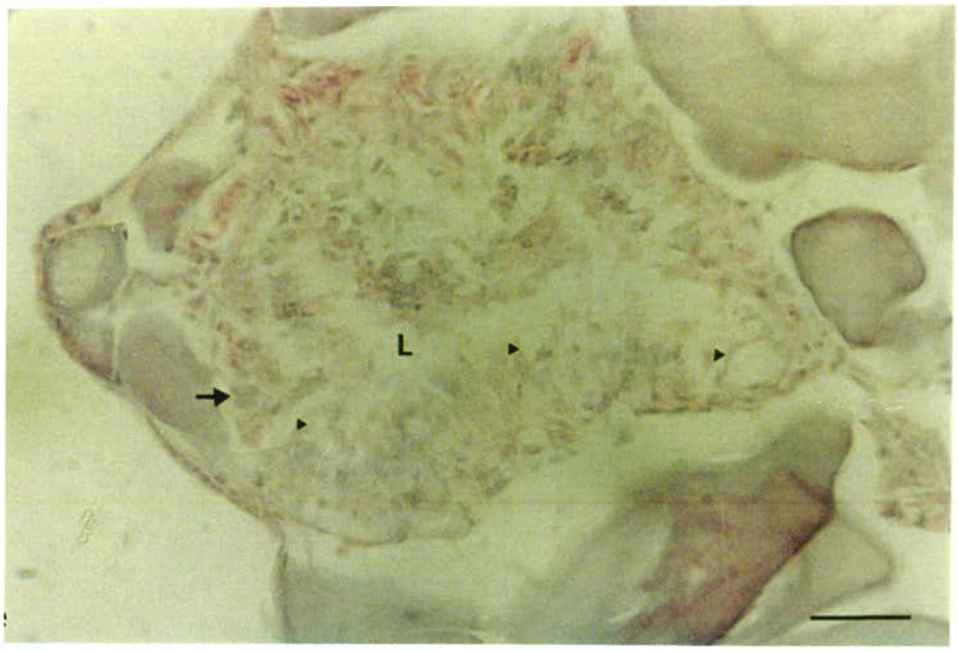


Figure 8.18 A light micrograph illustrating the morphological and histological appearance of a Day-1 post-ovulatory follicle (POF). Cell outlines are less obvious than in Day-0 POFs, but the lumen (L) remains distinguishable and vacuoles (arrowheads) are a common (Bars = 10 mm).

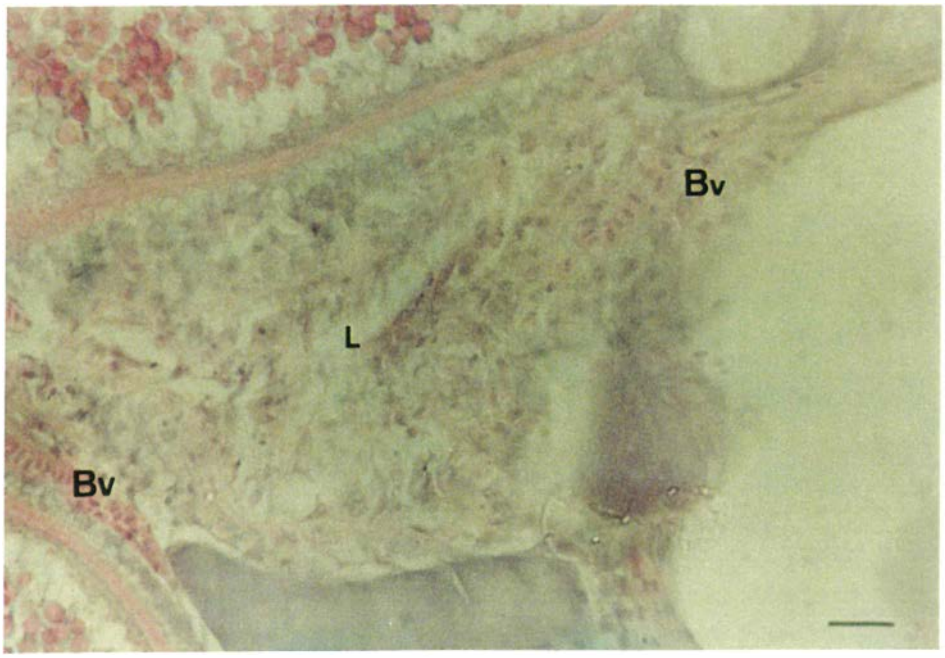


Figure 8.19 A light micrograph illustrating the morphological and histological appearance of a Day-2 post-ovulatory follicle (POF). The lumen (L) is much less distinct and the POF is similar in appearance to the surrounding ovarian stroma (Bars = 1.5μm).

CHAPTER 9. USE OF THE DAILY EGG PRODUCTIION METHOD TO ESTIMATE THE SPAWNING BIOMASS OF PILCHARDS IN SOUTH AUSTRALIAN WATERS.

M. Kinloch, F. Hoedt, T.M. Ward, G.K. Jones, G. Jackson , W. Dimmlich, and R. McGarvey

Objective (5): To evaluate the potential of using egg surveys for estimating the spawning biomass of pilchards in south-eastern Australia. Estimates of the spawning biomass of pilchards in waters of central and western South Australia were obtained from surveys conducted in 1995-97. Spawning biomass was estimated at approximately 59 000 t in 1995, 18 000 t in 1996 and 59 000 t in 1997. The low estimate for 1996 may reflect the mass mortality of pilchards that occurred in autumn 1995. Our confidence in the estimates obtained is limited by difficulties associated with acquiring reliable estimates of adult reproductive parameters, especially spawning fraction. This problem was mitigated by using a range of values for this parameter in calculations of spawning biomass and may recently have been overcome by the development of a fishery-independent sampling method.

9.1 Methods

Spawning Biomass Model

The equation for calculating spawning biomass using the daily egg production method (DEPM) is shown and discussed in Chapter 3.

Sample Collection

Plankton Surveys

The absence of spawning activity in Port Philip Bay precluded use of the DEPM for the Victorian fishery (see Chapter 7).

Egg surveys were conducted from the *FRV Ngerin* in 1995, 1996 and 1997 in continental shelf waters between Cape Jervis [138° 5′ E, 35° 36′ S] and the head of the Great Australian Bight [131° 09′ E, 31° 28′ S]. Plankton samples were collected from sites located at 5 to 10 nautical miles intervals along a series of parallel linear transects that were 10 to 20 nautical miles apart (Figure 9.1; Table 9.1). In 1995 and 1996, transects were orientated in a North-South direction but in 1997 they were aligned NE-SW in order to improve sampling efficiency. In 1995, surveys were conducted during 12-19 January and 4-10 March and were analysed as both two (independent) surveys and one (combined) survey. In 1996 and 1997, surveys were conducted between mid-January and late March (late summer) in two and three relatively contiguous sampling periods respectively.

Plankton samples were collected using paired conical (CALVET ®) plankton nets (internal diameter 0.285 m) deployed to within 5 m of the substratum (in waters <70 m deep) or to a depth of 70 m (in waters >70 m deep). The net was retrieved vertically at a speed of approximately 1 metre/second. The actual distance travelled by the net was calculated using calibrated flow meters (one in each net). The sample from each net was stored in 5% buffered formaldehyde. Preliminary analyses indicated that each net caught similar numbers, so at each site the samples from the two nets were placed into a single container.

At each egg sampling station sea surface temperature and salinity data were also collected in order to examine relationships between biological and environmental variables.

Adult Reproductive Parameters

Samples of adult pilchards used to estimate the reproductive parameters were collected at or around the same time as the egg surveys. Details regarding the sizes and sources of samples used to estimate each parameter are summarised in Table 9.2.

