Migratory dynamics and recruitment of snapper, *Pagrus auratus*, in Victorian waters

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Objectives:

1. Determine whether high annual variability in the abundance of pre-recruit (0-age) snapper in Port Phillip Bay is reflected by the entire western stock.
2. Determine the importance of spawning of snapper within Port Phillip Bay to the western stock as a whole.
3. Determine the movement patterns of snapper between Port Phillip Bay and offshore waters and whether they are changing over time.
4. To determine the proportion of the snapper population on the open coast that originates from Port Phillip Bay and how this changes with age.
5. To determine the proportion of the snapper population in Port Phillip Bay that originates from areas outside the bay and how this changes with age.

NON-TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

Initially this project demonstrated that high annual variability in recruitment of 0-age (< 4 months old) snapper in Victoria's largest estuary, Port Phillip Bay (PPB), was not reflected in other Victorian estuaries, indicating that recruitment variation is a local-scale process. The novel method of otolith chemistry was then successfully used to investigate spatial linkages between recruitment of 0-age snapper in PPB, and recruitment of older fish (1–5 years age) into the Victorian fishery. This component of the project demonstrated for two cohorts that most adult snapper recruiting into the major fisheries in PPB, Western Port, and western Victorian coastal waters originated from one major spawning/nursery area, PPB. Therefore, the strong fluctuations in juvenile recruitment that occur within PPB will dominate the population dynamics of the entire western Victorian snapper stock. There was no evidence for either cohort, or any of the age groups sampled, that 0-age recruitment occurring outside of PPB made a significant contribution to recruitment to the fishery within PPB. The otolith chemistry study revealed that young snapper (1–4 years of age) born in PPB can migrate large distances (100's km) to replenish populations in coastal waters and other bays. Recruitment of adults into the smaller eastern Victorian fishery, however, appeared derived from 0-age nursery areas in eastern Victorian estuaries, most likely Corner Inlet and Gippsland Lakes. While there was some evidence that snapper originating from PPB contributed to fishery recruitment in eastern Victoria, our results would support current ideas that the populations in eastern and western Victoria should continue to be treated as different 'stocks' for fisheries management purposes.

These findings have important implications for understanding, modelling and predicting population dynamics of snapper in Victorian waters. Firstly, the dynamics of the entire western Victorian snapper fishery, encompassing over 500 km of coastline from Portland to Wilsons Promontory, can now be linked to juvenile (0-age) recruitment variation in PPB. This finding provides a sound basis for restricting pre-recruit monitoring to the nursery areas in PPB. Fisheries Victoria now funds an ongoing 0-age recruitment monitoring program in PPB, which is based on the results and methods developed during this study. The data from this monitoring program are proving highly valuable in explaining fluctuations in the PPB and western stock fisheries, and for making longer-term forecasts of fishery productivity for the benefit of stakeholders. Managers now appreciate the consequences of long-term recruitment failure in PPB for the western Victorian fishery, and are considering adaptive management strategies to deal with such an occurrence. Secondly, this project has highlighted to managers and stakeholders the critical importance of maintaining the processes/habitats that support the production and survival of juvenile snapper in PPB. As a result, both managers and stakeholders have become increasingly interested in understanding what influences juvenile production and survival rates in PPB. This has encouraged Fisheries Victoria to fund further research into the process of 0-age recruitment variation in PPB. This recent work has indicated that local spawning and variable larval survival within PPB drive 0-age recruitment variation. In combination with the current study, these results have clearly demonstrated the importance of the spawning adults that migrate into PPB from coastal waters during spring/summer, and the potential implications of over-fishing of this spawning aggregation. Finally, this project has provided a basis for further exploring the use of otolith chemistry to study migration patterns of snapper between PPB and coastal waters based on variation in otolith barium levels. This project has demonstrated a link between barium levels in the water and incorporation of barium into snapper otoliths, and that the concentration of barium in PPB water is likely to be generally higher than coastal waters. A program of water chemistry monitoring has been initiated to explore the temporal consistency of barium enrichment in PPB. The otolith chemistry techniques applied to snapper in the current study are now being tested on other important species (i.e. King George whiting).

Managers and industry have responded positively to the new information provided by this project, particularly the clarification of the importance of spawning aggregations and 0-age nursery areas in PPB. Management has relied significantly on the results of this project for explaining the recent recovery of the snapper fishery in western Victoria, and in recent discussions with angler groups regarding stock status and revision of management strategies for the PPB fishery.

NON TECHNICAL SUMMARY

During the mid-1990s there were serious doubts over the status of the Victorian snapper fishery. In the major fishing area of Port Phillip Bay (PPB), adult snapper were perceived to be continually declining in abundance and the commercial catch was the lowest on record. The cause of declining catches and whether or not the decline in PPB was indicative of the entire Victorian fishery was difficult to determine due to poor understanding of the relationships between snapper in PPB and the populations in coastal waters and other bays.

It is thought that snapper in Victorian waters are comprised of two stocks; the 'western stock' that extends from approximately Kingston in South Australia to Wilsons Promontory in central Victoria, and the 'eastern stock' that extends from Wilsons Promontory into New South Wales. The PPB fishery is part of the wider western stock, however, there is uncertainty around the level of mixing among the populations in PPB and coastal waters. There is also uncertainty over the relationship between the localised fisheries in PPB and the adjacent Western Port, and between the eastern and western stocks. Understanding of juvenile recruitment variation is also poor, and it is unknown how different spawning/nursery areas contribute to replenishment of the snapper fisheries within and outside of PPB. These uncertainties are a significant impediment to both understanding and predicting changes in fishery productivity and to assessing the risks that localised habitat degradation and high fishing effort within PPB pose for the long-term sustainability of the fishery. A further requirement for understanding variability of the PPB fishery is a greater understanding of the migratory dynamics of snapper between PPB and coastal waters. To resolve these uncertainties this project proposed firstly to improve understanding of juvenile recruitment variation. Secondly, to use the novel approach of otolith (earbone) chemistry, to determine how juvenile recruitment occurring within and outside of PPB contributes to replenishment of the fisheries in PPB, Western Port and coastal waters. Thirdly, to explore the potential of the otolith chemistry technique to provide information on migration histories of individual snapper.

We used a purpose designed beam trawl to compare annual recruitment of 0-age snapper (less than 4 months age, ~2–8 cm total length) over 4 years across Victoria's 4 major estuaries; PPB, Western Port, Corner Inlet and Gippsland Lakes. This comparison revealed that recruitment of 0-age snapper varied by a factor of at least 10 times among years in PPB, however, the variation in PPB was not reflected in the other estuaries. Ongoing monitoring has further demonstrated high interannual recruitment variation in PPB. High and low recruitment years in PPB were obvious by the end of the larval settlement period (i.e. February), indicating that recruitment variation is related to processes occurring early in the life-history (i.e. first 1–2 months of life). Ongoing monitoring has further indicated that patterns of 0-age recruitment variation in PPB are not closely matched to those observed in Spencer Gulf, South Australia. Interannual variation of 0-age snapper recruitment therefore appears influenced by local (estuary)-scale processes. Monitoring and research on 0-age recruitment variation should be conducted at the scale of individual bays/estuaries as different processes may be driving recruitment variation in different areas. Finally, we found very few 0-age snapper in Western Port compared to PPB, and we could not confirm that 0-age snapper occurred in west Victorian coastal waters, although sampling effort in coastal waters was low.

To quantify linkages between 0-age recruitment areas and recruitment to the fishery we required a method that could track movement/connectivity between small juvenile and adult life-stages. Due to the inherent difficulties associated with manually tagging large numbers of very small fish and the biases associated with manual tag/recapture methods, we chose to use the novel approach of otolith chemistry. Otoliths are found in the inner ear of fish and grow by continuous deposition of calcium carbonate layers. Otoliths are used to age fish by counting their annual ring structures (i.e. similar to rings in tree trunks). Otoliths are particularly useful for studying fish movement because their chemical composition can be influenced by the physical and chemical properties of the water where the fish live. This means that fish living in different water bodies (eg. juveniles in different bay/estuary nursery areas) can develop different otolith chemistry. Geographic differences in otolith chemistry can be used to characterise natural 'tags' or 'signatures' that can in turn be used to study spatial linkages between juvenile and adult life-stages. Furthermore, variation in otolith chemistry can be related to fish age to reconstruct environmental and/or geographic migration histories.

Initially we used laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to compare otolith chemistry among small 0-age snapper collected from 6 bays and estuaries along the Victorian

coast in 2 months of 2 years (February-March of 2000 and 2001). While it was difficult to accurately distinguish among individuals from some of the estuaries based on otolith chemistry, irrespective of sampling month or year, 0-age snapper collected from PPB could be accurately (85–98% accuracy) distinguished from those collected in all the other estuaries. This result was largely due to the higher levels of barium in otoliths from PPB, linked to higher barium levels in PPB water. Although the chemical tags were similar for the 2000 and 2001 cohorts, otolith chemistry varied significantly among 0-age fish collected in PPB during 2000/2001 and archived samples collected 5 to 9 years prior. Therefore, the chemical tags from the 0-age fish of the 2000 and 2001 cohorts could only be applied to determine the origins of older fish that were born in these same years (i.e. fish of the same cohorts).

The next stage was to apply these chemical tags to determine the origins of older fish recruiting to the fishery. During summer/autumn of 2002, 2004, and 2005, with the help of recreational anglers, we sampled approximately 1000 snapper from the 2000 and 2001 cohorts as they aged from 1 to 5 years and grew from ~15 to 40 cm. At 3 years of age snapper recruit to the fishery and at 4 years of age they become sexually mature. These older fish were sampled from PPB, Western Port, and throughout coastal waters in western and eastern Victoria. We analysed the 0-age regions of otoliths from these older fish using LA-ICP-MS. The chemical tags characterised for the 0-age fish were used as baseline data to estimate the proportions of older fish that had originated from 0-age recruitment in PPB as opposed to areas outside of PPB. The results indicated that the majority of 3–5 year old snapper recruiting to the west Victorian fishery had originated from 0-age recruitment in PPB. This result was consistent for PPB, Western Port and west Victorian coastal waters up to 150 km east and 300 km west of PPB. There was no evidence that older snapper originating from 0-age recruitment outside of PPB contributed to fishery recruitment inside PPB. Recent work has further indicated that spawning within PPB, rather than in coastal waters, is the major source of 0-age recruits to PPB. We therefore conclude that spawning/0-age recruitment occurring in PPB is the primary source of population replenishment for the western Victorian snapper stock. There was evidence that a significant proportion of young snapper (<2 years age) in western Victorian coastal waters may have originated from coastal waters. However, by the age of 3 years, the high migration of young snapper from PPB to coastal waters resulted in the coastal population becoming dominated by fish that originated from PPB. Although replenishment of the fishery in eastern Victoria appeared to rely largely on 0-age recruitment in east Victorian estuaries, there was evidence that snapper born in PPB also migrated beyond the supposed stock boundary to contribute to the fishery in eastern Victorian.

Finally, we investigated whether otolith chemistry could be used to reconstruct migration histories of snapper between PPB and coastal waters, and if so, could this be used to examine variation in migration behaviour among fish and over time. Over two years we found that barium levels in PPB water were both consistent and significantly higher than in coastal waters. Barium levels in snapper otoliths were also positively correlated with levels in the water, and this relationship was similar for both juvenile and adult life-stages. Variation in otolith barium was also not significantly influenced by seasonal cycles in temperature and or growth. We therefore concluded that chronological variation in otolith barium could potentially be used to reconstruct movement histories between PPB and coastal waters. Strong variation in barium levels across otoliths of western, but not eastern Victorian snapper, and peaks in otolith barium corresponding to the time period when snapper move into PPB to spawn (i.e. spring/summer), appeared to confirm this potential. However, uncertainty over the longer-term (>5 years) consistency of barium levels in PPB and coastal waters prevented us from confidently reconstructing migration histories of adult snapper. Therefore, we could not make clear conclusions as to whether migration patterns had changed over time. Future application of this methodology will most likely require longer-term monitoring of barium levels in PPB and coastal waters so that chronologies of otolith barium can be interpreted in relation to any variation in water chemistry.

This project highlights the dependence of the west Victorian snapper stock on 0-age recruitment in PPB. The dynamics of the western Victoria snapper stock will be closely linked to juvenile recruitment variation in PPB. Pre-recruit (0-age) monitoring in PPB will provide a valuable indicator of variation in year-class strength for the entire western Victorian snapper stock. Development of spatial management strategies for the west Victorian snapper stock should focus on maintaining the habitats/processes important for spawning and 0-age recruitment in PPB, and the exploitation of adult spawning aggregations and juvenile fish within PPB.

Keywords: snapper, *Pagrus auratus*, 0-age, recruitment variation, migration, connectivity, natural tags, otolith chemistry, barium, laser ablation ICP-MS, Port Phillip Bay

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This project could not have been completed without the help of a number of enthusiastic recreational anglers and several commercial fisherman (listed below). Their interest in the project and willingness to voluntarily fill their freezers with snapper frames allowed us to collect over 1000 otolith samples across 700 km of the Victorian coastline. We would also like to thank Dr Patrick Coutin for access to otoliths from his earlier FRDC project (97/128). These otoliths were valuable in assessing the potential of otolith chemistry to reconstruct migration histories. Dr Greg Parry provided samples from the annual Port Phillip Bay trawl survey. Graeme Cottier, Ian Duckworth, Dave McKeown, Daniel Grixti and Grant Johnson also provided much appreciated assistance with otolith collections. Fisheries officers in the various regions also provided valuable assistance with otolith collections by providing us with confiscated catches.

Barry McKenzie and Rob Jenkins provided themselves, their local knowledge and boats for sampling at Gippsland Lakes. Barry also kept the pencils nice and sharp when in the field, and allowed Paul stay in his backyard caravan free of charge. A number of other people spent long nights on the water in the search for baby snapper, and to them we are eternally grateful for their enthusiasm and flexibility with fieldwork. These people include Brett Abbott, Sean Brodie, Sean Moran, Jeremy Hindell, and Anthony Plummer.

Recreational anglers involved in sample collections:

Alby Thomas	Andrew Joosen
Peter Wilson	Derek Hinchinson
Des Garnett	Maurice Hooenbach
Ken Graves	Bob O'Connell
Ray Beattie	Steve Burton
Ian Jones	Andy Orchard
Sean Brodie	Ross Winstanely
Chris Garner	Brad Herbertson
John Alsop	Bill Knibbs
Gordon Bannister	Daniel Kent
Garry Middleton	Russel Alerdice
Peter Jewell	Josh Lee

Commercial fisherman involved in sample collections:

Barry McKenzie
Eugene Garland
Neville Clarke
Bill Cull
White Fisheries

FINAL REPORT

1999/134 Migratory dynamics and recruitment of 0-age snapper, *Pagrus auratus*, in Victorian waters

1. Background

1.1 Snapper – biology

Snapper, *Pagrus auratus* (Sparidae) is a demersal/semi-pelagic species found in bays, estuaries and inshore coastal waters around New Zealand, Japan and the southern half of Australia (MacDonald 1982; Paulin 1990; Kailola et al. 1993; Fujita et al. 1996; Tabata and Taniguchi 2000). In each of these regions the species supports important fisheries. Snapper is a long-lived species (at least 40 years) that can spawn both in open coastal waters and within large sheltered bays and gulfs where seasonal spawning aggregations often occur (MacDonald 1982; Coutin et al. 2003; Fowler et al. 2003). The larval stages are pelagic for about 3–4 weeks (Francis 1994a; Fowler and Jennings 2003) before settlement into sheltered waters of bays and estuaries at about 12 mm length (Kingsford and Atkinson 1994; Trnski 2002; Fowler and Jennings 2003). Small 0-age juveniles (less than 1 year old, Fig. 2) are common in sheltered bays and estuaries throughout the southern half of Australia, but their occurrence in open coastal waters is poorly known (Lenanaton 1982; Gillanders 2002a; Fowler and Jennings 2003; Gaughan et al. 2003). Adults and older juveniles can be highly migratory, moving large distances in coastal waters, and between sheltered bays, estuaries and coastal waters (Sanders 1974; Coutin et al. 2003). However, in some regions, snapper can also show restricted home ranges and complex spatial population structure (Moran et al. 2003; Parsons et al. 2003; Sumpton et al. 2003). Understanding population structure and local population dynamics of snapper is complicated by their variable migration behaviour and the use of estuaries and sheltered bays at various life-stages.

1.2 Victorian snapper fishery

Snapper are continuously distributed in Victorian coastal waters and are found at all life stages within several large bay/estuaries; Port Phillip Bay, Western Port, and Corner Inlet (Fig. 1). Based on results of tagging studies, it is thought that there are two snapper stocks in Victorian waters, an eastern and a western stock (Sanders 1974; Coutin et al. 2003). The western stock is thought to extend from Wilsons Promontory to Kingston in South Australia and includes the fisheries in Port Phillip Bay and Western Port, and the eastern stock extends from Wilsons Promontory to the north coast of New South Wales (Fig. 1) (Sanders 1974; Coutin et al. 2003). The major fishery occurs within the sheltered waters of Port Phillip Bay (approximately 80% of the State catch), with smaller fisheries in Western Port and coastal waters (Coutin 2000; Coutin et al. 2003). Snapper recruit to the Victorian fishery at 27 cm total length (TL) and 3 years of age. The fishery is both recreational and commercial. Larger adult snapper are fished commercially with long-lines and both juveniles and adults are captured with haul seines. The commercial haul seine and recreational catch is dominated by sub-adult and younger adult snapper (27–45 cm TL), whereas most of the commercial long-line catch is of larger adult fish (>42 cm TL). Daily-catch limits of 10 fish, with only 3 fish allowed to be equal to or greater than 50 cm total length, apply for recreational anglers. The commercial snapper catch in Victorian waters for 2004/2005 was 114 tonnes, 95 tonnes coming from Port Phillip Bay (Anon 2006). A further 66 tonnes was taken from Commonwealth waters along the Victorian coast (Anon 2006). The total value of the commercial catch was close to $1,000,000 (Anon 2006). Recent indications are that the recreational snapper catch continues to grow and now significantly exceeds the commercial catch (Henry and Lyle 2003).

In the 1980–1990s a decline in the Victorian snapper catch stimulated much concern over the status of the fishery. The Victorian commercial snapper catch in 1980/81 was 200 tonnes, but in 1996/97 it had declined to only 49 tonnes. The catch in Port Phillip Bay (34 tones) was the lowest since catch records began in

1914. Several workshops involving managers, scientists and fishermen were convened during the mid-1990s to discuss the issue of declining catches. These discussions identified a number of key issues that inhibited management and understanding of snapper population dynamics in Victoria, and in particular the major Port Phillip Bay fishery (Newman 1998). Major areas of uncertainty included; poor knowledge of the early life-history and juvenile recruitment dynamics; lack of understanding of how different juvenile nursery areas contributed to fishery replenishment in different areas; and poor understanding of movement of snapper between Port Phillip Bay and coastal waters. There was a general consensus that future research should have particular emphasis on juvenile recruitment dynamics and defining the relationship between the localised Port Phillip Bay fishery and the wider population in coastal waters.

1.3 Juvenile recruitment dynamics

A key requirement for understanding and modelling fishery dynamics is knowledge of juvenile recruitment variation. This is particularly relevant to snapper fisheries where juvenile recruitment has been shown to vary greatly among cohorts (year-classes). Irrespective of the time period, age distribution data for Victorian snapper show clear domination by specific cohorts (Coutin et al. 2003). It is likely that much of this strong variation in cohort abundance is the result of factors that influence the production and survival of larval stages and/or small juveniles (0-age, less than 1 year old) (Fig. 2). Although previous studies have sampled larval and 0-age snapper in summer both in Port Phillip Bay, and at least one estuary in eastern Victoria (Rigby 1984; Ramm 1986; Jenkins 1986; Hamer et al. 1998), prior to this project there was little information on distribution and spatio-temporal variation in abundance of these early life-stages. Comparing recruitment levels over different spatial scales and across cohorts can indicate whether recruitment variation is a local or large-scale process. This is a fundamental first step towards understanding recruitment variation and ultimately for developing monitoring tools (i.e. pre-recruit monitoring) that can predict changes in productivity of the Victorian snapper fishery.