In 1995 and 1997, pilchards were obtained as frozen samples from the routine commercial catch monitoring program (see Chapter 4). In 1996, these samples were supplemented with adult fish collected between January and April from research-chartered commercial vessels and preserved in formaldehyde on-board the vessel (see Chapter 7).

In 1997, additional samples were also obtained using a mid-water trawl net deployed from the *FRV Ngerin* (Dotson and Griffith 1996). Trawling was conducted at night in areas where pilchard schools could be seen either at the surface or on the ship's echo sounder. The net was towed at approximately 4 knots for periods of 1 to 2 hours.

Sample Processing

Egg Production

Eggs were preserved in buffered formaldehyde solution. Pilchard eggs in each plankton sample were counted and staged according to the criteria of White and Fletcher (in press).

Adults

Methods used to process pilchards for the estimation of adult reproductive parameters are described in Chapter 7.

Data Analysis

Time of spawning

Pilchard eggs collected in 1995 and 1996 were aged using a temperature-development key (White and Fletcher, in press), assuming that seawater temperature recorded at the time of sampling was equal to the ambient temperature experienced by eggs during their development. Samples comprised only two age-groups of eggs, one from the previous night's spawning (day-0) and the other from spawning two nights previous (day-1). Estimates of age and the time of sample collection were used to calculate the time each batch was spawned.

Egg Production

The survey area was post-stratified into two areas based on egg distribution: Area 1 (A_1) which included all the positive (eggs present) stations with a few embedded negative (eggs absent) stations; and Area 0 (A_0) which contained all the negative stations outside of Area 1 (Picquelle and Stauffer 1985). The total area of each stratum and the area represented by each station were calculated using *MapInfo* computer software.

Ages presented for eggs are averages, obtained using regression analysis, and were therefore adjusted for each batch using information on sample collection time and population spawning time following the method of Lo (1985). The following formula was used to adjust the age of eggs:

$$\text{Final age} = \text{age (1)} + k + T \qquad (1)$$

Where age (1) is age determined from the temperature-development key, k is the time of the tow/sample, T is the expected time for observing eggs of a particular age (assuming that spawning occurred at 0130 hours).

Egg counts in each hourly age category at each station were converted to density units (number of eggs per $10m^2$ of sea-surface area) using the equation provided by Smith and Richardson (1985). These station egg densities were then weighted according to the area represented by each, as outlined in Picquelle and Stauffer (1985). Egg density at age is assumed to follow the exponential (constant) mortality model:

$$P_t = P_0 * e^{(-Z * t)} \qquad (2)$$

Where P_0 is daily egg production, P_t is egg abundance (density) at age t and Z is the instantaneous rate of daily egg mortality.

Weighted egg density was regressed against age using a non-linear fitting procedure to give estimates of P_0 and Z.
The best estimate of Z for 1995 was obtained using the combined (i.e. January and March) survey data, therefore this value was used to calculate egg production (P_0) for both the individual and combined surveys.

Before regressing egg density against age for Area 1 to obtain an estimate of P_0,, it was necessary to truncate the data for young and old eggs which are known to be under-represented in samples. Newly spawned (Stage 1 and Stage 2) eggs have not yet fully recruited into the plankton (Armstrong *et al.* 1988) and were extremely rare in the samples, Stage 3 eggs were present in some samples. Eggs reach stage 3 between 2.6 and 5.5 hours after spawning (at temperatures of 17.5 - 19.5° C). For the Pacific sardine (*S. sagax*), 3 hours is the minimum egg age used in the mortality model (N. Lo, personal communication) and this threshold was adopted for the current analysis also (although the bias probably extends to slightly older eggs). Eggs classed as stage 12 are near hatching and their abundance is biased by the loss of hatched eggs. Accordingly, in 1995, egg density data relating to eggs < 3 hours and >34 hours (age of the youngest stage 12 egg collected) old were not used in the exponential mortality model. In 1996, very few eggs under 8 hours of age were collected, again suggesting biased sampling of the younger eggs. Therefore, in 1996, data pairs where eggs were younger than 8 hours and older than 41 hours (the youngest stage 12 eggs) were truncated.

In 1997, due to problems with preservation of the egg samples on board ship, it was not possible to age the eggs by means of a temperature-development key as in previous years. This necessitated the use of a different analytical technique for estimating initial daily egg production, based on the mean density of eggs of all ages and an assumed value of egg mortality (Z). The mean density of eggs of all ages, which includes eggs from the first and the second days after spawning, can be expressed as:

$$\overline{P} = \frac{\int_{t=0}^{1} P_t \cdot dt + \int_{t=1}^{2} P_{t+1} \cdot dt}{\int_{t=0}^{1} dt}. \qquad (3)$$

$\overline{P}$ was calculated in the same way as were the mean densities of aged eggs described above. By substituting the exponential mortality equation describing the change in egg density with age (Equation 2) into Equation 3 and solving, the formula for initial egg production, P_0, is obtained:

$$P_0 = \frac{\overline{P}}{\int_{t=0}^{1} \left(e^{-Z \cdot t} + e^{-Z \cdot (t+1)}\right) \cdot dt}. \qquad (4)$$

By entering the estimate of $\overline{P}$ obtained from the survey data and an assumed value of Z, initial egg production can be calculated from Equation (4). In selecting a value of Z to input into this procedure, results from the previous two years were examined and the sensitivity of estimates of egg production to a range of Z values from 0.2 to 0.6. was tested (see Table 9.3).

Sex Ratio and Mean Female Weight

Sex ratio and mean female weight were estimated from samples obtained from the catches of commercial vessels taken during the spawning season (see Chapter 7 and Table 9.2). The average weight of reproductively active females (those having Stage II-V gonads) and the ratio of female weight to the total weight of males and females was calculated for each sample. The weighted mean values of both parameters were calculated according to the methods of Picquelle and Stauffer (1985).

Spawning Fraction and Batch Fecundity

Histological methods used to estimate batch fecundity and spawning fraction are described in Chapter 7. Linear and power curves were fitted to the regression of batch fecundity against whole weight (i.e. including gonads) and clean weight. Batch fecundity of each mature female and mean batch fecundity were estimated using the methods of Picquelle and Stauffer (1985). Spawning fraction was estimated from the mean occurrence of Day-0 and Day-1 POFs in each sample (Macewicz *et al.* 1996; Akkers *et al.* 1996). Mean spawning fraction was estimated from the unweighted mean of sample values (Chapter 7).

9.2 Results

Spawning Time

In 1995 and 1996, most spawning occurred in the period between midnight and dawn. In 1995, the highest number of batches were spawned between 0200 and 0500 hours and in 1996 there was a clear peak at 0130 hours. Data for the two years were combined to improve sample size and allow the estimation of a single spawning time. The number of egg batches spawned at hourly intervals for the

combined data for 1995 and 1996 are shown in Figure 9.2. This shows that pilchards spawn largely during the hours of darkness, with most batches originating between 1900 and 0900 hours. The highest number of batches originated between 0100 and 0200 hours (midpoint 0130 hours) (Figure 9.2). This time (0130 hours) is also the midpoint between 1900 and 0900 hours, the period of spawning and was therefore used as the 'time of spawning' in calculations of egg production and biomass.

Egg Production

The general pattern of spawning activity, and the density, extent and production of eggs on the spawning grounds varied between years (Figure 9.1; Table 9.1). In 1995, the spawning grounds covered 8.5 x 10^4 km^2, but in 1996 and 1997 spawning activity was restricted to areas of only 3.4 x 10^4 and 3.3 x 10^4 km^2 respectively. In 1995, the regression of egg density on age yielded estimates of daily egg production of 43 and 288.3 eggs/10m^2 for the surveys conducted in January and March respectively, and of 164.6 eggs/10m^2 for the pooled data (Z = 0.431 $\pm$ 0.61 SE) (Table 9.3). In 1996, daily egg production was slightly lower at 136.7 eggs/10m^2 and egg mortality (Z) was only 0.297. The highest levels of mean egg density and daily egg production were recorded in 1997 when egg mortality values of between 0.2 and 0.6 resulted in estimates of daily egg production ranging from 422.9 to 598.6 eggs/10m^2 (Table 9.3).