1.4 Linkages between juvenile sources and fishery recruitment

Knowledge of juvenile recruitment dynamics provides a key starting point for predicting fishery dynamics. However, to understand how the dynamics of local fisheries, such as Port Phillip Bay, are influenced by spatial and temporal variation in juvenile recruitment, linkages between different juvenile sources (i.e. nursery areas) and recruitment into the fishery need to be clearly defined. Defining these linkages is no simple task because it involves determining the spatial connectivity between the early juvenile and adult life-stages. Traditional methods for studying fish movement and population connectivity generally involve some form of manual tagging followed by recapture of tagged individuals (i.e. Sanders 1974; Begg et al. 1997; Jones et al. 1999; Sumpton et al. 2003). Such methods are difficult to apply effectively to early life-stages because of their high natural and tagging mortality. Furthermore, quantifying connectivity rates based on manual tagging and recapture can be confounded by tag shedding and spatial biases in mortality, and tagging and recapture effort (Crossland 1976; Kearney 1989).

1.5 Otolith chemistry – natural tags

The study of otolith chemistry promises to provide a new approach for studying population structure and movement of fish (Campana 1999; Campana and Thorrold 2001). Although fish otoliths (earbones) are primarily composed of calcium carbonate ($CaCO_3$) in a protein matrix (Degens et al. 1969), a variety of other chemical elements are incorporated in minute quantities (Campana 1999; Thresher 1999). It is estimated that these 'other' elements constitute approximately 1% of the otolith by weight (Campana 1999). Incorporation of these 'other' elements into otoliths, however, can be influenced by exogenous factors such as the physical and chemical properties of the water, and/or endogenous factors, such as growth, reproduction and stress (Kalish 1991, 1992; Radtke and Shafer 1992; Sadovy and Severin 1992, 1994; Campana 1999; Bath et al. 2000; Elsdon and Gillanders 2003, 2004). Otolith chemistry can therefore vary in space and time with a significant amount of this variation related to varying environmental conditions (Campana 1999; Bath et al. 2000; Elsdon and Gillanders 2003, 2004). Importantly, because otoliths are metabolically inert and continue to grow throughout life, they can provide permanent records of the chemical and or physical properties of the aquatic environments inhabited by fish (Campana 1999). Otolith chemistry can therefore be used as a 'natural tag' for identifying groups of fish that have resided in different environments or locations (Gillanders and Kingsford 1996; Milton et al. 1997; Thorrold et al.

2001; Gillanders 2002a). Furthermore, variations in chemistry within/across otoliths can potentially be matched to annual growth increments to reconstruct the environmental and or spatial migration histories of individual fish (Limburg 1995; Secor et al. 1995; Tzeng et al. 1997)

For investigating linkages between juvenile sources and recruitment into fisheries the use of otolith chemistry as a 'natural tag' is most promising. Spatial variation in the otolith chemistry of small juveniles can provide natural chemical 'tags' or 'signatures' that are specific to juveniles from different areas, such as individual estuaries or even ocean regions (Gillanders and Kingsford 1996, 2000; Milton et al. 1997; Thorrold et al. 1998ab; Rooker et al. 2001a). The chemical tags from juveniles of known origin can then be matched to the otolith chemistry of the juvenile region of adult otoliths to link adult fish back their geographic origin (Thorrold et al. 2001). This methodology has the potential to provide unprecedented information on linkages between juvenile sources and replenishment of snapper populations in Victoria.

1.6 Migratory dynamics

Adult snapper migrate from coastal waters into Port Phillip Bay during the late spring and summer (October–December) to spawn, and the majority of these immigrants are thought to return to coastal waters in the late summer and autumn (January–April). This spawning migration is the major focus of the Port Phillip Bay fishery (Coutin et al. 2003). Although this seasonal movement between the Bay and coastal waters occurs, snapper may also become resident either in the Bay or coastal waters. The proportion of adult snapper that exhibit migration and residence type behaviour and how this proportion changes over time is unknown. Variable migration behaviour of snapper between Port Phillip Bay and coastal waters is likely to be an important influence on the dynamics of the Port Phillip Bay fishery (Coutin et al. 2003). For example, it is possible that long-term changes in the abundance of adult snapper in Port Phillip Bay could be related to changes in the proportions of migratory and resident snapper.

Traditional tag/recapture methods cannot provide detailed migration histories of individual fish. Therefore, novel approaches to studying fish movement are required to investigate how migration varies with age, across years and among individuals. Chemical variation across otoliths can potentially provide information on life-history movement of fish among different water bodies (Secor et al. 1995; Campana 1999; Milton and Chenery 2003). Recent studies are beginning to indicate some consistencies in the response of otolith chemistry to physical and chemical properties of water (Thorrold et al. 1997; Bath et al. 2000; Secor and Rooker 2000; Elsdon and Gillanders 2003, 2004; Milton and Chenery 2001; Kraus and Secor 2004). If relationships between water chemistry and otolith chemistry are demonstrated to be clear and consistent across life-stages, chronological variation in otolith chemistry can potentially be used to reconstruct environmental and or movement histories of fish. This approach has particular promise for studying movement histories of individual snapper between Port Phillip Bay and coastal waters. This is because the chemical properties of sheltered marine bays with high anthropogenic inputs, such as Port Phillip Bay, are likely to differ from open coastal waters (Bruland 1983).

1.7 Report outline

In section 2 (objective 1) of this report we investigate spatial and temporal variation in recruitment of juvenile (0-age) snapper to determine whether recruitment variation in Port Phillip Bay is reflected in other bays and estuaries. In section 3 (objective 2, 4, 5) we investigate spatial and temporal variation in otolith chemistry of 0-age snapper, with the aim of identifying a natural tag for Port Phillip Bay snapper. This investigation provides the basis for using natural chemical tags in otoliths to quantify linkages between recruitment of small juveniles in Port Phillip Bay and replenishment of the Victorian snapper fishery. In section 4 (objectives 2, 4, 5) we sample sub-adult (1–2 years age) and young adult (3–5 years age) snapper from Port Phillip Bay and various other regions of the Victorian fishery. Using the otolith chemical tags from 0-age fish as baseline data we determine the proportions of these older fish that originated in Port Phillip Bay as opposed to areas outside the Bay, and how this proportion varies between cohorts and with age. The results of this investigation indicate whether the Port Phillip Bay fishery depends on locally derived recruitment irrespective of movement of snapper between the Bay and coastal waters. Furthermore, this investigation provides information on the contribution that spawning/0-age recruitment in Port Phillip Bay makes to replenishment of the coastal and Western Port fisheries. In section 5 (objective 3) we investigate the potential for chronological variation in otolith chemistry to provide information on the migration histories of snapper between Port Phillip Bay and coastal waters.

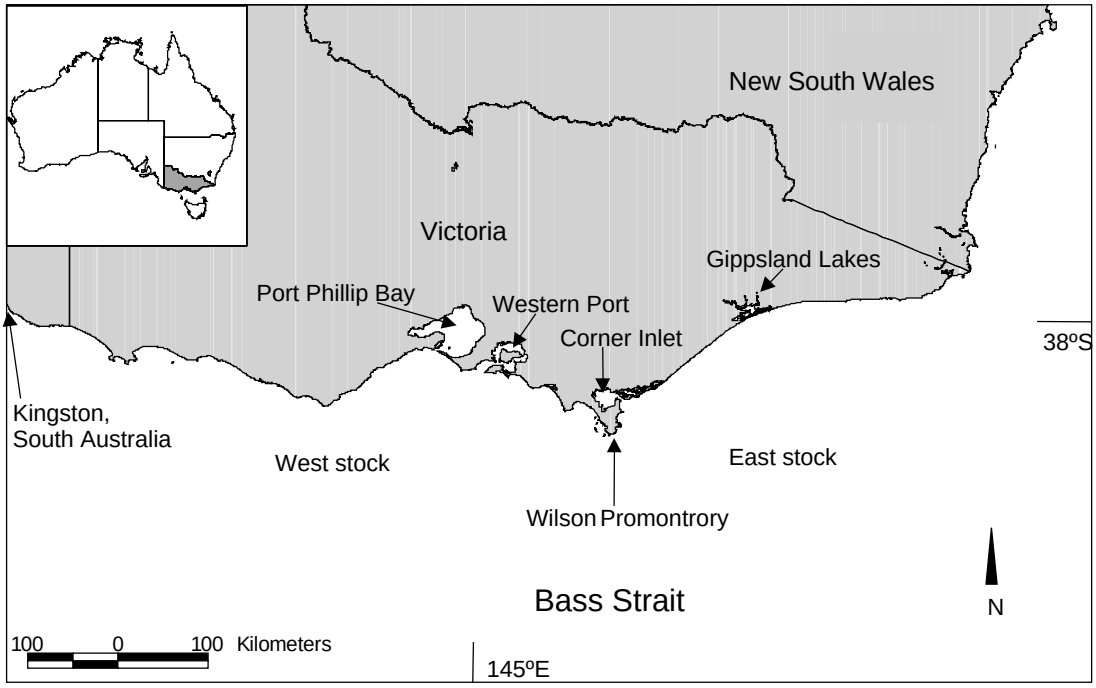

Figure 1. Map of Australia (inset) and the south-east coastal region (main) with major Victorian bays and estuaries and regions of the east and west snapper stocks indicated.

Figure 2. 0-age snapper (2-3 months old, 30-70 mm total length) left, and recently settled snapper (30 days old, 15 mm total length) right.

1.8 Need

From 1980 to the mid 1990s a significant decline in the Victorian snapper catch has occurred. The commercial catch in the major fishery, Port Phillip Bay, has declined from 207 t in 1980/81 to an historical low of 34 t in 1996/97. The status of the Port Phillip Bay fishery is in serious doubt because the relationship between the Port Phillip Bay fishery and the western stock as a whole is unknown. Whether catch declines in Port Phillip Bay are a result of local factors such as overfishing or local recruitment variability, or whether they are the result of changes in migratory dynamics of snapper between Port Phillip Bay and offshore waters, will not be known without new research that is novel in its approach. At present we cannot be certain whether the apparent decline of snapper in Port Phillip Bay mirrors a decline in the entire western stock. Imposing management controls is difficult in the face of such uncertainty, and therefore the snapper fishery in Port Phillip Bay is under threat. This project, endorsed by the workshop on snapper research in Victoria (Newman 1998), aims to clarify some aspects of the relationship between snapper in Port Phillip Bay and the greater western stock. Otolith chemistry is a novel technique that may prove very powerful in answering questions about the origin and migration patterns of snapper between Port Phillip Bay and coastal waters. In addition, pre-recruit sampling across the Victorian coast will indicate whether high recruitment variability observed in Port Phillip Bay is reflected in the entire stock.

1.9 Objectives

1. Determine whether high annual variability in the abundance of pre-recruit (0-age) snapper in Port Phillip Bay is reflected by the entire western stock
2. Determine the importance of spawning of snapper within Port Phillip Bay to the western stock as a whole
3. Determine the movement patterns of snapper between Port Phillip Bay and offshore waters and whether they are changing over time
4. To determine the proportion of the snapper population on the open coast that originates from Port Phillip Bay and how this changes with age
5. To determine the proportion of the snapper population in Port Phillip Bay that originates from areas outside the bay and how this changes with age

2. Spatial and temporal variation in recruitment of 0-age snapper in Victorian waters

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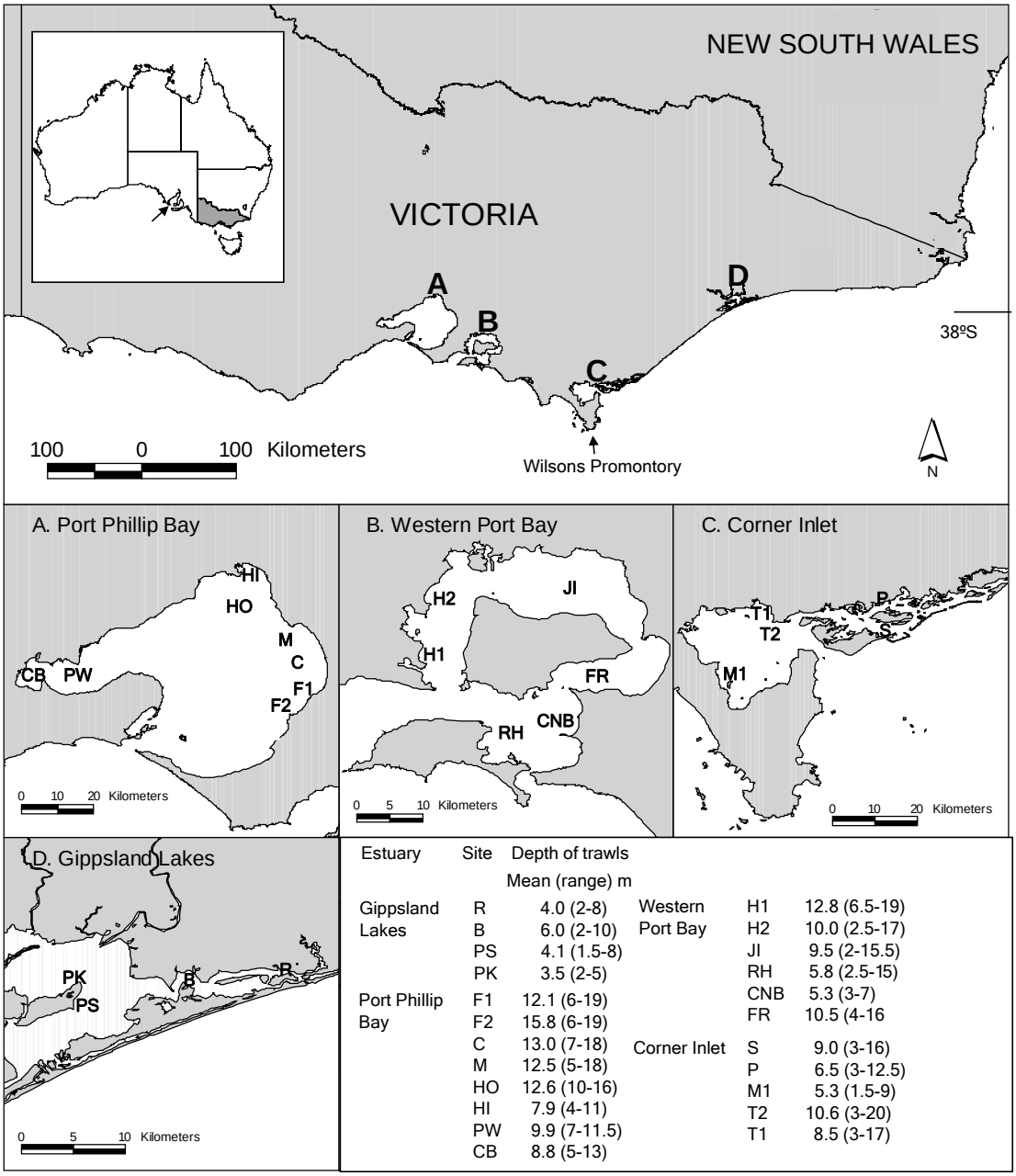
2.1 Introduction

Variable recruitment of young can be a major determinant of both the dynamics and demographics of fish populations (Mapstone and Fowler 1988; Sale 1990; Doherty and Fowler 1994). Studies in Australia (Fowler and Jennings 2003), New Zealand (Francis 1993), and Japan (Azeta et al. 1980) have demonstrated strong interannual variation in recruitment of 0-age (less than one year old, Fig. 2) snapper. High variation in recruitment of the early life stages is now considered to be a major influence on demographics and dynamics of snapper populations (Francis 1993; Fowler and Jennings 2003). Despite progress towards understanding variation in recruitment of 0-age snapper in Japan (Azeta et al. 1980; Tanaka 1985; Nakato and Hirano 1988) and New Zealand (Francis 1993, 1994a) the mechanism(s) driving recruitment variation remain poorly understood in most areas. Only recently has this topic received the close attention of Australian researchers (Fowler and Jennings 2003). Understanding and monitoring juvenile recruitment variation is now considered to be of significant importance to the future management of snapper fisheries in Australia (Ferrell and Sumpton 1996; Coutin et al. 2003; Fowler and Jennings 2003; Gaughan et al. 2003).

Recruitment of juvenile fish is a complex process where variation could stem from a range of factors that influence production, survival and movement of both the pre- and post-settlement stages (Sale 1990; Levin 1994; Hamer and Jenkins 1996; Jenkins et al. 1997; Shima 2001). Understanding recruitment processes necessarily involves obtaining information on the scales and patterns of spatial and temporal recruitment variation (Underwood et al. 2000). While previous studies have investigated interannual variability in recruitment of 0-age snapper (Azeta et al. 1980; Francis 1993; Fowler and Jennings 2003), they have not compared temporal dynamics across both large (100's km) and small (5–20 km) spatial scales. Determining whether the temporal dynamics of recruitment are consistent over different spatial scales is important for directing research on recruitment processes (Huston 1999; Sullivan et al. 2000). For example, if temporal patterns of recruitment were consistent across areas separated by 100's of kilometres it would suggest that large-scale processes (i.e. large-scale oceanography, climate variation) could be influencing recruitment variation. Alternatively, if temporal patterns of recruitment were not consistent across large spatial scales, but varied differently among discrete recruitment areas (i.e. bays/estuaries), it would suggest that local-scale processes (i.e. local hydrodynamics, river flow, habitat, food availability, predation) are likely to be important influences on recruitment variation (Sullivan et al. 2000). Further, this type of information is critical for the design of monitoring programs aimed at obtaining early estimates of year-class strength for particular stocks (i.e. pre-recruit monitoring). For example, where various discrete nursery areas, such as bays and estuaries, contribute recruitment to a widely dispersed adult stock, if the different areas have different dynamics of juvenile recruitment it may be necessary to monitor each area to obtain a recruitment index relevant for the entire stock.

The focus of this study is to improve understanding of spatio-temporal variability of 0-age snapper recruitment in Victoria. This knowledge is required to direct future research into causes of recruitment variation, and for designing ongoing pre-recruit monitoring programs for the Victorian fishery. There is also a distinct lack of knowledge on the habitats, distributions and variability of 0-age snapper recruitment in Victorian waters (Hamer et al. 1998). We used a small beam trawl to compare temporal variability in recruitment of 0-age snapper over four years across four Victorian bays and estuaries separated over a distance of approximately 500 km.



Estuary	Site	Depth of trawls Mean (range) m			
Gippsland Lakes	R	4.0 (2-8)	Western Port Bay	H1	12.8 (6.5-19)
	B	6.0 (2-10)		H2	10.0 (2.5-17)
	PS	4.1 (1.5-8)		JI	9.5 (2-15.5)
	PK	3.5 (2-5)		RH	5.8 (2.5-15)
Port Phillip Bay	F1	12.1 (6-19)		CNB	5.3 (3-7)
	F2	15.8 (6-19)		FR	10.5 (4-16
	C	13.0 (7-18)	Corner Inlet	S	9.0 (3-16)
	M	12.5 (5-18)		P	6.5 (3-12.5)
	HO	12.6 (10-16)		M1	5.3 (1.5-9)
	HI	7.9 (4-11)		T2	10.6 (3-20)
	PW	9.9 (7-11.5)		T1	8.5 (3-17)
	CB	8.8 (5-13)			

Figure 3. Map of Australia (inset, arrow indicates Spencer Gulf) and the Victorian coast showing locations of estuaries (A-D) and sites within estuaries where 0-age *Pagrus auratus* were sampled. Information on trawl depths at each site is included at bottom right. (Key to sites – CB, Corio Bay; PW, Point Wilson; HI, Hobsons Bay Inner; HO, Hobsons Bay Outer; M, Mordialloc; C, Carrum; F1, Frankston 1; F2 Frankston 2; H1, Hastings 1; H2, Hastings 2; JI, Joe's Island; FR, Freemans, CNB, Coronet Bay; RH, Rhyll; T1, Toora Channel 1; T2, Toora Channel 2; M1, Middle Channel 1; P, Pelican; S, Snake; PK; Point King; PS, Point Scott; B, Bancroft Bay; R, Reeves Channel).

2.2 Methods

2.2.1 Sampling locations

We sampled Victoria's four major bay/estuaries; Port Phillip Bay, Western Port, Corner Inlet, and Gippsland Lakes (Fig. 3). The limited information available on 0-age snapper suggested that recruitment was likely to occur in each of these areas. Importantly, for the aims of the study, these areas encompassed eastern and western Victoria and are separated over a large distance (~500 km). Port Phillip Bay is a large marine dominated estuary/bay with a narrow entrance region characterised by strong tidal currents and a large, low-energy, central basin area reaching a maximum depth of 24 m. Western Port is marine dominated, but is highly tidal throughout. It has two entrances to the ocean, and is characterised by channels of up to 20 m depth, interspersed with large areas of intertidal mudflats. Corner Inlet is marine dominated and highly tidal throughout. Corner Inlet has three major entrances to the ocean, and is also characterised by deeper channels, up to 25 m depth, and large areas of intertidal mudflat. Gippsland Lakes is an estuarine lagoon system with one entrance, and water depths generally less than 10 m.