Sex Ratio

Estimates of sex ratio ranged from 0.51 to 0.58 (Table 9.2).

Mean Female Weight

Mean female weight did not vary significantly between years and was estimated at 42.9 g in both 1995 and 1997 and 46.3 g in 1996 (Table 9.2).

Spawning Fraction

Difficulties collecting representative samples of mature fish prevented reliable estimation of spawning fraction in 1995 and 1996. In 1995, all samples were obtained from the commercial fishery were frozen on landing: no specimens were preserved in formaldehyde and none were suitable for examination of post-ovulatory follicles. In 1996, post-ovulatory follicles were not found in any of the mature females collected. Published values of spawning fraction were examined to obtain a range of values typical for temperate sardines. Estimates of spawning fraction of *S. sagax* ranged from 0.02 to 0.21. A spawning fraction of 0.18 was estimated for pilchard in Western Australia during the period

of peak spawning (Fletcher *et al.* 1996a, 1996b). In 1995 and 1996, spawning fractions between 0.08 and 0.2 were used to calculate the spawning biomass.

In 1997, two midwater trawl samples and three commercial fishery samples contained fish with post-ovulatory follicles. There was large variability in the spawning fraction values obtained for each sample (Chapter 7) and the estimate of mean spawning fraction (0.156) was thus imprecise (coefficient of variation = 0.99).

Batch Fecundity

Batch fecundity was not strongly correlated with female weight (see Chapter 7). Power curves had a slightly higher correlation coefficient than linear regressions when fitted to both whole and clean weight. The correlation coefficient was higher when fecundity was regressed against whole weight. The relationship between the two variables was similar in 1996 and 1997 so for 1997 a new relationship was derived from the pooled data:

$$F = 192.02 \times \text{weight}^{1.139} \qquad r^2 = 0.167 \qquad (n = 84)$$

Mean batch fecundity in 1997 of 13,947 eggs was calculated from 450 mature females.

Spawning Biomass

The parameters used in calculations of spawning biomass in 1995, 1996 and 1997 are given in Tables 9.1, 9.2, 9.3, 9.4 and 9.5. In 1995 spawning biomass was individually calculated for the January and March surveys and then summed to give a total for 1995. A wide range of possible estimates of spawning biomass were calculated for each year: approximately 38000 - 95 000 t in 1995, 11 000 - 28 000 t in 1996, 51 232 - 72 517 in 1997 (Table 9.6). Best estimates of spawning biomass for 1995, 1996 and 1997 were 58 613 t, 18 112 t and 58 725 t respectively. The effects of variation in egg mortality, spawning fraction and batch fecundity on estimates of spawning biomass for 1997 are shown in Tables 9.3, 9.4 and 9.5 respectively.

9.3 Discussion

Evaluation of the DEPM

One of the major logistical constraints of the DEPM is the large number of samples required to obtained precise estimates of egg production. In the large clupeoid fisheries off California and South Africa, between 200 and 900 plankton samples per annum are routinely collected. Sampling programs of this scale require a large vessel and extensive staff. Limitations in the numbers of plankton samples collected in the present study restricts our confidence in the estimates of spawning biomass. For example, areas south and east of Kangaroo Island were under-sampled due to the occurrence of bad weather and limitations in the availability of the FRV Ngerin. Furthermore, investigations of temporal variations in egg abundance that would have enhanced understanding of the precision of estimates of egg abundance were not be conducted because of the real and pressing need to (i) sample the entire spawning area and (ii) provide estimates of spawning biomass required for the management of a rapidly expanding fishery.

Egg mortality rates (Z) are needed to convert of estimates of egg density into estimates of daily egg production, but are difficult to estimate precisely. For example, Armstrong (1991) found that 95% confidence limits around Z often include zero (i.e. no egg mortality) and that uncertainty in estimates of Z introduce the largest source of variability into biomass estimates. Results obtained in this study confirm the difficulties associated with estimating egg mortality, but sensitivity analyses suggest that Z-values have relatively minor effects on estimates of initial egg density. For example, in 1997 a threefold increase in Z from 0.2 to 0.6 resulted in only a 41% increase in estimates of initial daily egg production from 422.9 to 598.6 (Table 9.4). A different approach to sampling may be required to reduce the effects of patchiness on the coefficient of variation of estimates of Z (see Lo et al. 1996). Pilot studies conducted in 1998 suggest that the collection of time series data from one or (preferably) several sites may allow the mean abundance of cohorts to be followed through time and allow more reliable estimation of egg mortality.

Interannual fluctuations in total egg production mainly reflected variations in spawning area and daily egg production. In 1995, the large spawning area and moderate level of daily egg production resulted in a total egg production value of 1.4×10^{12} eggs, whereas in 1996 the spawning area was 60% smaller, daily egg production fell by 17%, and total egg production was only 4.7×10^{11} eggs. In 1997, the spawning area was less than half the size it was in 1995, and slightly (1%) smaller than in 1996, but the high level of daily egg production (195% higher than 1995) resulted in a total egg production value of 1.6×10^{12} eggs. Confidence in estimates of spawning area and daily egg production would be

enhanced by the acquisition of data from cruises conducted from throughout the spawning season and by the use of continuous underway fish egg sampler (Lo 1997).

In this study, the acquisition of the samples of mature fish required for estimation of spawning fraction and batch fecundity posed significant problems. Pre- and post-spawning fishes were rarely caught by commercial purse-seine vessels. Mid-water trawling procedures routinely used in South Africa and California proved unsatisfactory, perhaps because of the relatively small size of most of the pilchard schools that were encountered during egg surveys. Future attempts to obtain representative samples of adult fish will benefit from the development of a new sampling method that involves the use of powerful surface and sub-surface lights and a multi-panel gillnet (T. Ward, in preparation).

The ratio of female weight to total weight (males and females) ranged from 51 to 58%. Workers in California and Western Australia have provided estimates of 54% and 58-65% respectively for this parameter. The higher relative weight of females mainly reflects their higher abundance in samples. It unknown whether this is due to differential catchability of males and females in purse seine nets or to real differences in the sex ratios of populations.

Estimates of mean female weight ranged from 42.9 to 46.3g. These are similar to estimates obtained from pilchards in Western Australian waters, i.e. 34.1 to 47.5g (Fletcher 1996a, 1996b), but are considerably lower than estimates of mean female weight from California (82.5g) or Namibia (135g) (Le Clus 1988; Macewicz *et al.* 1996). It is presumed that the smaller size of Australian pilchards can directly attributed to related to the relatively low nutrient levels which characterise Australian seas (Kailola *et al.* 1993).

No estimates of spawning fraction were obtained in 1995 and 1996, and a range of values (0.08-0.2) were used to calculate spawning biomass (Tables 9.2 and 9.6). In 1997, 6 samples provided estimates of spawning fractions between 0.01 and 0.375, with a mean of 0.156 and a coefficient of variation of 0.99 (Table 9.2). Values of 0.14 to 0.19 have been recorded for *S. sagax* in Western Australia (Fletcher et al. 1996) and Chile (Retamales and Gonzalez, 1983; Olivia *et al.* 1989) whereas estimates currently available for Californian and Namibian waters suggest that less than 10% of females spawn each night (Le Clus 1988; Macewicz 1996). Variations in spawning fraction between 0.12 and 0.20 resulted in estimates of spawning biomass for 1997 that ranged between 91 685 and 152 809 tonnes.