Based on discussions with local fisherman and the limited previous data (Rigby 1984; Ramm 1986; Hamer et al. 1998) we initially identified large areas within each estuary where 0-age snapper were likely to occur. Within these regions we allocated multiple sites of approximately 4.5 km^2, and ensured that sites were separated by at least 3–4 km to maintain their independence. To maintain separation between sites, and to obtain better estimates of recruitment at estuary wide scales, we varied the number of sites within each estuary in relation to overall area of the estuary. We selected eight sites in Port Phillip Bay, six in Western Port, five in Corner Inlet, and four in Gippsland Lakes (Fig. 3). Sampling was conducted at each estuary once in both February and March from 2000 to 2003. Sampling was timed to coincide with the expected end of the larval settlement period (based on Jenkins (1986)). Each sampling event consisted of five, non-overlapping trawls of 7 minutes bottom time within each site. The start co-ordinates of each trawl were randomly allocated prior to sampling.

2.2.2 Plumb-staff beam trawl

The small plumb-staff beam trawl we used in this study was designed based on similar trawls used by Gunderson and Ellis (1986) to sample small-benthic fishes and by Azeta et al. (1980) to sample small (12 – 100 mm standard length, SL; tip of snout to end of caudal peduncle) 0-age snapper in Japan. Unlike otter trawls, where the swept area can change depending on sampling conditions, habitats, and tow speeds, this type of trawl maintains a constant mouth width irrespective of sampling conditions or habitats (Gunderson and Ellis 1986). A detailed description of the trawl net is provided in figure 4. A tickler chain was attached between the bottom wing tips to scare fish off the bottom into the path of the net. The required warp to depth ratio for firm bottom contact in all conditions was 5:1 at a tow speed of 1.5–2 knots. To maximise catch rates we conducted all sampling at night (following Hamer et al. (1998)). The standard tow time was restricted to 7 minutes bottom time because of problems with algae fouling of the net at some sites. Latitude and longitude were recorded at the beginning and end of bottom contact for each tow using a differential GPS. All 0-age snapper were sorted from the net immediately after net retrieval and refrigerated or frozen for later measurement and extraction of otoliths. The number of 0-age snapper collected each trawl was standardised to number 1000 m^{-2} based on the distance of the tow and the mouth width of the net determined as 2.8 m when fishing. The average tow length was 350 m of bottom contact, but tow length did vary due to the influence of tidal currents and wind, hence standardisation of catches was required. Our main interest was in relative rather than absolute abundances. However, the range of species, sizes, and swimming abilities of fish captured with this gear (see Hamer et al. (1998)), including 1–2 year old snapper, would indicate that the plumb-staff beam trawl fished at night is an efficient sampler of 0-age snapper.

2.2.3 Water temperature

We obtained sea surface temperatures from satellite imagery data supplied by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Temperature estimates were obtained for the central region of Port Phillip Bay based on averages over four day intervals over the period of the study and long-term averages were determined from data collected over the period November 1989 to November 2003.

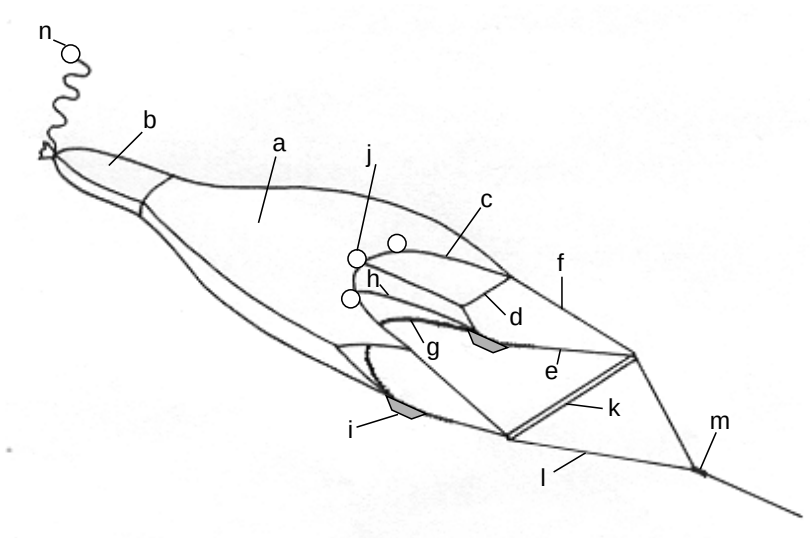


Figure 4. Diagram of the plumb staff beam trawl used to sample 0-age snapper. a) main body of net, 4 m long, 12 mm stretch, 4 mm² aperture knotless raschel mesh. b) codend bag, 1m long, 8 mm stretch, 3 mm² aperture knotless raschel mesh. c) 4.7 m head rope. d) 2.2 m breastlines. e) 1.7 m lower bridle, first metre of lower bridle is 2 cm x 3 cm x 0.5 cm chain. f) 1.8 m upper bridle. g) 4.8 m tickler chain, chain type as previous. h) 5.6 m footrope. i) 5.8 kg curved detachable lead weights (diving hip weights). j) 9.5 cm diameter foam floats. k) 3 m steel beam, 3 cm diameter pipe. l) 4.1 m beam bridle rope. m) stainless swivel. n) emergency retrieval line.

2.2.4 Data analyses

We used analysis of variance (ANOVA) to investigate spatio-temporal variation in catches of 0-age snapper among estuaries and years. Due to the random nature of the trawls within sites, habitat patchiness in some estuaries, and several years of low recruitment during the project, the catch data were highly variable among trawls, and even after transformations did not meet the assumptions of homogeneity of variances and normality required by ANOVA. In particular, the data for Western Port could not be included in any statistical analyses due to the extremely low catches (high frequency of zero data) from this estuary in all years. In order to meet assumptions of ANOVA we analysed the data from the three remaining estuaries (Port Phillip Bay, Corner Inlet, Gippsland Lakes) across the 4 years, with the data pooled across the trawls taken within each site. We then treated the sites as the replicates within each estuary. Spatio-temporal variability within estuaries was interpreted from graphical displays of the data. The data required transformation to $\log_{10}(x + 1)$ to meet the assumptions of ANOVA. If an interaction between year and estuary was found in the main analysis we then conducted planned one-way ANOVA followed by post-hoc Tukey's tests for each estuary to compare the patterns of variation across years, among the estuaries.

Comparison of size distributions among estuaries and years can provide further information on variation in recruitment processes. Post-settlement size distributions are a combination of variability in larval settlement times and rates, and post-settlement survival/growth. Similarity among size distributions of small 0-age juveniles can therefore be used to indicate similar dynamics of larval settlement and juvenile survival rates. We qualitatively compared size frequency distributions (standard length, SL, mm) among estuaries, months and years.

2.3 Results

2.3.1 Spatial and temporal variation among estuaries:

Over 1,500 0-age and 177 one year old snapper were collected during the study. Catch rates of 0-age snapper were generally higher and more variable in Gippsland Lakes than in Port Phillip Bay and Corner Inlet, although mean catch rates in Port Phillip Bay and Gippsland Lakes were similar in 2001 (Figs. 5, 6). We did not include Western Port in graphs or tables because of the low catches of 0-age snapper. Despite more than 250 trawls in a range of habitats over the four sampling years we only collected 12 x 0-age snapper in Western Port. Of these 12 specimens, 2 were collected in 2000, 4 in 2001, 3 in 2002, and 3 in 2003. They were only collected from the H1, H2, JI, and CNB sites (Fig. 3), and ranged in size from 14 to 57 mm SL. We also failed to catch 0-age snapper in all years at the CB site in Port Phillip Bay (Fig. 6).

Results from ANOVA showed significant year and estuary affects, and importantly, a significant year by estuary interaction (Table 1, Fig. 5). This meant that interannual recruitment variation was dependent on the estuary. This is demonstrated in figure 5, by the clear difference in the recruitment patterns among estuaries between 2001 and the other 3 years.

The month affect was also significant, with generally higher catches in March than in February (Table 1, Fig. 5). The interactions of year by month, month by estuary, and year by estuary by month, were however not significant. This suggested that spatial variation among estuaries was similar irrespective of the sampling month within each year and also that the patterns of interannual variation for each estuary were not dependent on the month in which sampling was conducted each recruitment season (Table 1, Fig. 5).

The results of planned one way ANOVA and Tukey's tests showed that for Port Phillip Bay the year effect was highly significant ($p < 0.001$), and that year 2001 had significantly higher recruitment than all the other years (Tukey's, $p < 0.001$) that were not significantly different from each other (Fig. 5, 6). For Corner Inlet the year effect was also significant ($p < 0.028$), but the only significant difference among years was that year 2001 was significantly higher than year 2003 (Tukey's, $p < 0.020$) (Fig. 5, 6). For Gippsland Lakes the year effect was not significant ($p > 0.05$) (Fig. 5, 6).

Table 1. Results of ANOVA comparing catch rates of 0-age snapper among three Victorian estuaries sampled in February and March of 4 years; 2000 to 2003.

Source	d.f.	MS	P
Year	3	0.598	0.007*
Estuary	2	0.715	0.001*
Month	1	0.491	0.027*
Year x Estuary	6	0.342	0.003*
Year x Month	3	0.141	0.236
Estuary x Month	2	0.115	0.314
Year x Estuary x Month	6	0.028	0.941
Residual	111	0.098	

*Significant results ($P < 0.05$).

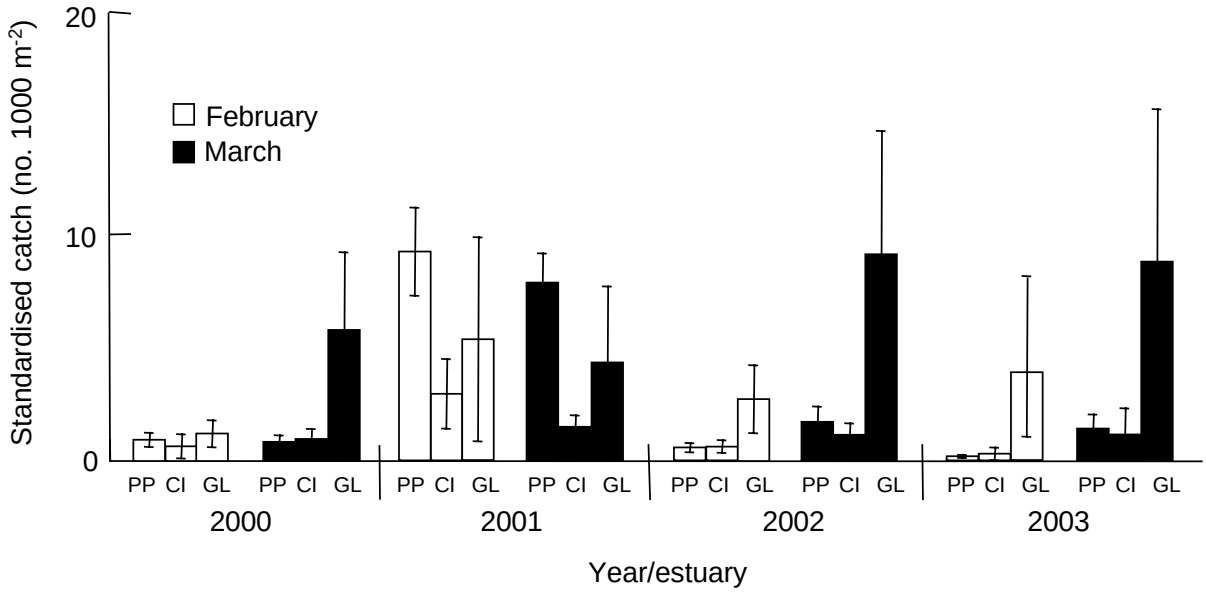


Figure 5. Mean catch rates (± 1SE) of 0-age snapper in three Victorian estuaries sampled in February and March and compared over four years. PP, Port Phillip Bay; CI, Corner Inlet; GL, Gippsland Lakes.

2.3.2 Variation within estuaries

In Port Phillip Bay 0-age snapper generally occurred in trawls deeper than 10 m, and they occurred at all sites except CB in each year (Figs. 3, 6). The pattern of spatial variation within Port Phillip Bay appeared to differ slightly among years, particularly between high and low recruitment years (Fig. 6). During the low recruitment years (2000, 2002, 2003) highest catches were generally at sites M, C, F1 and F2 in the north east part of the Bay (Fig. 6). In the high recruitment year of 2001, higher catches were observed at all sites with highest catches occurring at the HO, F1 and F2 sites (Fig. 6).

In Corner Inlet 0-age snapper were captured in depths from 3 to 15 m (Fig. 3). Variation among sites also appeared to depend on the year (Fig. 6). For example, variation among sites was similar in 2000 and 2002 but differed in 2001 (Fig. 6). In 2003 no 0-age snapper were taken at the M1, T1, and T2 sites situated in the Corner Inlet basin (Fig. 6). Similar to Port Phillip Bay, higher catches occurred at all sites during the highest recruitment year of 2001 (Fig. 6). In the low recruitment years the highest catches were always at the P site, and catches at the P and S site varied least among years, compared to the M1, T1, and T2 sites (Fig. 6).

In Gippsland Lakes 0-age snapper were captured in depths from 2 to 10 m (Fig. 3). Catch rates were more variable among trawls in Gippsland Lakes than the other estuaries (Fig. 6). Highest catches occurred at the two sites closest to the entrance, R and B (Figs. 3, 6). In 2001 no 0-age snapper were collected at the PK and PS sites situated most distant from the entrance and in 2003 no 0-age were collected at the PS site and only one individual was collected at the PK site (Fig. 6). Catches were similar at the two sites close to the entrance in all years except 2000 when catches were greater at site R (Fig. 6).

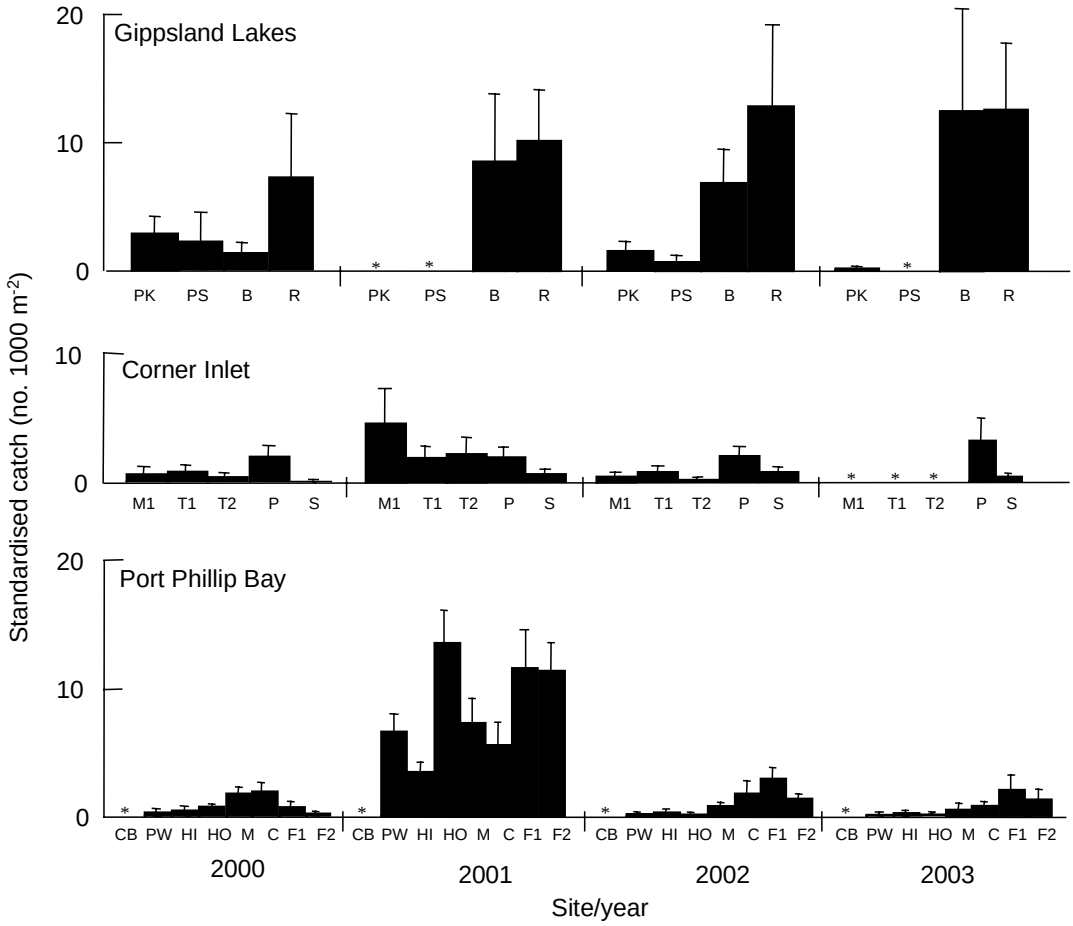


Figure 6. Mean catch rates (± 1SE) of 0-age snapper at different sites within three Victorian estuaries, compared over four years. Data are pooled across February and March sampling events, and * indicate that no snapper were collected during sampling. Site labels are as in Fig. 3.

2.3.3 Size distributions

We found newly settled snapper (< 15 mm SL) in all estuaries (Figs. 7–9), and the smallest snapper collected by the beam trawl in all estuaries were 10 mm SL (Figs. 7–9). In all years most 0-age remained under 110 mm SL in March samples (Figs. 7–9). The size distributions for February and March of each year were generally similar for the individual estuaries (Figs. 7–9). This suggested that early post-settlement processes such as mortality or migration did not modify the size structure of recruits.

Comparison of size distributions among estuaries and years showed distinct differences. Obvious differences among estuaries occurred for the 2001 and 2002 year-classes. The distribution for Port Phillip Bay in 2001 was bimodal (Fig. 7), whereas for Corner Inlet (Fig. 8) and, in particular, Gippsland Lakes (Fig. 9) the distributions were not strongly bimodal. In 2002 there appeared to be two size modes for Corner Inlet (Fig. 8) and Gippsland Lakes (Fig. 9), but only one for Port Phillip Bay (Fig. 7). In 2000 and 2003 size distributions were relatively similar among estuaries, with modes around 50 mm SL in March 2000, and around 20–30 mm SL in March 2003 (Fig. 7–9).

In March 2003 there were significant numbers of fish over 50 mm SL in Gippsland Lakes (Fig. 9), but fish of this size were rare in Port Phillip Bay (Fig. 7) and Corner Inlet (Fig. 8). Apart from 2001, Gippsland Lakes generally showed a greater spread of sizes than Port Phillip Bay (Figs. 7, 9). Recently settled recruits (<15 mm SL) occurred in Gippsland Lakes in March samples in all years (Fig. 9). They were, however, either not present or present in very low numbers in March samples from Port Phillip Bay (Fig. 7) and Corner Inlet (Fig. 8). This would appear to indicate that larval settlement finished later in Gippsland Lakes. In 2002, we found that one year old fish (1+) of the 2001 year-class were abundant in

Port Phillip Bay (Fig. 7). In contrast to Port Phillip Bay, 1+ fish of the 2001 year-class were taken in low numbers in Gippsland Lakes in 2002 (Fig. 9), and very few 1+ fish were taken in Corner Inlet in any year (Fig. 8).

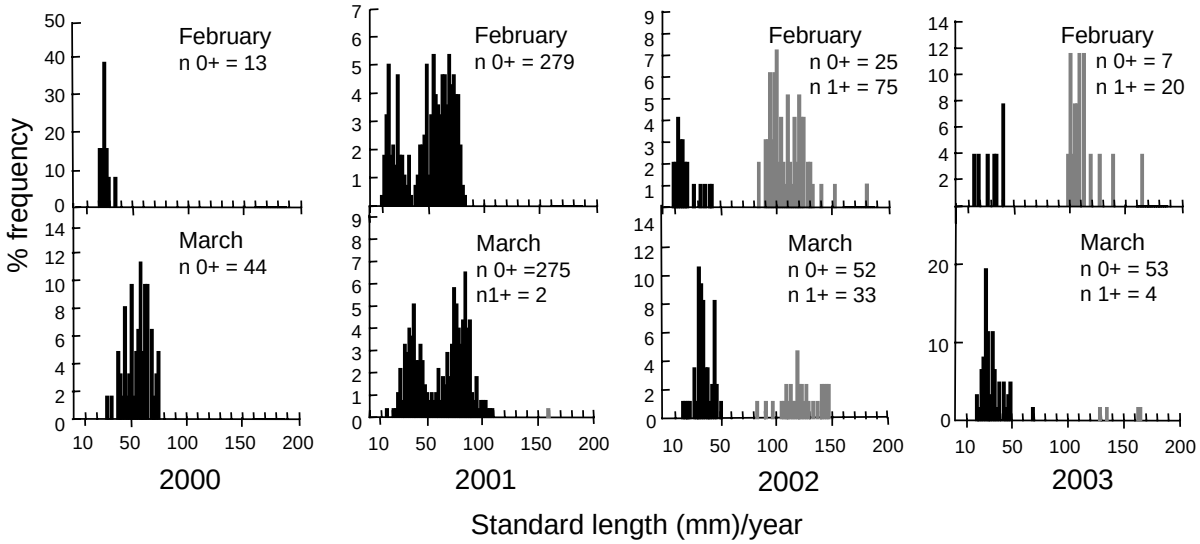


Figure 7. Port Phillip Bay: Size frequency distributions of snapper sampled during February and March from 2000 to 2003. Dark bars represent 0-age (0+) fish and light bars represent 1+ fish. Sample sizes (n) of each age class are indicated.

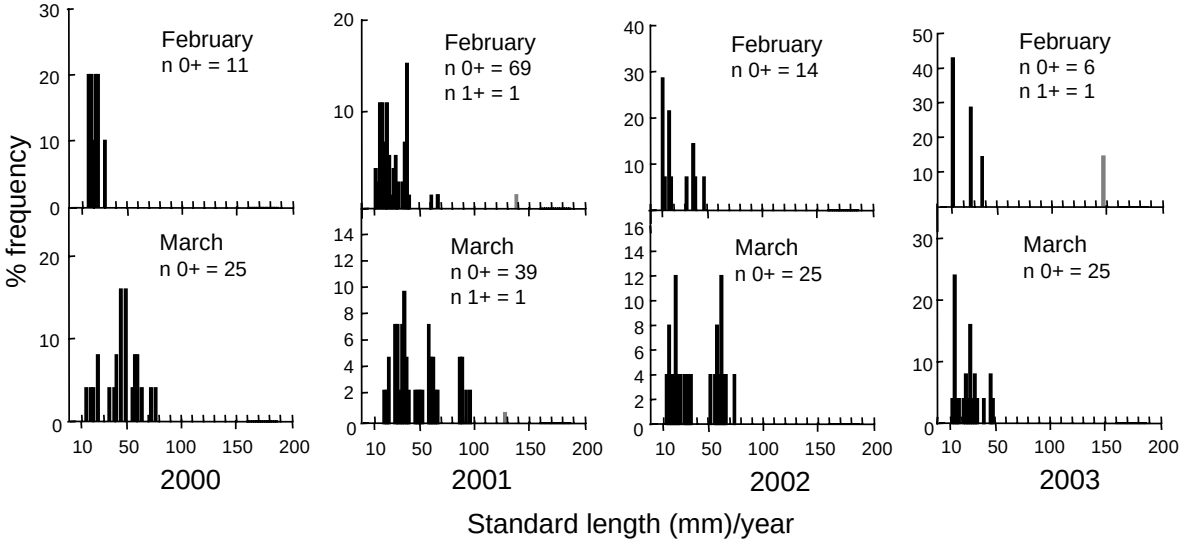


Figure 8. Corner Inlet: Size frequency distributions of snapper sampled during February and March from 2000 to 2003. Dark bars represent 0-age (0+) fish and light bars represent 1+ fish. Sample sizes (n) of each age class are indicated.

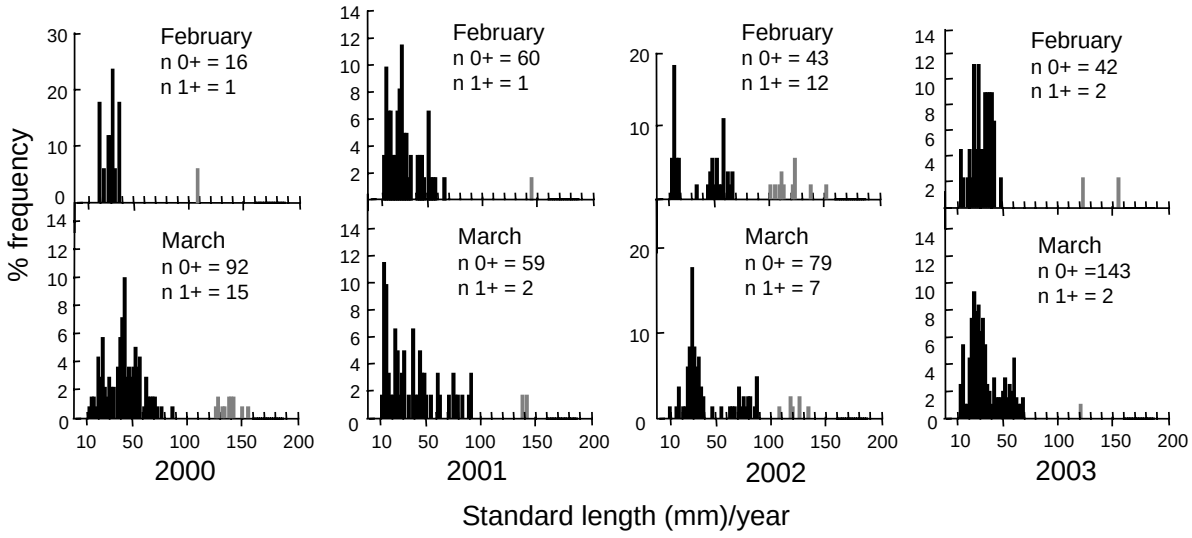


Figure 9. Gippsland Lakes: Size frequency distributions of snapper sampled during February and March from 2000 to 2003. Dark bars represent 0-age (0+) fish and light bars represent 1+ fish. Sample sizes (n) of each age class are indicated.

2.3.4 Water temperature

In all years sea surface temperature in Port Phillip Bay peaked in February, however, temperature profiles and maxima over the summer spawning/larval period (November to February) varied among years (Fig. 10). The summer profiles where similar to the long-term average for 1999—2000 and 2002—2003 (Fig. 10). In summer 2000—2001 the profile was characterised by a rapid increase (from 17 to 20°C) in early November and above average temperature over the summer period (Fig. 10). In contrast, water temperature in 2001—2002 was below average for the entire November to February period (Fig. 10).

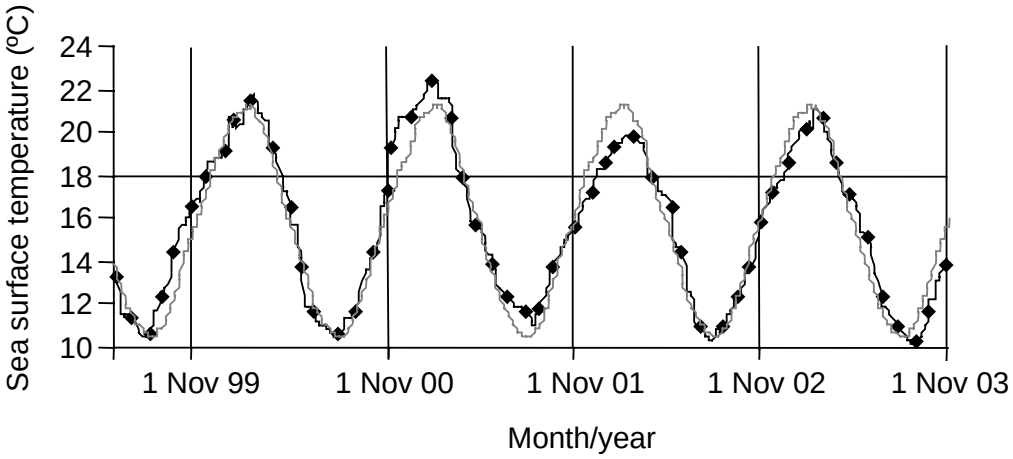


Figure 10. Daily (black line) and long-term average (grey line) sea surface temperatures for central Port Phillip Bay, from June 1999 until November 2003. Diamonds indicate the first day of each month. The long-term average is based on data collected from November 1989 to November 2003.

2.4 Discussion

2.4.1 Interannual recruitment variation

Prior to this study interannual recruitment variation of 0-age snapper had not been directly compared across multiple estuaries in southern Australia. We found that high interannual recruitment variation in Port Phillip Bay was not reflected in other Victorian bays and estuaries over a distance of 500 km. Recent comparisons of interannual (2000-2007) recruitment variation between Port Phillip Bay and Spencer Gulf (South Australia), approximately 1200 km west of Port Phillip Bay (Fig. 3), also indicate that major recruitment fluctuations are generally not synchronous among snapper stocks in southern Australia (P.A. Hamer unpublished data; A. Fowler unpublished data). Clear differences among 0-age size distributions for estuaries in Victoria further indicate that the processes influencing recruitment success within a spawning season also vary among different estuaries. These results clearly demonstrate that temporal patterns of 0-age recruitment differ among discrete snapper nursery areas in southern Australia. Variation in year-class strength is therefore unlikely to be closely related among southern Australian snapper stocks. Furthermore, different recruitment patterns among estuaries implies that understanding the dynamics of individual snapper stocks will require knowledge of how different recruitment areas contribute to stock replenishment. This will be particularly important for deciding the appropriate spatial design of pre-recruit monitoring programs.

High interannual variability in 0-age recruitment has now been demonstrated for most regions where important snapper fisheries occur. Studies in other regions of Australia (Fowler and Jennings 2003; M. Moran, personal communication), New Zealand (Francis 1993) and Japan (Azeta et al. 1980), have demonstrated that maximum year to year differences in recruitment can range from at least 7 to 20-fold. The 10-fold variation among years observed for Port Phillip Bay is therefore within the range experienced in other regions. Monitoring of one year old snapper in Port Phillip Bay in 2002 (i.e. the 0-age we sampled in 2001) showed that abundance was the greatest in 12 years of data collection and was approximately 4-fold greater than the next strongest year-class (Parry et al. 2003). This suggests that the 10-fold difference in 0-age abundance we observed over the four years of this study represented a major recruitment event in Port Phillip Bay. At the time of writing the 2001 cohort was becoming a major component of the recreational catches in western Victoria (S. Conron, unpublished data).

Differences in annual recruitment patterns between estuaries in eastern and western Victoria are likely to be related to different recruitment processes. Port Phillip Bay experiences a major immigration of larger reproductive adults during the late spring and summer and a restricted summer spawning period (Coutin et al. 2003). Because spawning is occurring within Port Phillip Bay, larval survival and recruitment of 0-age snapper to Port Phillip Bay is likely be strongly influenced by variation in local conditions. Unlike Port Phillip Bay, snapper are thought not to spawn in the smaller east Victorian estuaries and recruitment into east-coast estuaries is thought to depend on larvae being supplied from coastal spawning areas. Observations of snapper larval stages in ocean waters off far eastern Victoria coupled with the potential of the east Australian current to transport larval stages from spawning areas further north into New South Wales (NSW, Fig. 1, 3) (Ruello 1975; Montgomery 1990; Neira et al. 2000), suggests that ocean spawning over a wide area along the east Australian coast may contribute to larval recruitment in eastern Victorian estuaries. Combined with the more extended spawning season in the warmer waters off NSW (Ferrell and Sumpton 1996), this could result in a longer recruitment window for east-coast estuaries. Size distribution data support this idea by showing a generally longer period of larval settlement in Gippsland Lakes compared to Port Phillip Bay. Similar to the situation in Shijiki Bay Japan (Nakato and Hirano 1988), both local and large-scale hydrodynamic regimes, including variability in the flow of the east Australian current, are likely to be critical factors influencing the supply of larvae and the dynamics of 0-age recruitment in east Victorian estuaries.

We propose that negative and positive influences on larval production and survival are likely to produce greater extremes of recruitment when recruitment is largely dependent on one localised spawning source with a restricted spawning period, such as Port Phillip Bay. When recruitment is dependent on numerous spawning areas with a more prolonged spawning period, there will be an increased chance that spawning from at least some sources and times will produce recruitment in all years. This may result in less extreme variation in recruitment, such as observed for Gippsland Lakes and Corner Inlet.

The low abundance of 0-age snapper in Western Port is difficult to explain without further study. Large adult snapper are common in Western Port during the spawning season, but it is unclear whether the poor recruitment is a result of low spawning output, and/or poor larval or post-settlement survival. We sampled a wide range of habitats, depths and regions within Western Port, so localised areas of recruitment were unlikely to have been missed. Several other independent studies were also conducted in Western Port concurrent with this one and reported very few 0-age snapper using a variety of sampling techniques both in intertidal and sub-tidal habitats (Hindell and Jenkins 2004; J. Kemp unpublished data). These results, in combination, would suggest that Western Port is not a major recruitment area for 0-age snapper.

2.4.2 Variation within bays and estuaries

Small-scale spatial variation in juvenile snapper abundance can be related to habitat association (Kingett and Choat 1981; Francis 1995; Thrush et al. 2002) and/or food availability (Azeta et al. 1980; Kiso 1982; Sudo et al. 1983; Tanaka et al. 1987). We found considerable spatial variability in catch rates both among trawls and sites at each estuary. Differences in catch rate variability among trawls, sites and estuaries may have been related to differences in the scales of habitat or food variation. In Gippsland Lakes, high variation in catches among trawls (i.e. scales of 100 m's) appeared related to habitat association because the greatest catches were most often associated with shallower (< 4 m depth) seagrass (*Zostera tasmanica)* beds, a habitat that was spatially patchy at scales of 10 -100's metres (personnel observation). While this study was not designed as a study of habitat preference, we did observe that the largest catches of 0-age snapper occurred in different habitats depending on the estuary. For example, shallow (< 4 m) seagrass beds in Gippsland Lakes compared to deeper water (> 10 m) muddy habitats with sparse algae in Port Phillip Bay. Habitat association seemed to depend on the estuary, supporting the idea that habitat type *per se* is not the major influence on distributions and that food availability may be a more important factor influencing distributions of 0-age snapper (Tanaka et al. 1987, Jenkins and Hamer 2001).

Similar to the large-scale comparison among estuaries, the patterns of spatial variation in recruitment within estuaries appeared to differ among years. While the higher recruitment in Port Phillip Bay and Corner Inlet in 2001 was an estuary-wide phenomenon, the patterns of spatial variation within Port Phillip Bay and Corner Inlet differed between high and low recruitment years. Across years, however, certain regions in each estuary, for example, the sites in the north-east corner of Port Phillip (M, C, F1, F2), the Pelican (P) site in Corner Inlet and the Reeves (R) site in Gippsland Lakes (Fig. 3), consistently supported higher numbers of recruits. These sites may constitute areas where larval supply is most consistent among years and/or the habitat and food availability is most optimal for survival of recruits. In Gippsland Lakes the consistently greater abundances at the sites nearest the entrance was likely to have been driven by a combination of higher rates of larval settlement and habitat suitability. Newly-settled recruits (12 -15 mm SL) were common at sites near the entrance of Gippsland Lakes, particularly in seagrass habitat, but only larger individuals (>30 mm SL) were captured at the two sites furthest into the estuary. Recruitment to sites further into the system was therefore the result of post-settlement migration rather than larval settlement. While other studies have also shown that certain areas within large bays and gulfs consistently supported higher numbers of juvenile snapper (Azeta et al. 1980; Francis 1995; Fowler and Jennings 2003), it appears that identifying these areas within different bays and estuaries may not be a matter of simply *a-priori* identifying a specific type of habitat (i.e. seagrass, muddy unvegetated, reef).

2.4.3 Temperature and recruitment variation in Port Phillip Bay

Francis (1993) demonstrated a strong positive relationship between water temperature during the 0-age year and recruitment of one year old snapper in Hauraki Gulf, New Zealand. Fowler and Jennings (2003) have also suggested that a similar mechanism related to water temperature might explain strong 0-age recruitment variation in Spencer Gulf, South Australia. Previous studies have suggested that relatively small increases in water temperature can result in increased larval growth rates and subsequent reductions in larval duration (Gadomski and Caddell 1991; Francis 1994ab; Fowler and Jennings 2003), that in turn lead to higher larval survival rates and ultimately higher recruitment of juveniles (Houde 1987). Water temperature might also influence recruitment through its influence on spawning behaviour (Scott and Pankhurst 1992) and successful egg development, both of which are suggested to increase when temperatures exceed 18°C for Australian snapper (Battaglene and Talbot 1992; Coutin et al. 2003; Fielder and Allan 2003). The high recruitment observed in Port Phillip Bay in 2001 was coincident with the only summer where water temperature was both above average and reached 18°C by early

November. The presence of both larger and small newly-settled recruits in February of 2001 suggests that spawning and larval settlement began earlier and occurred over a longer period in this year. It is plausible that the early and prolonged increase in water temperature during the 2001 spawning season may have resulted in a longer period of spawning and higher larval growth and survival rates leading to the higher recruitment of 0-age fish to this cohort.

Earlier investigations of relationships between environmental variables and other indices of year-class strength (i.e. surveys of one year old fish and commercial catch data) suggest only a weak positive relationship between water temperature in November and year-class strength of snapper in Port Phillip Bay (Coutin et al. 2003). This research suggested that local-scale factors such as rainfall and river flow could also be important in influencing recruitment success (Coutin et al. 2003). The roles of other local-scale factors such as variability in planktonic food web dynamics, predation, and the abundance of spawning adults within the Bay have not been fully investigated, but are potential influences on recruitment that may or may not be independent of temperature. A recently published study of snapper larval dynamics in Hauraki Gulf, New Zealand, suggests that the positive correlation between 0-age snapper recruitment and water temperature previously mentioned, may be related to wind mixing rather than a direct temperature affect (Zeldis et al. 2005). Zeldis et al. (2005) suggested that increased wind mixing, correlated with temperature variation, results in higher water column productivity, and a superior feeding environment for larval stages which leads to higher larval growth and survival rates. They also found no clear evidence that variable egg production and predator abundance was driving major fluctuations in larval abundance (Zeldis et al. 2005). Although the mechanisms driving variation in larval food abundance are likely to vary among different areas, understanding variation in water column productivity may be critical in explaining high 0-age recruitment variation in Port Phillip Bay.

While the influence of temperature on recruitment success requires further study in Victoria, the results if this study indicate that year-class strength is set very early in the life-history, most likely the larval stage. This implies that research into the processes behind 0-age recruitment variation should be directed at the time period encompassing spawning and the first few months of life, i.e. late October – January. Daily ageing to compare spawning times, larval duration periods and larval growth rates among high and low recruitment years and with environmental parameters and planktonic food availability (ie. Fowler and Jennings 2003; Zeldis et al. 2005), along with continued recruitment and environmental monitoring is required to better understand causes of 0-age recruitment variation in Port Phillip Bay.

2.4.4 Conclusions

High annual variability in the abundance of pre-recruit (0-age) snapper in Port Phillip Bay is not reflected in other Victorian bays and estuaries, and does not appear closely matched to annual variations observed in South Australian gulfs. Monitoring of 0-age snapper recruitment and research into causes of variation should therefore be specific to individual bays/estuaries for Victorian snapper stocks. Spatial and annual recruitment patterns were similar across months in late summer/early autumn, therefore, annual monitoring of 0-age recruitment will only require one sampling event each year, and this should occur in March. High recruitment variation of 0-age snapper is most likely driven by processes influencing the survival of larval stages, which should be a focus of future research. The strong recruitment of 0-age snapper observed for Port Phillip Bay, coupled with the lack of recruitment observed in Western Port, suggests that Port Phillip Bay is an important spawning/nursery area for the western Victorian snapper stock. The occurrence of 0-age recruitment in coastal waters remains unclear and requires further more intensive sampling efforts.