Estimation of batch fecundity was hindered by difficulties in acquiring Stage IV females (i.e. ovaries with hydrated oocytes). Attempts to use tertiary oocytes and oocytes with migratory nuclei to estimate

fecundity were only moderately successful. Neither method has been shown to provide reliable estimates of the number of eggs in a batch. The low correlation between batch fecundity and fish weight in 1997 almost certainly reflects the small number and narrow size range of females with hydrated oocytes that were collected. In contrast, Macewicz *et al.* (1996) obtained a correlation coefficient (r^2) of 0.92 for the regression of batch fecundity against clean weight. This sample was based on 51 fish of broad range of sizes. Le Clus (1988) however was only able to obtain an r^2 of 0.31 based on a sample size of 212 fish and a weight range of 90–170 g. Mean batch fecundities of between 12 000 and 16 000 eggs caused estimates of spawning biomass for 1997 to fluctuate between 102 378 and 136 504 tonnes.

The fluctuations in annual spawning biomass suggested by data shown in Table 9.6 are typical of stocks of clupeoid fishes throughout the world (e.g. Kawasaki *et al.* 1991). Although biomass estimates provided are relatively imprecise, examination of the effects of poorly quantified parameters (egg mortality, spawning fraction and batch fecundity), suggests that the general trends in pilchard abundance are real. The fall in spawning biomass between 1995 and 1996 probably reflects the effects of the mass mortality event that occurred in late 1995. The increase in spawning biomass between 1996 and 1997 presumably reflects the stocks recovery from this event.

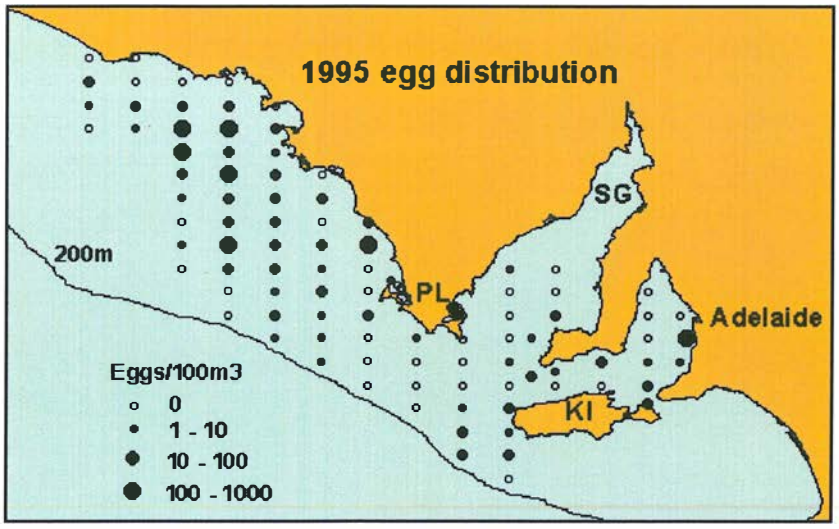
The DEPM is the method of choice for stock assessment in some of the world's largest fisheries (e.g. Parker 1985; Somerton 1990; Alheit 1993). It provides more reliable estimates of the biomass of clupeoid fishes than can be obtained using other methods, especially for newly exploited stocks for which little biological or ecological information are available. A major disadvantage of the method is that the accuracy and precision of estimates of some parameters are often low, and that confidence in estimates is further reduced by the multiplicative method used in spawning biomass calculations. Overseas studies have shown, however, that these problems can usually be overcome if the method is applied over several (5 to 10) years, and that uncertainty of estimates can be largely negated by the use of a range of values for unreliable parameters and by setting conservative exploitation rates.

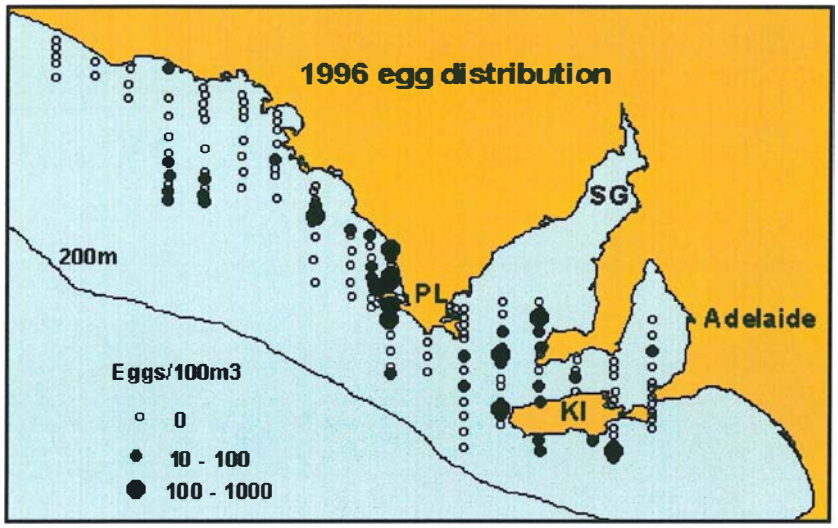
Sea Surface Temperatures

Results obtained in this study suggest the existence of a relationship between pilchard spawning patterns and surface water temperatures (Figure 9.3). Areas of high egg density were generally located in cooler waters, usually of less than 19.5° C. The difference between years in the patterns of egg distribution seem to reflect, at least in part, differences in the size of these areas of cool water. In 1995, a tongue of cool water extended over the entire continental shelf between south-eastern Kangaroo Island and the head of the Great Australian Bight, and pilchard eggs were found throughout

this region. In 1996, patches of eggs were located mainly found over discrete cells of cool water around Coffin Bay, west of Streaky Bay and off the western tip of KI. There was also a concentration of eggs in the frontal zone which forms at the mouth of Spencer Gulf in late summer (Bruce and Short 1993). In 1997, the distribution of cool waters and pilchard eggs were largely restricted to the coastal waters along western Eyre Peninsula and to small cells off the tip of Kangaroo Island and Yorke Peninsula.

The areas of cool water depicted in Figure 9.3 are thought to result from a poorly understood seasonal upwelling event that occurs in waters of central and western South Australia during late summer and autumn. It is well known that the spawning grounds of pilchards (and other clupeoids) are often located in upwelling regions where nutrient levels and primary productivity are high, presumably because these conditions maximise the growth rates and survivorship of larvae and juveniles (e.g. Curry and Roy 1988; Muck 1989). Future studies of the relationship between oceanographic patterns, levels of primary and secondary production, patterns of distribution and energy allocation of adult pilchards, and relative abundance and survivorship of pilchard eggs larvae and juveniles will enhance understanding of annual fluctuations in spawning biomass of pilchards and the function of the pelagic ecosystem of waters of central and western South Australia.





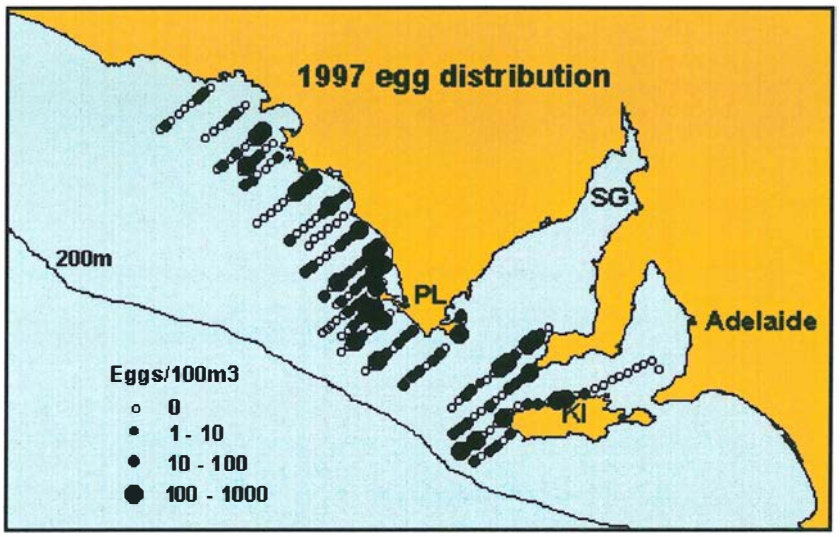


Figure 9.1 Location of sampling sites and eggs densities (eggs/100m^3) collected during 1995, 1996 and 1997.