3. Spatial and temporal variation in otolith chemistry of 0-age snapper in Victorian waters

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3.1 Introduction

For many species of marine fish the adults are continuously distributed over a broad area whereas the small juveniles are concentrated in specific habitats, such as estuaries. Determining the contributions of different juvenile areas (often termed nursery areas, see Beck et al. 2001) to local fish populations is important for understanding population structure and dynamics, and is essential for identification and protection of critical juvenile habitats. Traditional tagging methodologies have generally failed to provide this information due to difficulties in manual tagging of large numbers of small juveniles, high mortality of the juvenile stages, low recapture rates and biases in tagging and recapture effort. Study of the elemental composition of fish otoliths has demonstrated that incorporation of various elements into these predominantly calcium carbonate structures can potentially provide a mechanism for natural tagging of fish (Campana 1999; Campana and Thorrold 2001).

Recent research has taken advantage of spatial variability in otolith chemistry to characterise chemical 'tags' or 'signatures' in otoliths of juvenile fish that are specific to certain areas (Gillanders and Kingsford 1996; Thorrold et al. 1998ab). Development of fine-scale sampling techniques, such as laser ablation (Russo et al. 2002), have made it possible to chemically analyse specific regions within otoliths. By matching the chemistry of the juvenile region within adult otoliths to previously characterised chemical tags from juvenile otoliths, it is possible to retrospectively determine the geographic origins of older fish that may have migrated large distances from there place of origin (Thorrold et al. 2001). Otolith chemistry can therefore provide unprecedented information on the contributions that specific juvenile recruitment areas make to replenishment of local and widely dispersed fish populations.

The application of otolith chemistry as a natural tagging method depends firstly on finding spatial variation in juvenile otolith chemistry at the relevant scales. Secondly, knowledge of temporal variation in otolith chemistry, at a range of scales, will be critical in determining how chemical tags developed from juvenile otoliths are used to determine the origin of older fish (Gillanders 2002b). The aquatic environment can be both physically and chemically dynamic, and therefore otolith chemistry of fish collected in the same area might vary among years (Milton et al. 1997; Gillanders and Kingsford 2000; Rooker et al. 2001a; Gillanders 2002b), months (Thorrold et al. 1998a; Gillanders 2002b; Swearer et al. 2003), and potentially over even finer temporal scales. If temporal variation in otolith chemistry can confound patterns of spatial variation, this will have important implications for how chemical tags characterised from juvenile otoliths are used to determine the origins of adults (Gillanders 2002b). For example, if juvenile chemical tags differ strongly among cohorts (year-classes), then it may not be possible to use chemical tags from one cohort of juveniles to determine the origins of adults that are not from the same cohort.

Snapper, *Pagrus auratus*, are an ideal candidate for applying otolith chemistry to investigate spatial connectivity between juvenile recruitment areas and replenishment of adult populations. The life-history of snapper is generally thought to consist of a post-settlement and early juvenile phase (first year of life) within sheltered bays and estuaries, followed by migration of older juveniles and sub-adults (< 4 years age) into coastal waters (Kailola et al. 1993). In some large bay/estuaries, such as Port Phillip Bay, Victoria (Fig. 11), all life stages are found and adults actually migrate into the Bay from coastal waters on a seasonal basis to spawn (Coutin et al. 2003). In Port Phillip Bay, immigration of adult snapper from coastal waters occurs in the spring/summer (October–December) and these fish are the major focus of the Bay fishery (Coutin et al. 2003). Understanding what proportion of these fish actually originated from juvenile habitats within Port Phillip Bay is critical for defining the relationship between the localised Port Phillip Bay fishery and the wider snapper population in coastal waters.

Although, Port Phillip Bay is the largest known area in Victoria where recruitment of 0-age snapper occurs, a number of other smaller bays/estuaries along the coast provide recruitment habitat for 0-age snapper (section 2). These other areas are smaller in area compared to Port Phillip Bay and densities of 0-age snapper within them are generally similar to or less than Port Phillip Bay (section 2). Individually, the contributions of these other estuaries to population replenishment may be small; however, as a group their contribution may be of considerable importance to the Victorian fishery. There is a strong need to determine and quantify spatial linkages between juvenile and adult snapper recruitment in Victoria. Understanding these linkages will greatly improve models of population structure and highlight those juvenile habitats that are most important for sustaining fishery recruitment. Furthermore, recruitment of 0-age snapper into Port Phillip Bay is highly variable from year to year (section 2). Understanding the importance of this variability to fishery dynamics, particularly in Port Phillip Bay, depends on quantitative knowledge of connectivity between 0-age recruitment in Port Phillip Bay and recruitment into the local Bay fishery.

In this study we investigate spatial and temporal variation in otolith chemistry of 0-age snapper across a range of scales. We determine, for two cohorts (year classes), whether chemical tags can be identified in otoliths from 0-age snapper, with particular emphasis on finding a unique tag to identify 0-age snapper originating from Port Phillip Bay. We also assess both long- and short-term temporal variation in otolith chemistry of 0-age snapper, and discuss the implications of this for the future use of otolith chemical tags to determine the geographic origins of adults.

3.2 Methods

3.2.1 Fish collection

We sampled 0-age snapper (< 5 months old) of the 2000 and 2001 cohorts on two occasions separated by about one month during late summer/early autumn (February/March) of each year. Recruitment of 0-age snapper occurred in at least six estuaries along the Victorian coast; Port Phillip Bay, Western Port Bay, Corner Inlet, Gippsland Lakes, Snowy River estuary and Mallacoota Inlet (Fig. 11), although we only sampled the Snowy Estuary in 2001. Several other small estuaries along the Victorian coast were closed to the sea by sandbars during summer/autumn of 2000 and 2001 and therefore could not receive 0-age recruitment and were not sampled. At the time of sampling there was no evidence for recruitment of 0-age snapper in open coastal waters. We did conduct two pilot sampling trips to coastal locations but failed to find 0-age snapper (Appendix 3).

Within each estuary we collected snapper from multiple sites. The number of sites varied with size of the estuary, from nine in Port Phillip Bay, to two in the Snowy Estuary (see Table 2, Fig. 11). In the deeper larger estuaries; Port Phillip Bay, Corner Inlet, Gippsland Lakes and Western Port Bay, fish were collected with the small plumb-staff beam trawl (see section 2.2.2). In the Snowy Estuary and Mallacoota Inlet we used a 10m x 2m, 1mm^2 mesh seine net fitted with 10 m hauling ropes. To minimise any school related biases, recruits used for otolith chemical analyses were haphazardly selected from multiple randomly-placed hauls of the seine or trawls of the beam trawl within each site.

Future use of chemical tags from juveniles to determine origins of adults will involve a random sample of adults from each cohort, and not a random sample of adults originating from a specific period of larval settlement and 0-age recruitment within each cohort. For this reason we chose not to restrict otolith analyses to fish of the same size or otolith weight as this could introduce possible biases to tag compositions due to short-term variations in otolith chemistry. Further, if short-term temporal variation in otolith chemistry occurred, tags based on one sampling event would require highly accurate temporal matching between analyses of the juvenile otoliths and juvenile regions within and adult otoliths (see discussion). We therefore chose to characterise chemical tags based on haphazard samples of fish collected across two months after the larval settlement period. This would both allow assessments of short-term variation in otolith composition and produce chemical tag data that are more representative of the populations of 0-age fish that could eventually contribute to adult recruitment.

We further investigated longer-term (> 5 years) among cohort variation in 0-age otolith chemistry for Port Phillip Bay by comparing the chemistry of archived otolith samples collected from the 1993, 1995, and

1996 cohorts with those from the 2000 and 2001 cohorts. We made two long-term comparisons, the first involved 0-age otoliths from the 1995 and 2001 cohorts collected from the same 6 sites (Fig. 11; PW, HI, HO, M, CR, BB). The second involved otoliths collected from two sites (Fig. 11; HI, M) compared over five cohorts (1993, 1995, 1996, 2000 and 2001). Collection times for the archived samples were similar to those for the 2000 and 2001 cohorts (March). Archived samples were collected by otter trawl as part of an ongoing monitoring program in Port Phillip Bay (Hobday et al. 1999).

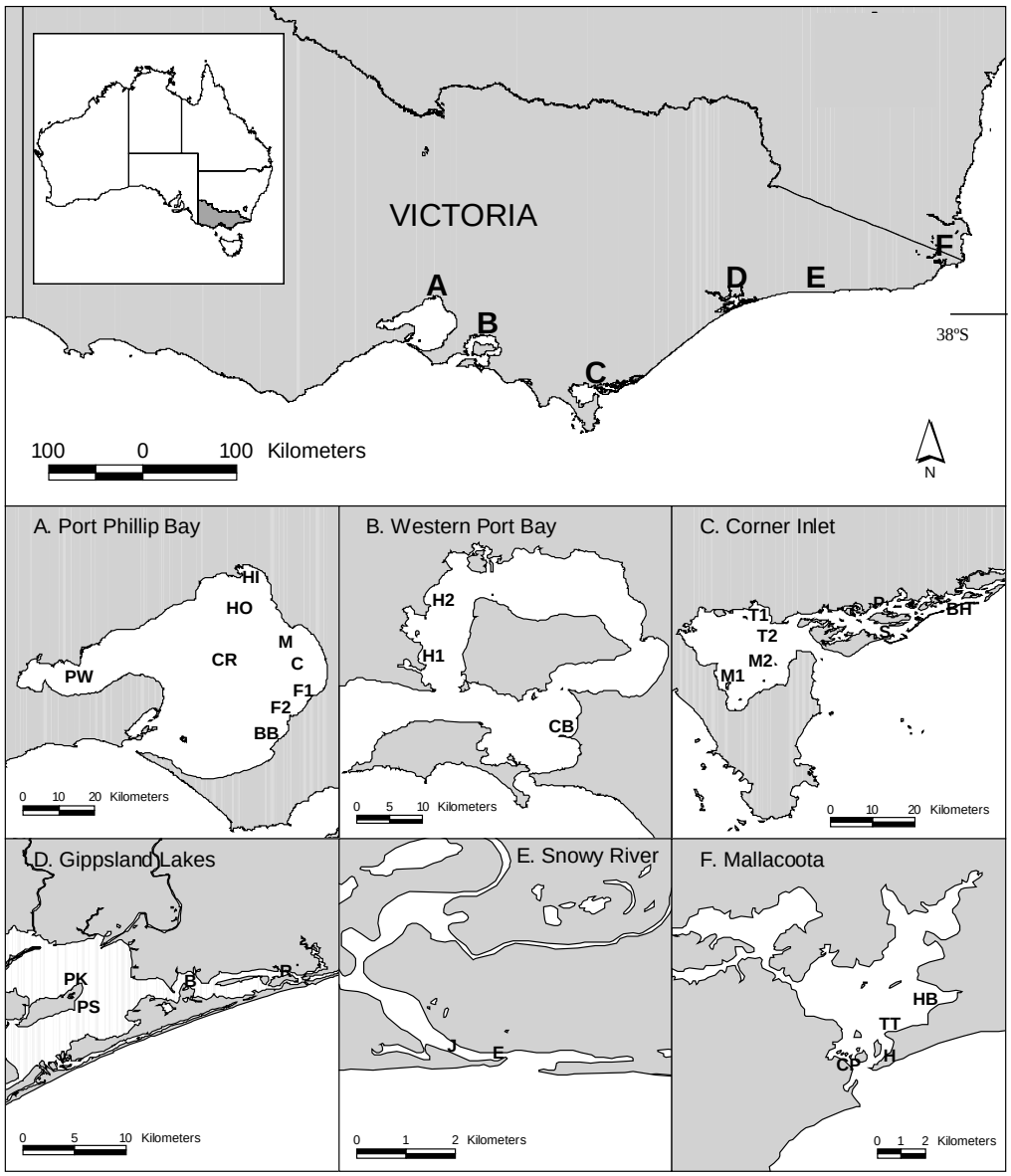

Figure 11. Map of Australia (inset) and the Victorian coast showing location of estuaries where *Pagrus auratus* samples were collected (A-F) and locations of sites within estuaries indicated by letter labels. (Key to sites - PW, Point Wilson; HI, Hobsons Bay Inner; HO, Hobsons Bay Outer; CR, Central; M, Mordialloc; C, Carrum; F1, Frankston 1; F2 Frankston 2; BB, Balcombe Bay; H1, Hastings 1; H2, Hastings 2; CB, Coronet Bay; T1, Toora Channel 1; T2, Toora Channel 2; M1, Middle Channel 1, M2, Middle Channel 2; P, Pelican; S, Snake; BH, Boat Harbour; PK; Point King; PS, Point Scott; B, Bancroft Bay; R, Reeves Channel; J, Jetty; E, Entrance; CP, Caravan Park; H, Harrison's; TT, Tea Tree; HB, Howe Bight).

Table 2. Details of the sizes and otolith weights of 0-age *Pagrus auratus* analysed for otolith chemistry from various estuaries along the Victorian coast and two cohorts. Temperature and salinity were measured at the same sites and times as fish were collected and are averaged across sites within each estuary. SL=standard length, OW=otolith weight, T=temperature, S=salinity

Cohort Estuary	Month	N Sites	N Fish	SL (mm) mean (range)	OW (mg) mean (range)	T(°C)	S(ppt)
2000							
Port Phillip Bay	February	4	14	32.04(23-61)	1.25(0.4-4.9)	20.5	36.5
	March	5	26	53.94(30-74)	4.10(1.4-8.0)	18.7	37.0
Western Port Bay	February	1	2	17.50(15-20)	0.25(0.2-0.3)	22.1	37.0
Corner Inlet	February	2	16	31.44(16-53)	1.34(0.2-3.4)	21.1	37.2
	March	5	24	46.34(20-75)	2.95(0.5-7.3)	18.5	37.6
Gippsland Lakes	February	3	13	30.54(21-39)	0.93(0.3-1.6)	21.4	32.4
	March	4	22	56.54(39-79)	4.35(1.9-7.9)	19.4	31.2
Mallacoota Inlet	March	2	20	40.97(29-56)	2.70(0.3-1.6)	24.0	34.4
2001							
Port Phillip Bay	February	8	53	55.27(15-82)	4.28(0.5-8.5)	22.4	36.7
	March	9	56	64.09(27-100)	7.37(1.0-17.6)	18.7	36.5
Western Port Bay	March	2	4	44.75(25-47)	3.23(0.9-5.0)	16.7	37.0
Corner Inlet	February	6	39	38.02(15-68)	1.83(0.2-5.5)	20.1	36.5
	March	6	25	54.72(21-94)	5.19(0.4-13)	16.5	36.8
Gippsland Lakes	February	2	13	40.92(23-60)	2.21(0.4-4.2)	21.2	31.7
	March	2	14	53.58(26-93)	5.39(0.8-13.6)	18.0	28.8
Snowy Estuary	February	1	6	34.80(25-42)	1.27(0.6-1.8)	21.8	30.8
	March	2	10	52.50(22-74)	4.41(0.5-8.0)	18.1	32.9
Mallacoota Inlet	February	3	15	53.20(28-90)	4.97(0.9-15.9)	22.9	27.5
	March	4	16	51.95(17-100)	5.37(0.5-20.5)	21.8	29.8

3.2.2 Otolith preparation

The sagittal otoliths were generally dissected from chilled fish on return from the field; however, we received some frozen specimens from fishermen. Otoliths in the archived samples were dissected from fish that had been chilled for periods of up to 10 h. Comparison between freshly dissected (chilled for 8 h) and frozen (frozen for 80 days) otoliths from the same sites showed no significant effect of freezing on the concentrations of Mn, Sr, and Ba near the otolith margins (t-test, df 10, $p > 0.05$ for each element). Other studies have similarly shown no difference between Ba, Sr and Mn concentrations in otoliths that were either dissected fresh or after freezing (Rooker et al. 2001b). Otoliths were cleaned of adhering tissue in Milli-Q water, air dried for at least 24 hours, and stored in plastic vials until mounting.

The otoliths were weighed to the nearest 0.1 mg prior to mounting in epoxy resin (Struers epofix). Transverse sections of approximately 350 μm thickness were taken through the primordium using a continuous flow of Milli-Q water to lubricate the diamond blade. Sections where polished with three grades of aluminium oxide lapping film (30, 9, 3 μm) lubricated with Milli-Q water. The polished sections were fixed to acid cleaned (10% HNO_3) microscope slides with epofix resin. The resin used for mounting and fixing was doped with approximately 30 ppm indium (In) as a resin indicator. Final cleaning involved a three-minute immersion in Milli-Q water in an Ultrasonic bath, followed by triple rinsing with Milli-Q water and drying inside a class 100 laminar flow cabinet. Pre-ablation was also used to eliminate any chance that surface contamination might influence results.

3.2.3 Otolith analysis

Otoliths were analysed with a Merchantek LUV 266™ Nd:YAG ultraviolet laser microprobe operated in Q switched mode in conjunction with a Finnigan MAT ELEMENT™ 1 high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS). Ablation was conducted in helium that was mixed with argon for transport to the plasma. A more detailed description of the system used in this study is provided by (Lahaye et al. 1997). Typical operating conditions of the laser and HR-ICP-MS are outlined in Table 3. After a preliminary investigation we decided to focus on the isotopes ^{55}Mn, ^{88}Sr, and ^{138}Ba, along with ^{44}Ca which was used as the internal standard, and ^{115}In, the resin indicator.

We chose to sample near the margins of otoliths to represent the most recent period of elemental incorporation as this is most relevant to the site of capture. We made three ablations of 90-100 μm on each otolith (Fig. 12ab). Each ablation sampled about 15–20 days of otolith growth. The three ablations were situated one at the dorsal and ventral tip, and one adjacent to the ventral side of the sulcal groove (see Fig. 12a). Variation within otoliths for all elements was less than among otoliths. ANOVA of a random sample of 30 otoliths from two randomly selected bays showed that the % of variation explained among/within otoliths was; Mn 58 % / 42 %, Sr 67 % / 33 %, Ba 91 % / 9 %. The high variation within otoliths for Mn was due to a consistent difference between the spot adjacent to the sulcus groove (i.e. lower values) and the spots at the dorsal and ventral tip areas (i.e. similar and higher values). Standardisation of sampling locations within otoliths has been previously suggested to account for the possibility of consistent within otolith variations (Campana 1999). We therefore analysed all otoliths in similar areas, and averaged data from the three ablations to provide the elemental concentrations used in statistical analyses.

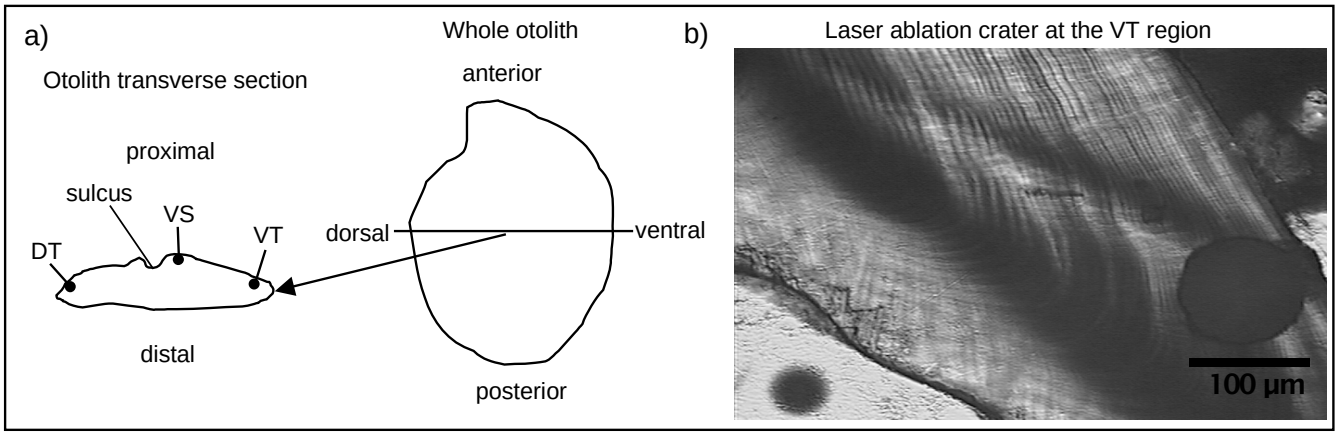

Figure 12. a) Schematic showing how otoliths were sectioned and the regions where laser ablation samples were taken; DT – dorsal tip, VS – ventral sulcus, VT – ventral tip. b) Example of ablation crater.

To eliminate the influence of any biases due to instrument drift we randomised and blocked each daily analysis sequence with respect to bay/estuary. At the time of this study no certified otolith-based standard suitable for laser analyses was available. We used the National Institute of Standards (NIST) 612 glass standard reference material (SRM) for quantification of elemental concentrations. We analysed this standard every 10–12 otolith ablations to further eliminate possible short-term drift effects. The concentration of Ca in otoliths was taken as 38.8 % by weight or 388,000 ppm following the determination of otolith Ca concentration presented in Yoshinaga et al. (2000) and a previous study of *Pagrus auratus* otolith chemistry (Edmonds et al. 1989). The average counts of a 20-scan blank acquired prior to each ablation were subtracted from the average sample counts before concentration calculations. Samples were acquired for 50 scans of each isotope (approx. 40 secs) with the initial 10 scans being ignored to allow for pre-ablation and signal stabilisation. The ablation cell was purged with He for 20 seconds prior to each blank/sample acquisition to remove any residue from previous samples. Data reduction was conducted offline. If indium spikes occurred in the data we removed the associated scans from the data prior to averaging of counts. We found that indium counts were rarely above background and indium spikes were generally associated with the laser ablating through some samples that were sectioned too thin. We chose to convert raw data (counts s^{-1}) to concentrations ($\mu g\ g^{-1}$) using the equation of (Ludden et al. 1995):

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

where; C_x = concentration of the element being quantified; $I_{m,x}$ = intensity at mass x of the element being quantified; $I_{m,\,Is}$ = intensity at the mass used for the internal standard; C_{Is} = concentration of the internal standard element; std = std; samp = sample.

A blank of 70 scans was acquired at the start and end of each session. The standard deviation of these blanks was used in calculations of detection limits. Detection limits also depend on the amount of material ablated and so were adjusted for each ablation based on ablation yield estimates (Lahaye et al. 1997). Average detection limits ($\mu g\ g^{-1}$) were, ^{55}Mn: 0.32, ^{88}Sr: 0.39, ^{138}Ba: 0.02, ^{115}In: 0.015. Both accuracy and precision were estimated on a daily basis for the NIST SRM 612 (analysed as an unknown) and precision was also estimated for a pressed pellet made of ground snapper otolith. Precision estimates for concentrations of individual elements measured as the mean relative standard deviation (RSD) in 2000/2001 for the NIST SRM 612 were; Mn: 10 / 4 %, Sr 5 / 2 %, Ba: 5 / 3 %. Precision estimates for the pressed pellet in 2000/2001 were; Mn: 25.3 / 19 %, Sr: 4.5 / 3.9 %, Ba: 7.0 / 5.1 %. Accuracy for individual elements measured as mean percentage recovery for the NIST SRM 612 in 2000/2001 were; Mn: 97.2 / 100.2 %, Sr: 99.2 / 100 %, Ba: 98.8 / 100 %.

As the external standard we used was not matrix matched we further assessed precision between analyses performed in different years by re-analysing (in 2001) otoliths of 25 randomly selected otoliths that were previously analysed in 2000. These otoliths were re-analysed by ablations immediately adjacent to the previous ones. Repeat analyses showed no significant differences in Ba and Sr determinations between the two years (t-test, df = 23, $p > 0.05$). There was a small but significant difference in Mn determinations between years (t-test, df = 23, $p = 0.031$), with slightly higher Mn concentrations (mean difference = 0.896 $\mu g\ g^{-1}$) in year 2000 than 2001. Therefore, interannual comparisons for Ba and Sr would not be influenced by any systematic differences in instrument performance between the years. However, for comparisons between 2000 and 2001 we chose to adjust the Mn data by subtraction of 0.896 $\mu g\ g^{-1}$ (the mean difference) from the determinations for each otolith analysed in 2000.

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

Laser		**HR-ICP-MS**	
Wavelength	266nm	Resolution	300 (low)
Mode	Q switched	Gas flow	
Repetition rate	6 Hz	Coolant (Ar)	14.00 L min^{-1}
Energy	1 mJ	Auxillary (Ar)	1.55 L min^{-1}
Spot size	90–100 μm	Sample (Ar/He)	1.50 L min^{-1}
Mixing Chamber	He (0.4 L min^{-1})	Cone	Nickel
		Detection modes	Analogue (Ca, Sr)
			Pulse counting (Mn)
			Both (Ba)
		Dwell time	10 ms
		Channels/peak	4 (22% of mass window)
		Magnet settling time	1 ms per amu + 5 ms

3.2.4 Data analysis

Individual elements: We used univariate analysis of variance (ANOVA) to investigate temporal and spatial variation in concentrations of individual elements in otoliths. We performed several ANOVA to investigate variation among otoliths sampled a month apart within each cohort and between the two cohorts (i.e. year effects). Due to the lack of samples from some sites and one estuary, in particular during the February sampling of the 2000 cohort, we restricted comparisons of the 2000 and 2001 cohorts to the March sampling period of each year. For the 2000 cohort, due to a lack of samples from Mallacoota Inlet in March, we excluded this estuary from the month comparison for 2000. Otolith chemistry data were averaged across fish from each net haul within each site to provide a more realistic measure of within site variation (as opposed to using fish as replicates within sites). Western Port Bay was not included in any univariate analyses due to the low number of samples, but is displayed graphically. For longer-term among cohort comparisons involving only Port Phillip Bay we performed two separate ANOVA (i.e. six sites compared across two cohorts and two sites compared across five cohorts). Estuary, month and cohort were treated as fixed factors in all ANOVA. For all analyses, data for Ba and Mn required ln (x + 1) transformation to meet the assumption of homogeneity of variances. We chose not to make adjustments to significance levels to account for multiple ANOVA tests, although it would be expected that at $\propto = 0.05$, one in 20 tests would be significant by chance alone. P-values are included in the results to indicate the strength of significant results.

Multi-element chemistry: We used multi-variate analysis of variance (MANOVA) to investigate spatial differences in otolith multi-elemental chemistry (Mn, Sr, Ba), and quadratic discriminant function analysis (QDFA) to determine if 0-age snapper from Port Phillip Bay could be distinguished from those collected in other estuaries based on multi-element otolith composition. We initially analysed the data separated by individual bay/estuary, however, although Port Phillip Bay was well discriminated from the other estuaries (results), the other estuaries were poorly discriminated from each other in both years (50 - 60 % classification accuracy). We therefore chose to group the data into two groups; samples from Port Phillip Bay and samples from all the other estuaries. This grouping was consistent with our main aim of discriminating Port Phillip Bay recruits from those that recruited in all other Victorian nursery areas. However, grouping of the other estuaries was also important to produce similar sample sizes among groups (Quinn and Keough 2002). Classification accuracies from QDFA were determined for each cohort and for the different months within each cohort. Classification accuracies were determined using the leave-one-out approach (i.e. the observation being classified is removed from the data set). F-to- remove statistics, which provide a measure of the contribution that individual variables make to discrimination, were used to assess which elements contributed most to discrimination (Wilkinson et al. 1996). Canonical discriminant function plots of the data, with 95% confidence ellipses around the data for each grouping (i.e. Port Phillip Bay v other estuaries), were used to display temporal and spatial variation in the multi-elemental chemistry among the groupings. To further examine the similarity in otolith tag composition

between the 2000 and 2001 cohorts, we used data from one cohort as a training set to classify the data for the other cohort being investigated.

For all MANOVA and QDFA, Ba and Mn data were ln (x +1) transformed. While individual elements showed approximate univariate normality, examination of within-group scatterplot matrices suggested some deviation from the assumption of equality of covariance matrices. QDFA was chosen as opposed to linear discriminant function analysis as it does not require homogeneity of within-group covariance matrices (Quinn and Keough 2002). We used the Pillai trace statistic to test for significance in MANOVA as it is the most robust to deviations from multi-variate normality (Quinn and Keough 2002).

3.3 Results

3.3.1 Fish size and otolith weight

Differences in temporal recruitment patterns among estuaries resulted in some variation of fish sizes and otolith weights (Table 2). Although size or age variation could potentially influence variation in otolith chemistry (i.e. Fowler et al. 1995; Bath et al. 2000), inclusion of otolith weight as a co-variate in a previous study of the same species and developmental stage made no difference to results of spatial comparisons (Gillanders 2002b). Likewise, we found no evidence for relationships between otolith weight and elemental concentrations based on correlation analyses, and inclusion of otolith weight made no difference to spatial comparisons. We therefore elected not to include otolith weight as a co-variate in the formal statistical analyses. We also suggest that the inclusion of co-variates such as otolith weight in laser based studies would complicate the use of juvenile otolith tags in retrospective studies of adult origins. This is because it would be difficult to accurately determine the value of the co-variate (i.e. otolith weight) appropriate to the time (position) of laser sampling within the juvenile portions of adult otoliths.

3.3.2 Individual elements

Spatial variation: We found significant spatial variation in otolith elemental concentrations both among estuaries and sites within estuaries for Ba and Mn in 2001, and for Ba among estuaries in 2000 (Table 4a, Fig. 13). Sr levels varied significantly among estuaries only in 2001, but the month by estuary interaction was also significant (Table 4a, Fig. 13). The important result, in relation to finding an elemental tag unique to Port Phillip Bay, was the higher Ba levels in Port Phillip Bay otoliths compared to those from the other estuaries in both years (Tukey's post hoc test, $p < 0.001$) (Fig. 13).

Variation between adjacent months: Mn showed a small but significant difference between months across all estuaries in 2001 (Table 4a, Fig. 13), however, there was no significant difference between months in 2000, although fewer sites and estuaries were included in this analysis (Table 4a, Fig. 13). In 2001 Mn concentrations were generally higher across all estuaries in the first sampling month (Fig. 13). In 2001 there were significant interactions between month and estuary for Sr and between month and site nested within estuary for Ba (Table 4a).

Variation between adjacent cohorts: Consistent differences between the 2000 and 2001 cohorts occurred for Sr levels only (Table 4b). Sr levels were generally higher in otoliths from the 2000 cohort (Fig. 13). There were no between cohort effects or spatio-temporal interactions for Mn (Table 4b). There was, however, a strong cohort by site nested within estuary interaction for Ba (Table 4b).

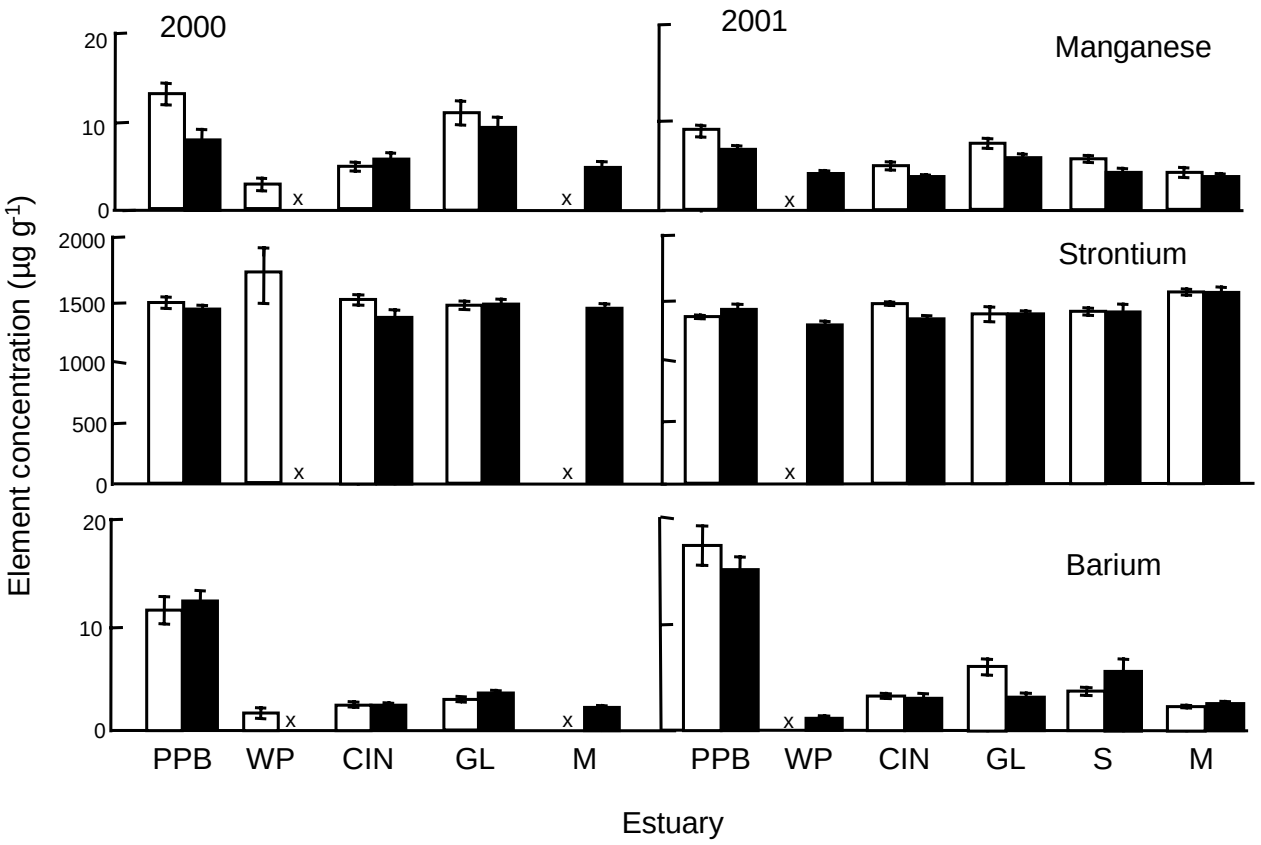

Figure 13. Mean concentrations (± SE) of Mn, Sr and Ba in otoliths from 0-age *Pagrus auratus* collected in six estuaries along the Victorian coast in two adjacent months (February, clear bars and March, dark bars) for two adjacent cohorts/years (2000 and 2001). Data are pooled across sites within estuaries, see Table 1 for details of fish numbers and number of sites sampled within estuaries. ×, no samples collected.

Table 4. Results from ANOVA comparing concentrations of elements in 0-age *Pagrus auratus* otoliths, a) within and b) between adjacent cohorts for different Victorian estuaries; c) across two cohorts at six sites, and d) across five cohorts at two sites in Port Phillip Bay only. ***p<0.001, **p<0.01,*p<0.05.

Source	df	Ms Mn	Ms Sr	Ms Ba
Months within cohort comparisons				
2000				
Estuary	2	0.232	1909	1.203***
Month	1	0.145	27569	0.007
Month x Estuary	2	0.027	2206	0.010
Site(Estuary)	7	0.051	31968	0.031**
Month x Site(Estuary)	7	0.016	10582	0.008
Residual	30	0.041	22328	0.007
2001				
Estuary	4	1.504**	26345*	12.051**
Month	1	0.413*	19227	0.072
Month x Estuary	4	0.095	49290***	0.323
Site(Estuary)	14	0.246***	6938	1.541***
Month x Site(Estuary)	14	0.083	5189	0.162*
Residual	63	0.066	5221	0.070
Cohort (among years) comparisons				
2000 and 2001 cohorts all estuaries				
Estuary	3	0.708	21366	10.281***
Cohort	1	0.420	353877***	0.003
Cohort x Estuary	3	0.076	21366	0.078
Site(Estuary)	9	0.288	48169	0.323***
Cohort x Site(Estuary)	9	0.241	14165	0.463***
Residual	45	0.265	22760	0.073
c) Port Phillip Bay, six sites, 2001 and 1995 cohorts				
Site	5	0.522*	6770	3.683***
Cohort	1	0.108	443547***	28.060***
Cohort x Site	5	0.562*	4206	0.763*
Residual	84	0.214	15977	0.240
d) Port Phillip Bay, two sites, 2001, 2000, 1996, 1995 and 1993 cohorts				
Site	1	0.855	9381	3.394
Cohort	4	0.625	233905***	31.308***
Cohort x Site	4	0.306	7820	2.256***
Residual	81	0.291	18278	0.261

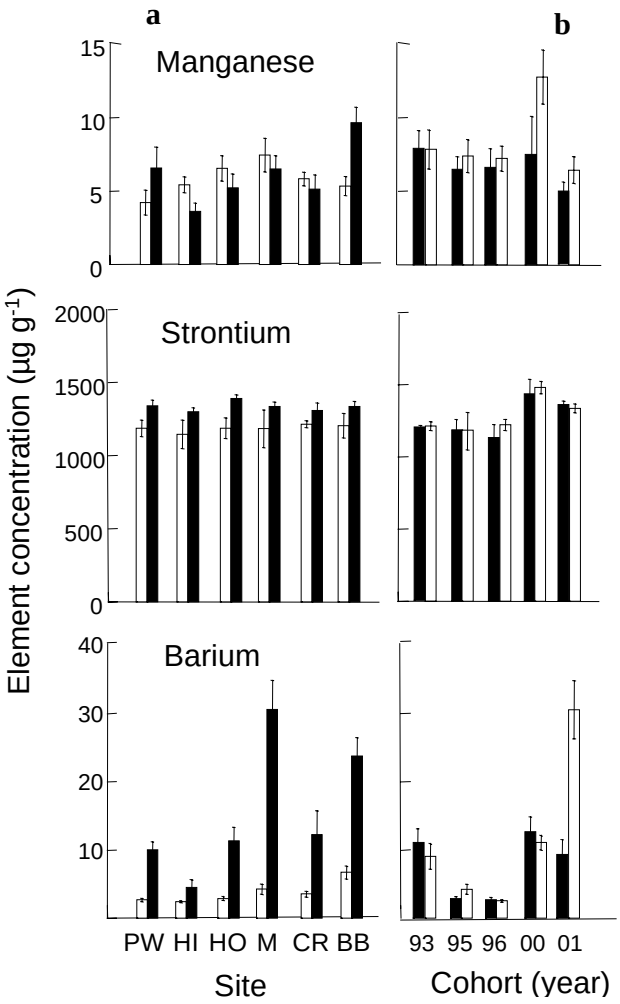

Figure 14. Mean (± 1SE) concentrations of Mn, Sr, and Ba, in otoliths from 0-age *Pagrus auratus*; a) collected at six sites (see Fig 11) within Port Phillip Bay and compared between two cohorts (1995 clear bars, 2001 dark bars) separated by six years, and b) collected at two sites HI (dark bars) and M (clear bars) (see Fig 11) for five cohorts separated across nine years.

Long-term among cohort variation for Port Phillip Bay: Otolith chemistry varied among cohorts over longer time scales for both Ba and Sr (Table 4cd, Fig. 14ab). Variation among cohorts was significant in both the analysis involving six sites compared across two cohorts (1995 and 2001) (Table 4c, Fig. 14a) and two sites compared across five cohorts (1993, 1995, 1996, 2000 and 2001) (Table 4d, Fig. 14b). Ba and Sr concentrations were generally higher in otoliths from the 2000 and 2001 cohorts than the other cohorts (Fig. 14ab), however, Ba levels in the 2000 cohort were similar to the 1993 cohort (Fig. 14b). There were weak cohort by site interactions for Ba and Mn in the comparison involving six sites across two cohorts (Table 4c), and a strong cohort by site interaction for Ba in the comparison involving two sites across five cohorts (Table 4d).

3.3.3 Multi-element otolith chemistry

Spatial discrimination – multi-element tags: We found highly significant differences in the multi-element otolith chemistry (Ba, Mn, Sr) between Port Phillip Bay and the other estuaries for both the 2000 and 2001 cohorts (MANOVA, $p < 0.001$). Differences between Port Phillip Bay and the other estuaries were also highly significant in both sampling months for each cohort (MANOVA, $p < 0.001$). Overall accuracy of discrimination (QDFA) between Port Phillip Bay 0-age snapper and those from the other estuaries based on Ba, Sr and Mn was high at 99 % for 2000 and 88 % in 2001 (Table 5a). Ba made the greatest contribution to discrimination power in both cohorts (F to remove statistics: 2000 – Ba = 381.2, Sr = 16.7, Mn = 0.5; 2001 – Ba = 189.14, Sr = 12.44, Mn = 0.07). Canonical discriminant function plots showed no overlap of the 95% confidence ellipses (representing the distributions of the multi-elemental chemistry data) for the 2000 cohort but some overlap for the 2001 cohort (Fig. 15a). This was irrespective of whether data were separated by months or pooled across months (Fig. 15 ab). The lower classification accuracy for Port Phillip Bay otoliths in 2001 was largely due to the site within estuary by cohort interaction for Ba (Table 4a). Closer inspection of the 2001 data revealed that misclassified samples were from one or two sites within Port Phillip Bay that had lower Ba levels compared to the 2000 cohort.