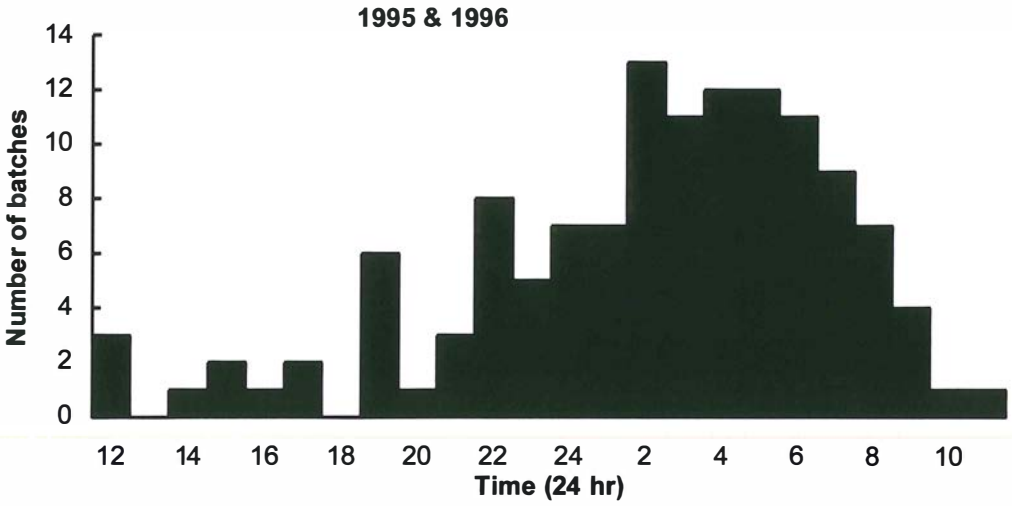


Figure 9.2 Numbers of pilchard egg batches for hourly intervals from the 1995 and 1996 surveys. Total number of egg batches was 127.

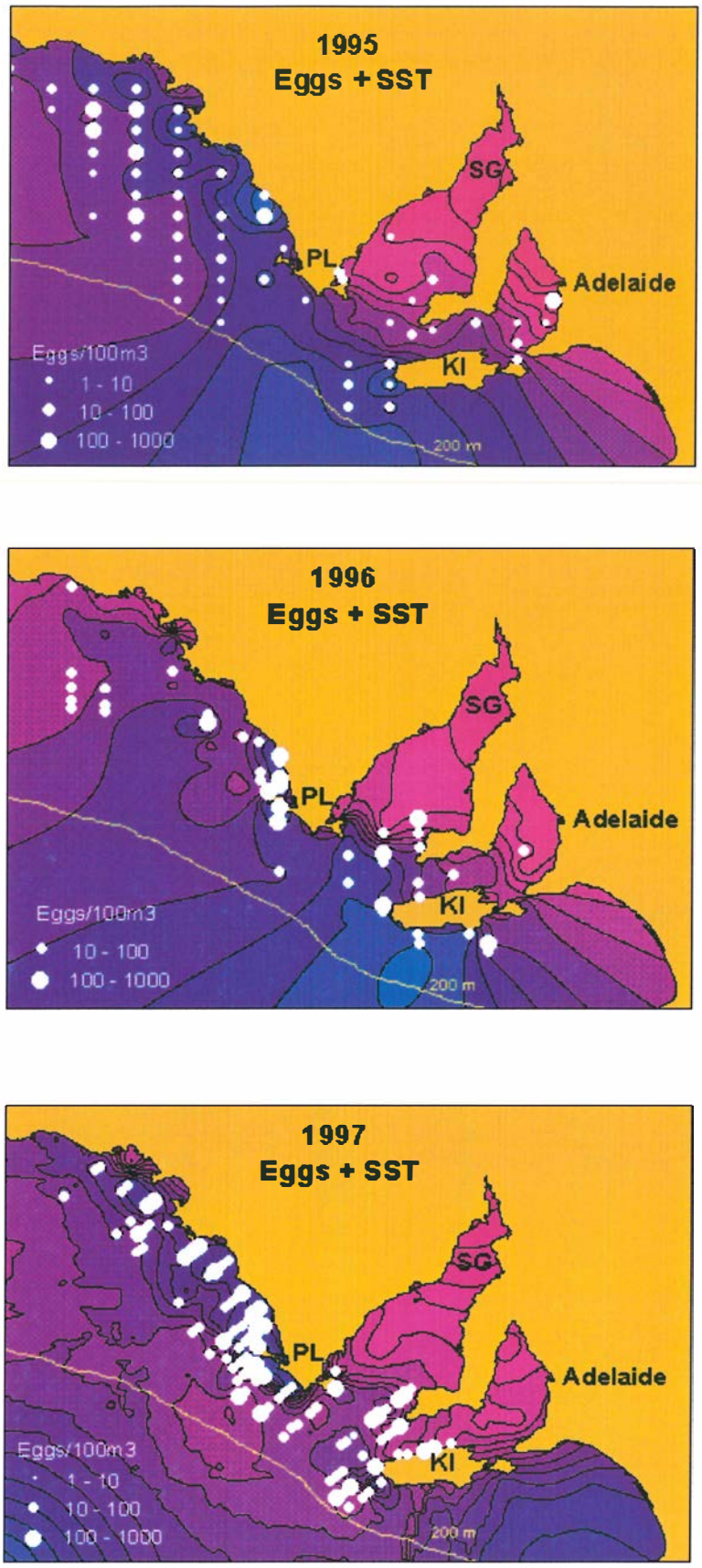


Figure 9.3 Sea surface temperature information obtained from *in situ* readings and egg densities at each site.

Table 9.1 The total area surveyed, number of stations sampled, number and percentage of positive stations sampled, and patterns of egg production in 1995, 1996 and 1997.

	1995	**1996**	**1997**
Total Area Surveyed (km^2)	11.2×10^4	7.6×10^4	4.5×10^4
Number of Stations Sampled	105	154	189
Number of Positive Stations	61	43	97
(%)	(58.1)	(27.9)	(51.3)
Eggs per $100m^3$			
Range	2 - 395	21 - 583	10 - 888
(mean ± SD)	(39.3 ± 72.3)	(53.6 ± 97.5)	(83.9 ± 165.7)
Spawning area (km^2)	8.5×10^4	3.4×10^4	3.3×10^4
(% change)		(-60%)	(-1%)
Daily egg production	$164.62/10m^2$	$136.67/10m^2$	$484.8/10m^2$
(% change)		(-17%)	(+ 195%)
Total egg production	1.4×10^{12}	4.65×10^{11}	1.6×10^{12}
(% change)		(- 67%)	(+ 15%)

Table 9.2 Sources, sizes of samples and estimates of adult reproductive parameters.