Variation in multi-element chemistry between months: The multi-element chemistry for Port Phillip Bay otoliths varied significantly between February and March in both the 2000 and 2001 cohorts (MANOVA, $p < 0.001$ for 2000, $p < 0.05$ for 2001) (Fig. 15b). In both the 2000 and 2001 cohorts, Mn was most important in driving differences between months (greatest F to remove). For the other estuaries group there was no significant difference in the multi-element otolith chemistry between months in 2000 (MANOVA, $p > 0.05$) (Fig. 15b), however, there was a significant difference between months in 2001 (MANOVA, $p < 0.001$) (Fig. 15b). Mn was again the biggest contributor to this difference between months (largest F to remove).

Table 5. Classification accuracies from QDFA of 0-age *Pagrus auratus* otolith elemental composition (Ba, Sr, Mn); a) for each cohort using the leave one out approach and b) cross validation using discriminant functions from one cohort (the training data set) to classify the other cohort treated as of unknown origin.

a)	Accuracy of predicted group membership (%)		
Sampling period	Cohort	Other estuaries	Port Phillip Bay
February	2000	100	100
March	2000	100	100
Months Pooled	2000	100	98
February	2001	90	90
March	2001	90	88
Months Pooled	2001	90	85

b) Cross validation accuracy of predicted group membership (%)		
Cohort used as training set	Other estuaries	Port Phillip Bay
2001	98	100
2000	90	83

Variation in multi-elemental chemistry between adjacent cohorts: For both Port Phillip Bay and the other estuaries group, the elemental tags varied significantly between the 2000 and 2001 cohorts (MANOVA, $p < 0.001$ for all tests) (Fig 15a). These differences were largely driven by differences in Sr levels between the two years (largest F to remove for both tests).

Long-term among cohort differences in multi-element chemistry for Port Phillip Bay: We found highly significant among cohort differences in the chemistry of 0-age otoliths from Port Phillip Bay. Differences were highly significant for both comparisons (MANOVA, $p < 0.001$). Separation among 95% confidence ellipses in the canonical variate plot was almost complete between 2000/2001 and 1996, indicating strong differences in otolith chemistry (Fig. 16). While 1995 and 1996 had considerable overlap, they only overlapped slightly with 2000, 2001 and 1993 (Fig. 16). For both comparisons Ba was most influential on differences among cohorts (F to remove, 6 sites for 95, 2001: Ba=51.18, Mn=16.53, Sr=11.78; F to remove, 2 sites for 93, 95, 96, 2000, 2001: Ba=16.83, Sr=9.94, Mn=3.02).

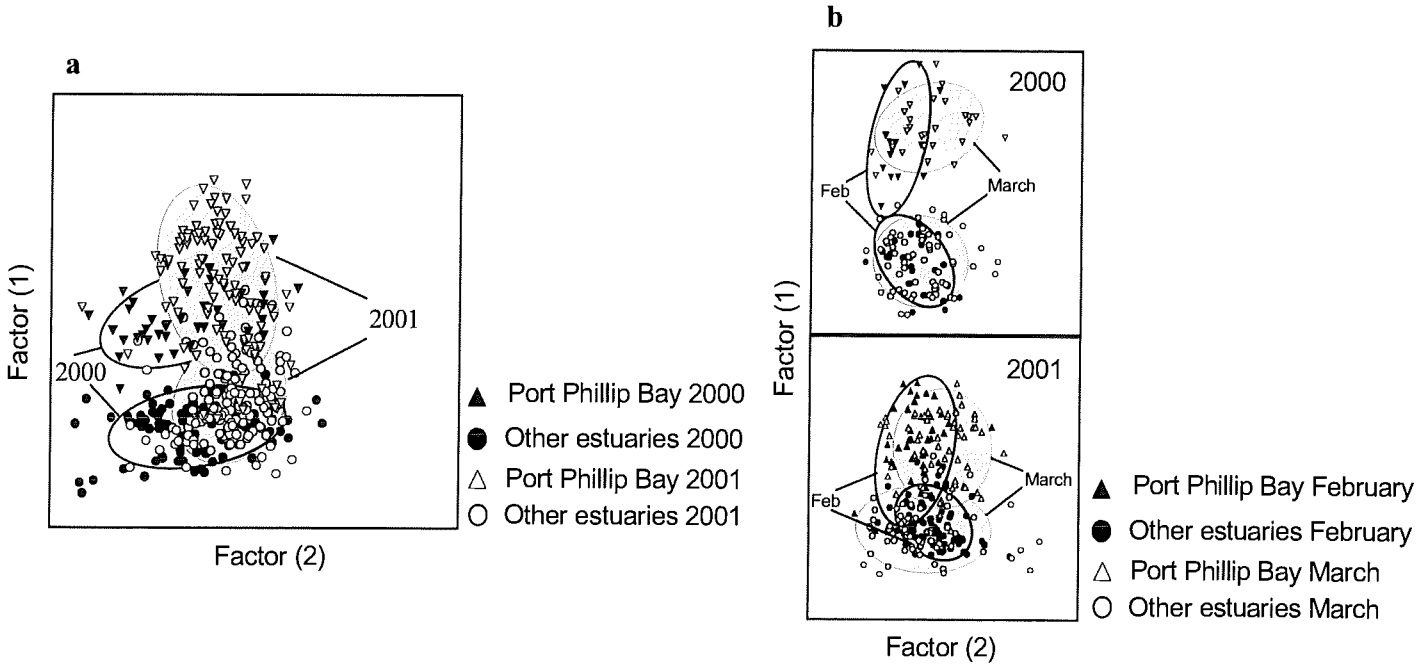

Figure 15. Canonical variate plots displaying; a) spatial differences in multi-elemental tags of 0-age *Pagrus auratus* from the Port Phillip Bay and other Victorian nursery areas for two adjacent cohorts, and b) spatial differences in multi-elemental tags of 0-age snapper from the Port Phillip Bay and other Victorian nursery areas for two adjacent months within two adjacent cohorts. Ellipses represent 95% confidence intervals around the data, and data points represent individual fish.

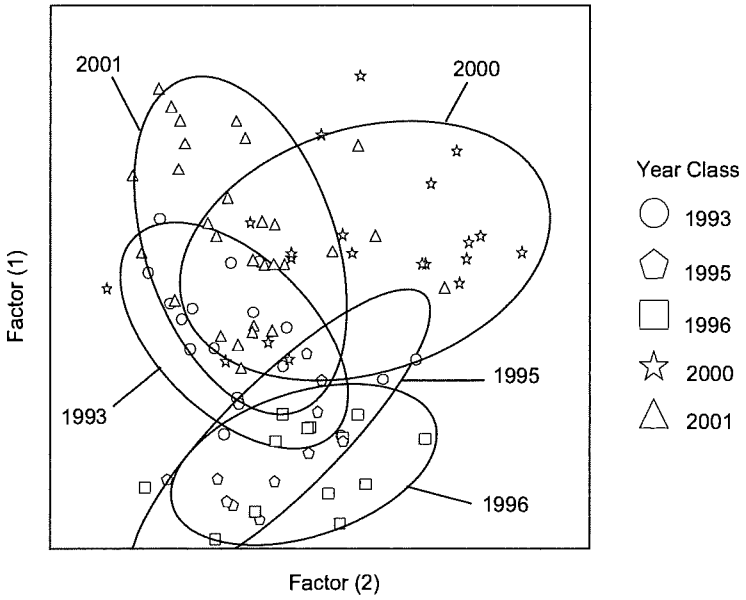

Figure 16. Canonical variate plot displaying among cohort variation in multi-elemental tags of 0-age *Pagrus auratus* collected at two sites within Port Phillip Bay for five cohorts over a nine year period. Ellipses represent 95% confidence intervals around the data, and data points represent individual fish.

3.3.4 Influence of temporal variation in multi-elemental otolith chemistry on spatial discrimination

Discrimination of 0-age snapper from Port Phillip Bay was highly accurate for adjacent years and months even though both MANOVA results and canonical variate plots showed some temporal variations in multi-elemental otolith chemistry (Fig. 15ab). The canonical variate plots show that separation between 95% confidence ellipses for the two groups was maintained irrespective of which month or year is compared (Fig. 15ab). This means that short-term changes in otolith elemental composition were not strong enough to confound discrimination between Port Phillip Bay and the other estuaries. This is further demonstrated by the accuracy with which data from the 2000 cohort classified fish from the 2001 cohort and visa versa (i.e. 99% and 82% respectively) (Table 5b). Unfortunately we could not obtain otoliths from other areas to compare to Port Phillip Bay for the long-term among cohorts comparison. The significant differences in otolith composition among cohorts over longer time periods for Port Phillip Bay, however, would suggest that elemental tag compositions are unlikely to be stable over long time periods.

3.4 Discussion

Spatial variation in multi-element otolith composition (Mn, Sr, Ba) allowed 0-age snapper from Port Phillip Bay to be accurately discriminated from those collected in other Victorian estuaries in two cohorts. Spatial differences in otolith chemistry, particularly in Ba levels, provided the basis for a natural otolith tag that was unique to juveniles from Port Phillip Bay. Variation in otolith composition across months during the recruitment season resulted in negligible difference in the accuracy with which Port Phillip Bay 0-age could be discriminated from and those from the other areas. Similarly, variation between adjacent cohorts resulted in minor differences in the accuracy of discrimination. However, longer-term (> 5 year) among cohort variation in the elemental composition of 0-age snapper otoliths from Port Phillip Bay was highly significant. This implied that it would be inappropriate to use chemical tags identified for one cohort of Port Phillip Bay juveniles to determine the origins of adults from other cohorts.

Previous studies of 0-age snapper otolith chemistry have demonstrated spatial variation among estuaries along the east Australian coast separated over a wide latitudinal range with concomitant variations in temperature and rainfall regimes (Gillanders 2002b). The current study involved estuaries and marine embayments along a narrow latitudinal range and also found considerable spatial variability. We found strong differences in otolith chemistry among areas with negligible differences in temperature and salinity (see Table 2). For individual elements, spatial variation in Ba and Mn occurred both at small scales (< 10 km) within, and larger scales (> 50 km) among estuaries, whereas for Sr, spatial variation was low and observed only at larger scales of among estuaries. Gillanders (2002b) also found significant estuary and site within estuary variation for Ba and Mn, however, unlike the current study, only found spatial variation in otolith Sr within individual estuaries and not among estuaries.

We found temporal variation in otolith chemistry at a range of scales. Temporal variation in otolith multi-elemental tags can have significant implications for their application in retrospective determinations of adult origins (Gillanders 2002b). This is particularly the case if temporal differences in otolith composition can confound spatial differences (Gillanders 2002b). If this occurs it is essential that otolith chemical tags used to determine adult origins are derived only from juveniles of the same cohorts as the adults. Differences in otolith chemistry between adjacent cohorts were driven predominantly by one element, Sr. However, Sr variation was consistent across all estuaries and so did not result in confounding of spatial differences among estuaries. The differences among cohorts observed for Port Phillip Bay over longer time scales (> 5 years), were driven predominantly by Ba and Sr variation. As Ba was important for discriminating 0-age snapper from Port Phillip Bay this meant that for future classification of adults we would have to use elemental tags characterised from juveniles of the same cohorts as the adults. Our observations of long-term variation in otolith chemistry are similar to those reported by Campana et al. (2000) for Atlantic cod in the Gulf of St. Lawrence. In their study whole otolith chemistry was similar among fish collected in adjacent years but differed markedly between fish from the same areas compared over intervals of 4-13 years.

Temporal variation in otolith chemistry at monthly scales has been indicated in previous studies (Thorrold et al. 1998a; Thorrold and Shuttleworth 2000). Similarly, seasonal and monthly variation in the incorporation of certain elements across adult otoliths has been observed (Kalish 1989, 1991). Similar to among year or cohort differences, variations in otolith chemistry at the monthly scale could have important implications for the way in which otolith tags are both characterised from juvenile otoliths and applied to determining the origins of older fish. The implications of short-term temporal variation in elemental tags for retrospective analyses of adult origins have perhaps not been adequately addressed by previous studies. Many species of fish have extended recruitment seasons. For example, 0-age snapper can recruit to Victorian estuaries over a period of at least three to four months (section 2), and there is potential for environmental variation in estuaries over these small time scales (Elsdon and Gillanders 2006). Therefore, juveniles sampled at different times during the recruitment season could display differences in otolith composition. If temporal variation at this scale confounds spatial discrimination, the accurate classification of adults based on juvenile otolith tags will require highly accurate matching between the time periods sampled by laser probe in adult and juvenile otoliths.

In our study variation in otolith chemistry between months was driven by variation in Mn that was general across sampling areas. This variation was possibly related to ontogeny rather than environmental variation (Fowler et al. 1995). Variation in Sr occurred between months at some estuaries and, for Ba, variation between months did occur at some sites within estuaries. These short-term variations were, however, relatively minor compared to the larger-scale spatial differences between Port Phillip Bay otoliths and those from other estuaries, and therefore had negligible confounding affect on discrimination of Port Phillip Bay juveniles. These results are similar to a previous study of American shad where otolith chemistry of juvenile fish differed between samples taken from the same locations several months apart yet spatial classification accuracy varied little between sampling times (Thorrold et al. 1998a). The results for 0-age snapper in this study suggest that the use of otolith chemical tags from small juveniles to determine the origins of adults should not be significantly confounded by small-scale (month) temporal variation in otolith chemistry.

The influence of short-term environmental variation on otolith chemistry will rarely be known a-priori. To account for this possibility, it would be wise to develop otolith chemical tags from juveniles so that they incorporate any short-term temporal variations in otolith chemistry. It is also important that tags are representative of the entire cohort of recruits that could eventually contribute to the adult population. For

this reason we recommend that chemical tag data should be based on multiple samples of recruits across time from the particular cohort, and should not be restricted to fish of the same size (i.e. settlement group). Otolith tags developed in this way should not require highly accurate temporal matching between sampling of adult and juvenile otoliths, thus reducing the risk that classifying adults to juvenile origins is influenced by small variations in the position of the laser sample within the juvenile region of the adult otolith.

It is clear from previous studies that a suite of factors including temperature (Radtke et al. 1990; Townsend et al. 1992), salinity (Kalish 1990; Secor 1992; Secor et al. 1995), growth (Sadovy and Severin 1992, 1994), ontogeny (Kalish 1989, 1991), and ambient concentration (Farrell and Campana 1996; Gallahar and Kingsford 1996; Milton and Chenery 2001; Elsdon and Gillanders 2003, 2004) may be important influences on elemental incorporation into otoliths. Although detailed understanding of the proximal causes of variation in elemental incorporation into otoliths is not essential for the use of otolith chemistry as a natural tag, a brief discussion related to Ba incorporation is warranted in this case.

Otolith Ba levels varied among sites and estuaries in the absence of significant variations in temperature or salinity. Previous studies have demonstrated that ambient Ba concentrations can influence levels of Ba in otoliths (Thorrold et al. 1998a; Bath et al. 2000; Milton and Chenery 2001; Elsdon and Gillanders 2003, 2004). Analyses of water samples has indicated elevated levels of Ba in Port Phillip Bay compared to other major Victorian estuaries and inshore coastal waters (see section 5.3.1). Port Phillip Bay has a highly developed catchment, with two large cities, a major port and several minor and one major rivers, and significant industrial development around its shores. This range of possible sources coupled with the long flushing time of Port Phillip Bay (approximately 1 year) (Walker 1999) compared to the other more tidal estuaries, offers clear potential for enrichment of elements such as Ba in its waters.

3.4.1 Conclusions

While spatial variation in otolith chemical composition may not be found in all situations (Gillanders et al. 2001), this and other studies cited herein indicate that characterisation of estuary-specific chemical tags in juvenile otoliths is likely to be possible for many species in many areas. Knowledge of temporal variation in otolith chemistry will be important for determining how otolith chemical tags from juveniles can be applied to studying spatial connectivity between juvenile and adult recruitment areas. The characterisation of elemental tags for the 2000 and 2001 cohorts will enable investigation of connectivity between the Port Phillip Bay nursery area and the replenishment of the wider snapper population in Victorian coastal waters and other bays and estuaries. In the following section we use the elemental tags from this study to determine the contribution of 0-age recruitment in Port Phillip Bay to recruitment into the Victorian fishery, and whether this varies among geographic areas, cohorts and with age.

4. Application of otolith chemistry to quantify linkages between 0-age recruitment areas and fishery recruitment: the role of Port Phillip Bay in sustaining the Victorian snapper fishery

Data for 1 and 2 year old fish published: Canadian Journal of Fisheries and Aquatic Sciences 62: 623–630 (2005)

4.1 Introduction

The use of sheltered bays and estuaries for spawning and/or as juvenile habitats is a common attribute of coastal fish species (Blaber 1980; Loneragan et al. 1989; Potter et al. 1990; Potter and Hyndes 1999; Beck et al. 2001). The importance of sheltered bays and estuaries as sources of population replenishment for coastal fisheries is often assumed based on the abundance of early life stages within these environments (Lenanton and Potter 1987; Beck et al. 2001; Able 2005). For species where the use of sheltered bays and estuaries as spawning and/or juvenile habitat is obligatory, this assumption is valid, and of greater importance is determining the relative importance of different estuaries to population replenishment. For other species where the use of estuaries is facultative or opportunistic, the relative importance of bays, estuaries and open coastal habitats to population replenishment may vary both temporally (among cohorts) and spatially (among populations or sub-populations). Although the role of sheltered bays and estuaries as juvenile fish habitat is well established (Blaber 1980; Potter et al. 1990; Able and Fahay 1998; Beck et al. 2001; Gillanders et al. 2003), quantifying how juvenile recruitment in bays and estuaries contributes to population replenishment is increasingly important as these environments continue to be affected by population growth and coastal development (Gillanders 2005; Ray 2005). Furthermore, there is an increasing need for finer-scale management of fisheries resources (i.e. closed area management regimes, protection of critical habitats). This requires a more detailed understanding of connectivity levels among juvenile recruitment sources and replenishment of local fisheries (Caley et al. 1996; Botsford et al. 2001; Sale and Kritzer 2003; Kritzer and Sale 2004).

Due to the difficulty of spatially linking adult fish back to there place of origin, the degree to which most local fish populations depend on specific juvenile recruitment sources or 'nursery areas' is unknown. To assess the dependency of local fish populations on specific juvenile sources, methods of tracking the dispersal/movement of fish between the larval/juvenile and adult life stages are necessary. Traditional methods, such as manual tagging and recapture (Sanders 1974; Begg et al. 1997), are unlikely to be suitable for studying spatial connectivity between the juvenile and adult stages. This is because of the difficulty in tagging large numbers of very small juveniles, high juvenile mortality rates, and potential for strong spatial biases in tagging and recapture effort (Crossland 1976; Kearney 1989; McGlennon and Partington 1997). Several studies have now shown that juvenile fish collected from different geographic areas can be distinguished based on the chemistry of their otoliths (Thorrold et al. 1998ab; Gillanders and Kingsford 2000; Rooker et al. 2001a). Furthermore, development of fine-scale sampling techniques, such as laser ablation (Fowler et al. 1995; Thorrold and Shuttleworth 2000; Russo et al. 2002), has enabled otolith chemical tags characterised from small juveniles to be identified within the juvenile regions of otoliths from older fish that may have migrated large distances from there place of origin. This methodology promises to provide unprecedented information on spatial connectivity between juvenile recruitment areas and replenishment of fisheries (Thorrold et al. 2001; Gillanders 2005).

Snapper, *Pagrus auratus*, support one of the most important coastal fisheries in south-eastern Australia (Kailola et al. 2003) (see sections 1.1,1.2). In Victoria, the fishery is highly localised within Port Phillip Bay, although important fisheries also occur in coastal waters and the adjacent Western Port bay (Coutin et al. 2003) (Fig. 17). Port Phillip Bay is a known spawning area and is the largest of a number of Victorian estuaries where recruitment of 0-age snapper is known to occur (section 2; Jenkins 1986; Coutin et al.

2003). Due to the significant migration of snapper between Port Phillip Bay and coastal waters, and between Port Phillip and Western Port bay (Coutin et al. 2003), it is uncertain whether larval settlement/juvenile recruitment in Port Phillip Bay is the primary source of snapper that eventually recruit to this major fishery. Furthermore, the contribution of juvenile recruitment in Port Phillip Bay to replenishment of populations in coastal waters and Western Port is unknown.