	1995			1996			1997		
	m	***n***	***N***	***m***	***n***	***N***	***m***	***n***	***N***
							Source		
Sex Ratio (R)	15	0 – 34	293	32	9 – 23	79	18	10	180
	(Commercial PS – March)			(Commercial PS – April)			(Commercial PS - Feb-Apr)		
	0.51			**0.58**			**0.54**		
Mean Wt of Females (W)	11	2 – 30	132	38	3 – 16	357	23	10-134	418
	(Commercial PS – March)			(Commercial PS – Jan- Mar)			(Commercial PS - Feb-Apr)		
							2	12-20	32
							(Midwater Trawl - March)		
	42.9			**46.3**			**43.0**		
Batch Fecundity (B)	-			20	30	62	14	1-15	22
				(Chartered PS - Feb)			(Commercial PS - April)		
	15 366			**16 422**			**13 947**		
Spawning Fraction (S)	-			20	30	30	3	60	180
				(Chartered PS – Feb)			(Commercial PS - April)		
							2	12-22	34
							(Midwater Trawl - March)		
	0.08 –0.2*			**0.08 – 0.2***			**0.156**		

Table 9.3 Results of sensitivity analysis of estimates of initial daily egg production (P_0) and spawning biomass in 1997 to variation in the estimate of daily egg mortality (Z). Values tested embraced the range of estimates obtained in previous years.

Egg Mortality (Z)	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**
Egg Production/10m^2	422.9	463.6	506.4	551.5	598.6
Spawning Area (m^2)	3.3E+09	3.3E+09	3.3E+09	3.3E+09	3.3E+09
Female Weight	42.98	42.98	42.98	42.98	42.98
Spawning Fraction	0.156	0.156	0.156	0.156	0.156
Batch Fecundity	13947	13947	13947	13947	13947
Sex Ratio	0.54	0.54	0.54	0.54	0.54
Biomass	**51 232**	**56 162**	**61 347**	**66 811**	**72 517**

Table 9.4 Effect of variability of spawning fraction on estimates of spawning biomass

Egg Mortality (Z)	0.35	0.35	0.35	0.35	0.35
Egg Production/10m^2	484.8	484.8	484.8	484.8	484.8
Spawning Area (m^2)	3.3E+09	3.3E+09	3.3E+09	3.3E+09	3.3E+09
Female Weight	42.98	42.98	42.98	42.98	42.98
Spawning Fraction	**0.120**	**0.140**	**0.160**	**0.180**	**0.200**
Batch Fecundity	13947	13947	13947	13947	13947
Sex Ratio	0.54	0.54	0.54	0.54	0.54
Biomass	**76 404**	**65 490**	**57 303**	**50 936**	**45 842**

Table 9.5 Effect of variability of batch fecundity on estimates of spawning biomass

Egg Mortality (Z)	0.35	0.35	0.35	0.35	0.35
Egg Production/10m^2	484.8	484.8	484.8	484.8	484.8
Spawning Area (m^2)	3.3E+09	3.3E+09	3.3E+09	3.3E+09	3.3E+09
Female Weight	42.98	42.98	42.98	42.98	42.98
Spawning Fraction	0.156	0.156	0.156	0.156	0.156
Batch Fecundity	**12000**	**13000**	**14000**	**15000**	**16000**
Sex Ratio	0.54	0.54	0.54	0.54	0.54
Biomass	**68 252**	**63 001**	**58 501**	**54 601**	**51 189**

Table 9.6 Estimates of spawning biomass for 1995, 1996 and 1997. Estimates for 1995 based on assumed mortality (Z) values of 0.35, 0.2 and 0.6.

Spawning Biomass	**1995**	**1996**	**1997**
Best Estimate (t)	58 613	18 112	**58 725**
Minimum (t)	38 000	11 320	**51 232**
Maximum (t)	95 000	28 298	**72 517**

CHAPTER 10. POTENTIAL YIELDS OF PILCHARDS STOCKS OF SOUTHERN AND EASTERN AUSTRALIA.

T.M. Ward and G.K Jones

Objective: To discuss issues associated with estimating the potential yield(s) of pilchards in southern and eastern Australia. This chapter discusses the implications of literature reviews and research discussed in this report for future estimates of the potential yields of pilchard fisheries in South Australia and Victoria. No biomass estimate was obtained for Victorian waters and estimation of that fishery's potential yield remains difficult. Investigations of spawning by pilchards in Victorian waters are continuing as part of a study of the ichthyoplankton assemblage in Bass Strait. The estimate of the 1997 spawning biomass obtained for central and western South Australian waters was used to set the Total Allowable Catch for 1998. The current high level of demand for pilchards as fodder for the South Australian tuna mariculture industry suggests that there will be considerable pressure to increase the exploitation rate and/or the Total Allowable Catch. The pilchard industry in South Australia has agreed to fund DEPM surveys between 1998 and 2001 in order to ensure a quantitative basis for establishing future quotas. The value of these studies will be maximised if they are conducted in conjunction with integrated studies of factors that control natural fluctuations in the size of pilchard stocks (e.g. sea surface tempratures), the effects of the pilchard fishery on other components of the pelagic ecosystem (e.g. predators such as seals and penguins), and research programs that utilise hydroacoustic and classical stock assessment approaches to investigate cheaper and more convenient methods for obtaining indices of pilchard abundance

10.1 Introduction

Factors limiting the potential yields of fisheries can be grouped into four categories: (i) economic constraints, including costs of capitalisation and harvesting, the size and reliability of markets and effects of competition; (ii) biological and ecological constraints, especially the size, distribution patterns, age structure, growth rates and reproductive characteristics of populations, but also unpredictable catastrophic events (e.g. mass mortality events and environmentally-induced changes in species abundance); (iii) intellectual and technical constraints, including the status of knowledge on stocks, the type/reliability of research conducted, and the availability of suitable harvesting and handling procedures; and (iv) management constraints, including issues associated with harvesting options (e.g. short-term versus long-term rewards), conflicts over allocation issues, especially the need for ecological allocations to ensure conservation objectives. These categories are closely linked, and in some cases overlap, but provide a convenient framework in which to discuss issues regarding the estimation of sustainable yields for pilchard fisheries in south-eastern Australia. No estimate of stock

size are available for New South Wales or Victorian waters, so this chapter necessarily focuses on South Australian stocks. Estimates of spawning biomass of pilchards in southern Queensland will discussed in a report that will be submitted when that project is completed.

10.2 Economic Constraints

Clupeoid fisheries of southern and eastern Australia are driven by local demand (Chapter 4). The rapid development of the South Australian pilchard fishery reflects the change in the exploitation strategy of the southern bluefin tuna in South Australian waters from a simple pole fishery to a value-adding mariculture industry. Juvenile southern bluefin tuna caught in the Southern Ocean are now towed to Port Lincoln where they are 'grown out' in 'sea cages' on a diet that consists largely of pilchards. Annual demand for pilchards by the South Australian tuna mariculture industry is currently in the vicinity of 28 000 t and thus exceeds the entire Australian catch (Figure 10.1). Imported pilcharda are used to overcome shortfalls.

Catches of pilchards from South Australian waters have increased from 3 t in 1990/91 to 3428 tonnes in 1996/97 (Figure 10.1). The quota for 1997/98 has been set at 11500 t, i.e. 10% of the spawning biomass estimated in 1997 (Chapter 8). Minimal processing is required as the entire catch is used as fodder for tuna. Fish are usually stacked in bins or crates prior to delivery to the sea cages. Under the current handling regime, the product is unsuitable for use as bait or for human consumption. Total dependence on the tuna mariculture industry places the fishery in a vulnerable position. Scientists at South Australian Research and Development Institute (Aquatic Sciences) are confident that a synthetic food source for tuna will soon be available (Steve Clark, personal communication). Such a product will be cheaper and more convenient than fresh pilchards, and if it produces higher growth rates whist maintaining product quality (as recent results suggest it may), it will almost inevitably replace pilchards as the preferred food source. The effects on the South Australian pilchard fishery would be dramatic. As the likelihood of this scenario is high, there is a clear and pressing need to: (i) identify alternative markets for South Australian pilchards and (ii) develop processing methods that will satisfy the requirements of those markets.

There is a large market for pilchards as baitfish for tailor (*P. saltatrix*) in northern New South Wales and southern Queensland. Pilchards from Western Australia have historically been used to supply this market but recent quota reductions and increases in demand for human consumption have decreased the availability of pilchards from this source. This bait market requires high-quality frozen pilchards. A Queensland fisher has recently made large a large impact on this market by providing prime quality pilchards produced using Individual Quick Freeze procedures conducted at sea.

There is also a large international market for pilchards (sardines) for human consumption. This market is currently being exploited by a single Western Australian company which supplies canned and crummed sardines. Sardines are also sold in various forms both locally and interstate. Pilchards sold for bait or human consumption fetch a considerably higher price than those sold as tuna fodder or pet food.

Access to markets for bait and human consumption will require improvements to processing equipment and handling methods. This will necessitate increased capitalisation of the fishery and may also require a change in fishing strategy, notably the targeting of adult fish outside Spencer Gulf rather than immature fish in locations near the sea cages (Chapter 4).

10.3 Biological and Ecological Constraints

Australian seas are characterised by low levels of nutrients and productivity (Kailola *et al.*1993). Stocks of pilchards and other clupeoids are subsequently small by international standards. Catches will never match the size of overseas clupeoid fisheries, although our 'clean image' (low pollution) considerably enhances the potential value of the product.

The world's large pilchard fisheries are invariably located near regions of nutrient enhancement (Cury and Roy 1989). For example, the Californian pilchard fishery is linked to the California Current system. Upwelling events and spawning are spatially and temporally coincident, and there are strong correlations between upwelling strength, phytoplankton (diatom) and zooplankton abundance, and recruitment success of pilchards (Wickett 1966; Hemmingway 1979; Ware and Thomson 1991, Millan-Nunez *et al.* 1996). Long-term connections between alongshore wind stress, upwelling and pilchard condition have been identified (Hsieh *et al.* 1996). Similar patterns have been described for the South African and Japanese pilchard fisheries e.g. (Barrange and Van der Lingen 1996;Yamanaka *et al.* 1988).

Over the past three years we have identified a correlation between the geographical distribution of pilchard eggs and low surface temperatures in coastal waters of central and western South Australia (Chapter 8). The cool water is believed to emanate from an upwelling event that occurs in waters around the west coast of Kangaroo Island and Eyre Peninsula and may be generated by persistent SE winds that occur during late summer (see Wenju *et al.* 1990). Factors causing this upwelling, spatial and temporal variations in its intensity and possible effects on patterns of plankton abundance and the survivorship, condition, reproductive outputs and recruitment of pilchards, are poorly understood.

Pilchard stocks of South Australia and other regions display large inter-annual fluctuations in abundance. These fluctuations can result from 'normal' variations in oceanographic conditions and recruitment success, or may be associated with unpredictable catastrophic events like the 1995 pilchard kill. These variations can limit economic viability of fisheries by reducing the reliability of income and the predictability of supply.

10.4 Intellectual and Technical Constraints

Pilchard stocks of South Australian waters have only been the subject of dedicated research since 1994. Information concerning stocks are subsequently sparse. Results obtained during this project indicate that inter-annual fluctuations in stock size are large and suggest that spawning success of pilchards may be largely controlled by climatic and oceanographic factors. As discussed in Chapter 3, pilchards are thought to be the major prey item of many of the predatory species that occur in this region, e.g. southern bluefin tuna, Australian salmon and little penguins. Better knowledge of the relationships between physical factors, the abundance of pilchards, and the abundance and breeding success of predators is essential for better understanding of the dynamics of this valuable and poorly understood pelagic ecosystem.

In this study, spawning biomass estimates were only obtained for central and western waters of South Australia. Estimates of spawning biomass in Port Phillip Bay (Victoria) were not obtained becausethere are few data on spawning in Vistorian waters. Results of this and other studies indicate that pilchards more commonly spawn in shelf waters than embayments (Chapter 8). This study has also shown the potential value of satellite imagery for identifying pilchard spawning areas. Satellite images of surface water temperatures and chlorophyll concentrations suggest that upwelling may occur in waters of eastern South Australia and western Victoria (Lewis 1983). This may be a potentially-important spawning area for pilchards and may be a source of pilchards for the Victorian fishery. Further studies are needed on the stocks and spawning patterns of pilchards in Victoria.

Egg surveys are generally acknowledged as the most reliable method of estimating spawning stock biomass and are the methods of choice in the clupeoid large fisheries of South Africa and California (CALCOFI 1996; Barrange and Van der Lingen 1996). The major disadvantages of these methods are the relatively high costs, including time spent at sea and processing samples. Some scientists have recommended the use of hydroacoustic surveys to augment and partially replace the use of egg surveys (see Chapter 4). Such replacement can only be justified after several years use of both methods to order in determine the precision of data obtained using hydroacoustic procedures.

Several studies have attempted to quantify the structure of Australia's pilchard stocks (see Fletcher *et al.* 1997). None have provided particularly conclusive results. For example, Dixon *et al.* (1993) concluded that there were as series of 'contiguous quasi-independent pilchard sub-populations'. The major problems encountered in these studies appear to be the relatively high levels of genetic variation observed within sampling units and logistical difficulties associated with collecting and processing sufficient spatial and temporal replicates to adequately investigate variation within and between sampling units. It seems unlikely that genetic studies will provide the data required to identify suitable management units. Current (state) management arrangements are probably appropriate for most stocks, but recent reults suggest their may be a future need for integrated management of the Queensland and New South Wales fisheries.

Several authors have noted the difficulties and costs associated with ageing pilchards using counts of otolith annuli. Fletcher (1995a) recommended the use of otolith weight, but results obtained in this study suggest that this method can also be unreliable. A major factor contributing to the relatively successful study of age and growth conducted during this study was the acquisition of monthly samples. Length frequency data obtained allowed cohorts to be tracked over time, and when used in conjunction with annuli counts and otolith weight data, provided valuable insights into spatial and temporal variations in the growth rates of pilchards. It is recommended that future examinations of the age structure of the South Australia fishery also utilise monthly samples and examine length frequency, as well as annuli counts and weights. The use of the otolith weight method to produce catch at age curves, as has been conducted in Western Australia since 1991, also requires additional investigation.

Methods used to catch pilchards are adequate but improvements in handling methods and processing equipment could substantially increase the value of the fishery. It is clear that returns from these resources would be maximised by developing world's best practice in handling and presentation, and by developing processing strategies that add value to the raw product.

10.5 Management Constraints

Several aspects of the current harvesting strategy in South Australia are controversial. For example, immature fish comprise a large component of the catch and there are ongoing debates regarding allocation of quotas. These issues are primarily the concern of the local management agency (Primary Industries Resources of South Australia) and the pilchard working group and are not discussed herein.

The proportion of estimates of spawning biomass that have been set as exploitation levels and Total Allowable Catches for pilchards and other small pelagic teleosts are shown in Table 10.1. Exploitation rates above 25% of spawning biomass appear to have consistently resulted in signs of overfishing. Levels of 10% or less have usually been set when stocks were recovering and/or environmental conditions were poor. Exploitation rates that are consistently sustainable probably range from 10% to 20% of the spawning biomass.

In South Australia, the DEPM has only been applied since 1995. Estimates obtained between 1995 and 1997 should be treated with some caution, mainly because of difficulties in obtaining reliable estimates of spawning fraction (see Chapter 8). Possible effects of uncertainty have been partially been overcome by use of conservative values of problem parameters and by setting the Total Allowable Catch for 1998 at 10% of the spawning biomass. Pilchard fishers of South Australian have agreed to fund DEPM surveys in 1998-2001 in order to provide a quantitative basis for establishing Total Allowable Catches.

The high level of demand from the tuna mariculture industry suggests that there is likely to be considerable pressure from fishers to increase the exploitation rate and/or the Total Allowable Catch in future years. There are compelling arguments for such increases, these include:

(i) Maximising economic returns to fishers;

(ii) Economic and practical advantages of reducing reliance on imported fodder;

(iii) Reduction of risk of introducing an exotic pathogen;

(iv) Advantages of fishing down the food web (Chapter 3), i.e. reductions in stocks of predators may increase the quatities of pilchards are available for harvesting.

The major problem with increasing the exploitation rate and/or the Total Allowable Catch for pilchards is the potential effects on other components of the pelagic ecosystem and the highest risk is clearly in areas around Spencer Gulf where most of catch is currently taken. It is likely, however, that such increases would probably cause the fishery to expand into areas outside its current limits and potentially increase the regional significance of these ecosystem effects. There are, therefore, compelling arguments for not increasing exploitation rate beyond the current level:

(i) Increased risk of over-exploiting stock and reducing future catches;

(ii) Potential effects on economically-important stocks (especially southern bluefin tuna).

(iii) Potential effects on species with significant conservation values (e.g. Australian sea lions and little penguins).

It is clear that the industry-funded DEPM surveys that will be conducted in South Australian waters during 1998-2001 will provide most valuable results if they are conducted in conjunction with quantitative and integrated studies of: (i) factors which control natural fluctuations in the size of pilchard stocks; (ii) cheaper an more convenient methods for obtaining indices of pilchard abundance; and (iii) effects of the pilchard fishery on other components of the pelagic ecosystem.

10.6 Summary and Conclusions

1. Dependence on a single market (as tuna fodder) is a threat to the long-term viability of the South Australian pilchard fishery.
2. Access to other markets is currently limited by relatively crude processing procedures.
3. There are large markets for baitfish in Australia and human consumption overseas.
4. Potential value of product on world markets is maximised by 'clean' unpolluted image.
5. Stock and potential yield is relatively small by international standards.
6. Knowledge of the South Australian stock is limited by lack of research prior to 1994.
7. Biomass estimates obtained between 1995 and 1997 should be used with caution.
8. South Australian pilchard fishery to fund DEPM surveys in 1998-2001.
9. Need for research on factors controlling inter-annual fluctuations in pilchard abundance.
10. Need to develop cheaper, more convenient methods for monitoring pilchard abundance.
11. Need for research on the effects of the pilchard fishery on predatory species.

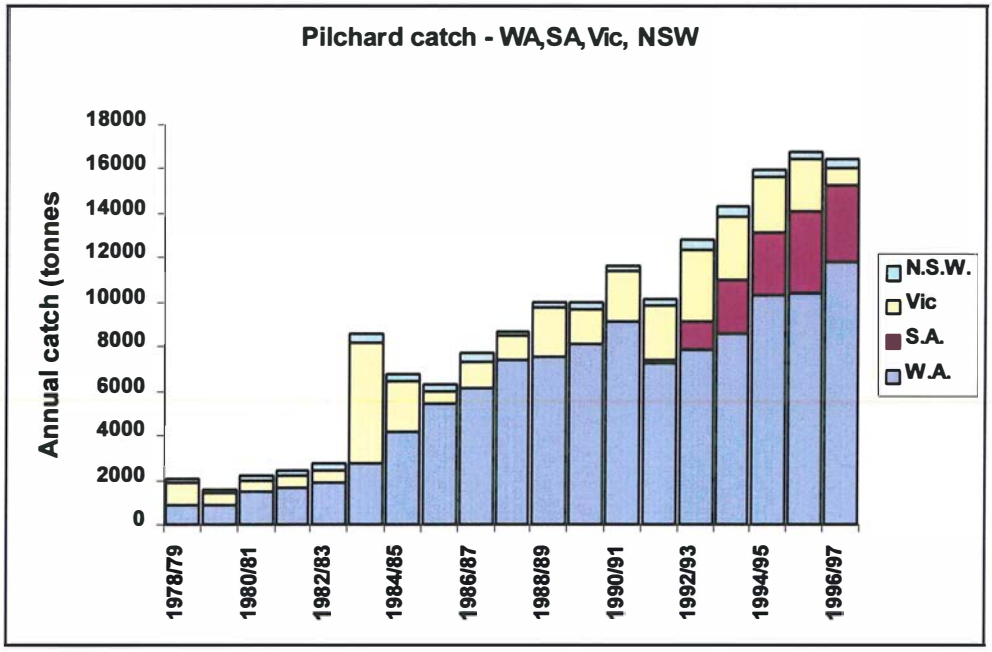


Figure 10.1 Australian annual pilchard catches by state between 1978/79 and 1996/97. No data are available for Queensland. Data for South Australia does not include all pilchards caught as live bait in the pole and line fishery for southern bluefin tuna.

CHAPTER 11. BENEFITS

T.M. Ward and G.K. Jones

The review of methods for estimating the biomass of clupeoids confirmed the suitability of the DEPM for assessing pilchard stocks, and emphasised the synergistic advantages of current use of classical fisheries models and hydroacoustic techniques. This integrated approach will be taken in industry-funded stock assessments to be conducted in South Australian waters between 1998 and 2001.

The review of the potential effects of the fisheries for small pelagic teleosts on predator populations highlighted the need for ecosystem research in South Australian waters and facilitated the development of a proposal for funds from the National Heritage Trust to investigate the effects of the South Australian pilchard fishery on the population sizes and breeding success of Australian sea lions and little penguins.

Information collated as part of the baitfish surveys was used to produce stock assessment reports for Victoria (Neira *et al.* 1997a, b) and will be used to produce a similar report for South Australia. Studies of fishing patterns and catches (catch rates and age composition) provided baseline information that will be vitally important for the future management of South Australia's rapidly developing pilchard fishery and that will help to identify areas that may be potentially susceptible to localised stock depletion.

Studies of age and growth provided valuable information on the age composition of catches in both states and assisted the location of areas commonly used by juvenile and mature pilchards in South Australian waters (i.e. Spencer Gulf and Coffin Bay, respectively). Such information will be important if the fishery is to expand into markets for bait or human consumption that require adult fish. Results of otolith studies indicated the advantage of obtaining monthly samples and of using several independent techniques (e.g. length frequency, otolith weight and otolith annuli) to monitor age and growth.

Investigations of reproductive biology and spawning patterns discounted the possibility of significant pilchard spawning in Port Phillip Bay and identified the need for additional research on potential spawning areas in the waters of eastern South Australia and western Victoria. Investigations in western South Australian facilitated the acquisition of preliminary data on the upwelling event that occurs in those waters. Data on this phenomena were presented at the Australian Society for Fish

Biology conference in Darwin during 1997 (Kinloch *et al.* 1997a) and will provide the basis for future applications for funds to investigate factors controlling productivity and natural fluctuations in pilchard abundance in these waters.

Genetic studies confirmed the view that Australian pilchard stocks may not be divided into discreet units. Information obtained in a concurrent study (Staunton-Smith and Ward 1998) suggests that there may be a strong case for joint management of some stocks, e.g. southern Queensland and northern New South Wales.

Results from South Australia confirmed the benefits of using egg surveys to provide estimates of spawning biomass for clupeoid fishes (see Fletcher *et al.* 1996a, b). Results obtained have been presented to industry in the form of (written) stock assessment reports (Hoedt *et al.* 1996; Kinloch *et al.* 1997b) and seminars. Knowledge of stock size have been used by the South Australian Pilchard Working Group to establish the Total Allowable Catch for 1998. Benefits to the industry are indicated by the decision of South Australian fishers to fund egg surveys in 1998-2001.

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