Lack of knowledge of connectivity rates between juvenile recruitment in Port Phillip Bay and local fishery recruitment places major uncertainty around the importance of Port Phillip Bay as a snapper breeding and nursery area (Coutin 2000). This also impedes efforts to understand local population dynamics and develop fine-scale management strategies. In Port Phillip Bay, over the past 15 years, the deeper habitats where larvae settle and the small 0-age snapper are found have been impacted by introduced pest species, while pollution from industries and the large population base around the Bay provide ongoing threats to habitat quality. These factors, coupled with growing concerns over high exploitation of sub-adult and adult spawning snapper and strong annual variation of 0-age recruitment within the Bay (section 2), underpin the importance of determining how the Victorian snapper fishery depends on juvenile recruitment within Port Phillip Bay.

This study has two aims. Firstly, to demonstrate the use of natural chemical tags in otoliths for quantifying connectivity between 0-age recruitment areas and local fishery recruitment, and secondly, to assess the contribution of 0-age recruitment in Port Phillip Bay to replenishment of the Victorian snapper fishery, and how this varies geographically and with age. In section 3 of this report we characterised chemical tags in the otoliths of recently settled snapper (approximately 1 to 3 months age post-settlement, hereafter termed 0-age) of two cohorts (year-classes) that distinguished individuals from Port Phillip Bay from those collected in all other known Victorian nursery areas. The elemental tag for small juveniles from Port Phillip Bay was largely based on their higher levels of Ba relative to the other areas. The elemental tag for 0-age snapper in Port Phillip Bay, however, varied among cohorts over longer time scales (> 5 years). This meant that elemental tags for 0-age fish could only be used to determine the origins of older fish from the same cohort (birth year). Here we use the elemental tags from the 0-age snapper to determine the proportions of older snapper (1–5 years age) of the 2000 and 2001 cohorts that were derived from 0-age recruitment in Port Phillip Bay. By sampling older fish of these two cohorts in Port Phillip Bay, Western Port bay and various locations along the Victorian coastline (approximately 700 km) over a 4 year period (2002, 2004, 2005), we quantify the importance of 0-age recruitment in Port Phillip Bay to replenishment of the Victorian snapper fishery. Specifically, we investigate how this contribution varies among regions of the fishery and with age over the first five years of life.

4.2 Methods

4.2.1 Otolith chemical tags

The chemical tags used to estimate the origins of older snapper were characterised from otoliths of 0-age fish collected from all bays and estuaries along the Victorian coast where 0-age snapper were known to occur (section 3). The major spatial differences in otolith chemistry of 0-age snapper were between Port Phillip Bay and all the other known recruitment areas that were relatively poorly discriminated from each other (section 3.2.4). In this section we have used two sets of baseline otolith chemistry data with which to determine the origins of older snapper from the 2000 and 2001 cohorts. One data set represents elemental compositions of 0-age snapper that had recruited (originated) within Port Phillip Bay, and the other represents those that had recruited in areas outside Port Phillip Bay.

4.2.2 Sampling of older snapper

Older (1–5 years age) snapper were collected on an opportunistic basis during summer/autumn (January – May) of 2002, 2004, 2005. Snapper recruit to the fishery in Victoria at 3 years of age (27 cm TL) and become sexually mature at around 4 years of age (30–40 cm TL) (Coutin et al. 2003) We employed several collection methods, including line fishing, fish traps and trawling. A number of recreational fisherman (see acknowledgments) voluntarily provided fish frames from specified locations. For Port Phillip Bay, to ensure we took a sample that was representative of the entire Bay fishery, we collected older fish from multiple areas in the north and south of the bay (Fig. 17). We used length as an indicator of age to focus sample collection on the two cohorts (based on Fig. 18) The age of each fish was determined during preparation for laser ablation sampling based on validated annual increments (Francis et al. 1992). Details of sample numbers and collection sites are included in Table 6. Western Victoria refers to west of Wilsons Promontory, and eastern Victoria, east of Wilsons Promontory (Fig. 17). To reduce any school related biases, samples were comprised of fish from at least two separate sampling events (dates) in each sampling area. Unfortunately, owing to the poor recruitment of the 2000 cohort, the samples sizes and number of collection locations are substantially lower for this cohort (Table 6a).

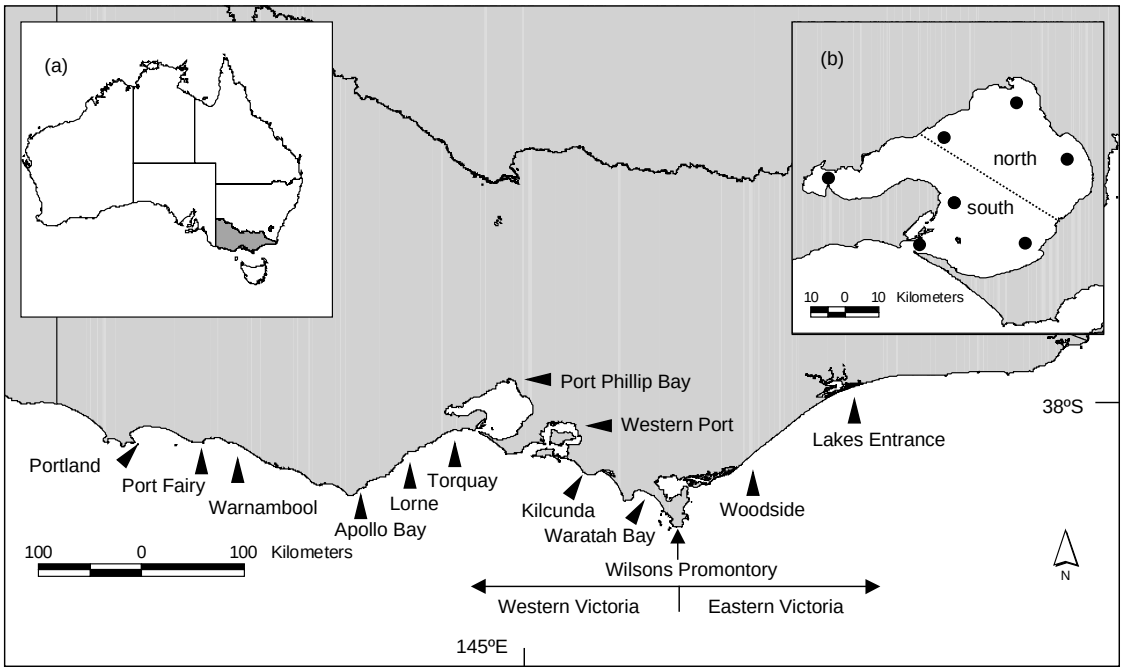

Figure 17. Map of the Victorian coast with inset of Australia (a) and Port Phillip Bay (b) showing locations where older snapper were collected for otolith chemical analyses. Dots in inset (b) indicate approximate collection areas in the north and south of Port Phillip Bay.

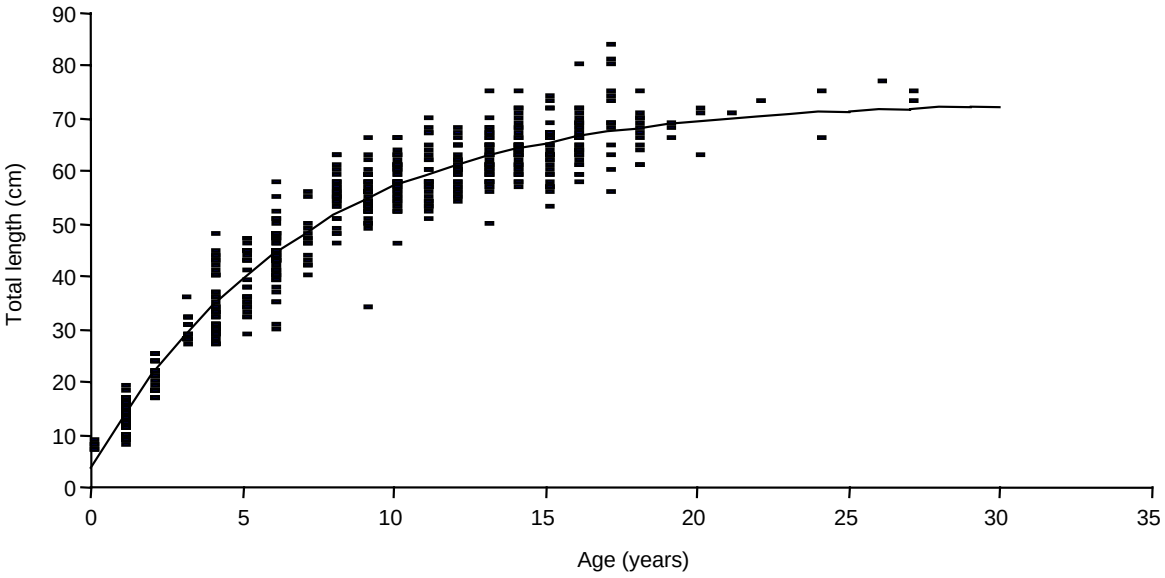

Figure 18. Age and length of *Pagrus auratus* from Victorian waters with von Bertalanffy growth function ($L\infty = 83.610$, $K = 0.100$, $T_0 = 1.147$), n = 646. (data provided by Central Ageing Facility, Primary Industries Research Victoria, Marine and Freshwater Systems, Queenscliff).

4.2.3 Otolith preparation and analysis

We took particular care to ensure that all otoliths from the older fish were stored and prepared using the same methods that were applied to the 0-age fish used for the baseline data (see sections 3.2.2, 3.2.3). Otoliths of older fish were analysed using similar instrument and calibration methods (i.e. Nist612 glass) as used for the 0-age otoliths (see section 3.2.3). However, analyses of adult otoliths collected in 2004 and 2005 was conducted using a new ThermoElectron Element 2 HR -ICP-MS and New-wave 213 nm laser, situated at Primary Industries Research Victoria, Marine and Freshwater Systems, Queenscliff, whereas previously we had used an Element1 and a 266 nm laser at Monash University, Melbourne. We checked precision between analyses of the 0-age and adult otoliths by re-analysing (adjacent ablations) 20 randomly selected otoliths from the 0-age samples on the new instrument at Queenscliff. As a regular precision check we also routinely analyse a pressed pellet of ground snapper otolith. We compared the results between 40 ablations of this pellet using the new and old instruments. For the re-analysed otoliths the mean difference, and mean percentage difference between concentrations determined from the repeated analyses were; $\mu g\ g^{-1}$ (±SD) / % difference (±SD), Mn – 0.88(0.75)/15(13), Sr – 120(82)/8(6), Ba 0.86 (0.93)/10(8). For the pressed pellet the mean (±SD) values for concentrations determined with the old/new instrument were ($\mu g\ g^{-1}$); Mn – 2.15 (0.45)/1.86(0.26), Sr – 1905(74)/1943(77), Ba - 5.35(0.28)/5.30(0.51).

Otoliths of the older fish were analysed with three 100 μm ablations, and similar to the 0-age fish, we averaged the elemental concentrations across the three ablations. The positions of the three ablations were standardised so as to approximate the same area (time) of otolith deposition that was analysed for tag characterisation in 0-age otoliths (Fig. 12). To estimate this area we measured the distances from the cores to the ablation craters of 100 randomly selected otoliths from the 0-age fish. These measurements provided minimum and maximum distances along the three standardised growth axis, within which would be the region of otolith relevant to the period when 0-age tags were incorporated. While temporal matching between sampling of the otoliths from the older and 0-age fish may not be exact, the results in section 3 of this report suggested that small-scale temporal variation in otolith chemistry had little affect on otolith composition.

Table 6. Summary of samples of sub-adult (age 1–2 years) and adult (age 3–5 years) snapper analysed for otolith chemistry; a) 2000 cohort, b) 2001 cohort.

a) 2000 cohort

Collection period	Age (years)	Collection region	Collection location (number of samples analysed)	Total number samples per region
2002	2	East Victoria	Woodside (21)	21
		Port Phillip Bay	South (33), North (10)	43
		West Victoria	Lorne (23), Apollo Bay (23)	46
2004	4	East Victoria	Lakes Entrance (7)	7
		Port Phillip Bay	South (2), North (28)	30
		West Victoria	Waratah Bay (4), Western Port (3), Torquay (4), Lorne (2), Apollo Bay (6), Portland (5)	24
2005	5	East Victoria	Woodside (1), Lakes Entrance (12)	13
		Port Phillip Bay	South (8), North (2)	10
		West Victoria	Lorne (4), Apollo Bay (2), Portland (2)	10
				Total = 204

b) 2001 cohort

Collection period	Age (years)	Collection region	Collection location (number of samples analysed)	Total number samples per region
2001	1	East Victoria	Woodside (10), Lakes Entrance (20)	30
		Port Phillip Bay	South (33), North (66)	96
		West Victoria	Kilcunda (20), Western Port (21), Torquay (21), Lorne (32), Portland (26)	120
2004	3	East Victoria	Woodside (20), Lakes Entrance (20)	40
		Port Phillip Bay	South (57), North (33)	90
		West Victoria	Waratah Bay (22), Kilcunda (8), Western Port (30), Torquay (12), Lorne (12), Apollo Bay (11), Warrnambool (12), Port Fairy (11), Portland (12)	130
2005	4	East Victoria	Woodside (21), Lakes Entrance – 22	43
		Port Phillip Bay	South (60), North (40)	100
		West Victoria	Waratah Bay (28), Western Port (30), Torquay (14), Lorne (15), Apollo Bay (6), Warrnambool (10), Port Fairy (13), Portland (12)	128
				Total = 777

4.2.4 Data analysis

Estimating the proportions of older snapper originating from Port Phillip Bay: We used a maximum likelihood estimation approach (MLA) (Millar 1987; Millar 1990ab) to determine the contributions of the different 0-age sources to the mixed samples of older fish. Analyses were performed using the HISEA program developed by Millar (1990b) and available at; http://www.stat.auckland.ac.nz/~millar/. The baseline otolith chemistry data for these analyses were the data (Mn, Sr, Ba) from the 0-age fish of the two cohorts (presented in section3). Data for all elements were transformed to $\ln(x + 1)$ to meet univariate normality and to reduce the weight of the Sr data (Millar 1990a). For each cohort we initially used the simulation mode to determine the accuracy with which the two baseline groups (Port Phillip Bay versus other areas) could be distinguished. We used 1000 simulations of the data to estimate the variability of the estimator, and compared the estimated mean contributions with the known contributions of each source in the simulated mixture (Table 7). Bootstrapping, with 1000 re-samplings (sample size the same as original sample size), of the baseline (0-age) and mixed sample data (older fish), was then used to estimate the mean and standard deviation of the proportions of the older snapper originating from Port Phillip Bay (Millar 1987). We conducted MLA with the samples of older snapper grouped at different spatial scales. For the 2000 cohort, because of the low sample sizes, we chose to group the data into three regions, east and west Victorian coastal waters and Port Phillip Bay. For the 2001 cohort we conducted two sets of MLA. The first was a broad scale analysis involving data grouped into eastern and western Victorian (including Western Port) waters, and Port Phillip Bay (i.e. similar to the 2000 cohort). The second was a finer scale comparison involving the data grouped into 6 regions; eastern Victoria, western Victorian coastal waters between Kilcunda and Waratah Bay, Western Port, Port Phillip Bay, western Victorian coastal waters between Torquay and Apollo Bay, and western Victorian coastal waters between Warrnambool and Portland. For the 2001 cohort we also compared MLA estimates between samples from the north and south regions of Port Phillip Bay (Fig. 17).

Comparison of 0-age baseline data with 0-age otolith compositions of older fish: Confidence in the accuracy of the MLA estimations of the origins of adult snapper requires the assumption that all possible 0-age sources that could contribute to adult recruitment have been included in the baseline data (Wood et al. 1987; Campana 1999). While all currently known 0-age recruitment areas were included in the baseline data, it is possible that unknown recruitment areas may have been missed during sampling of 0-age fish, and therefore not included in the baseline data set. If unknown 0-age recruitment areas contributed to recruitment of the older age classes we sampled this may show up as elemental compositions of the 0-age regions of these otoliths that do not closely match the 0-age baseline data. To investigate this possibility we used canonical variate plots to compare the multi-elemental otolith compositions of the 0-age region of the otoliths from the older fish with the baseline data from the 0-age fish of known origins.

Table 7. Results of maximum likelihood analyses comparing the otolith chemistry from 0-age snapper of know origin (i.e. baseline data).

Source (sample size)	Actual contribution to simulated mixture (%)	MLA estimated contribution to simulated mixture (%)	Standard deviation of estimation (%)
2000 cohort (140)			
Port Phillip Bay	31	30	4
Other estuaries	69	70	4
2001 cohort (252)			
Port Phillip Bay	57	63	4
Other estuaries	43	37	4

4.3 Results

4.3.1 Maximum likelihood analysis - 2000 cohort

Age 2: The 2 year old snapper sampled in eastern Victorian waters were predominantly (>90%) derived from 0-age recruitment areas outside of Port Phillip Bay (Fig. 19). For Port Phillip Bay and western Victorian coastal waters, >90% of the 2 year old samples were attributed to 0-age recruitment in Port Phillip Bay (Fig. 19). Standard deviations of the mean percentage contributions ranged from 3 to 7%, indicating a high level of precision in the estimations of the mixed sample compositions (Fig. 19).

Age 4: The results for the 4 year old fish were consistent with the 2 year old samples. Although we only had a small sample size for eastern Victoria, none of these samples appeared to have originated from Port Phillip Bay (Fig. 19, 22). For Port Phillip Bay and western Victorian coastal waters, there was a slight reduction in the estimated percentages originating from Port Phillip Bay, however, the results for both regions still indicated that >80% of the samples had originated from 0-age recruitment in Port Phillip Bay (Fig. 19). Standard deviations of the mean percentage contributions ranged from 0 to 7%, indicating a high level of precision in the estimations of the mixed sample compositions (Fig. 19).

Age 5: There was little difference between the results for 5 year old fish and those for the 2 and 4 year olds (Fig. 19). There was no clear indication that 0-age recruitment in Port Phillip Bay contributed significantly to samples from eastern Victoria (i.e. low mean with high standard deviation), and samples from both Port Phillip Bay and western Victorian coastal waters were again comprised largely (~90%) of fish attributed to 0-age recruitment in Port Phillip Bay (Fig. 19). Standard deviations of the mean percentage contributions ranged from 9 to 12%. This was slightly higher than for the other ages, but again indicative of a high level of precision in the estimations of the mixed sample compositions (Fig. 19).

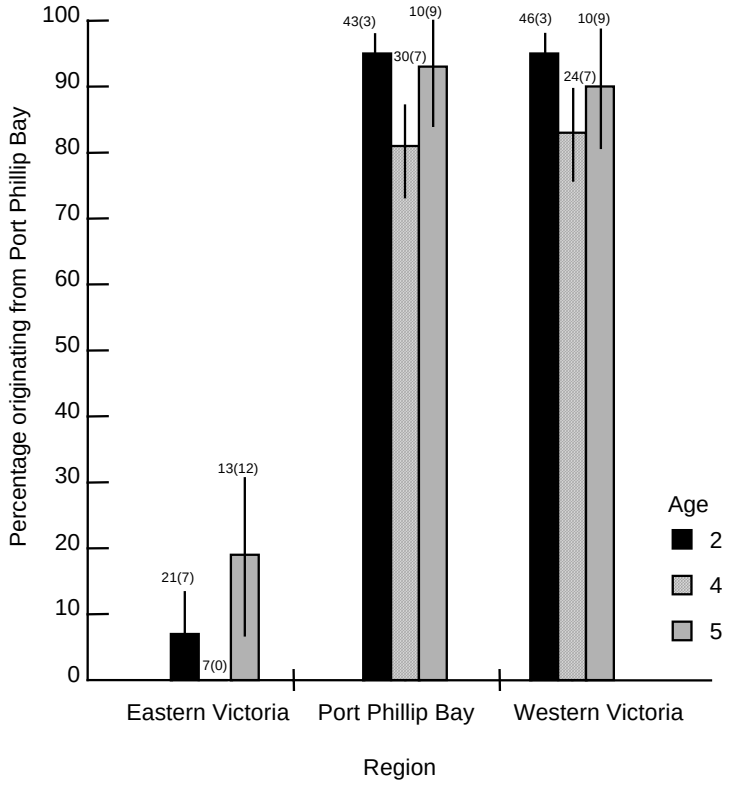

Figure 19. 2000 cohort - Maximum likelihood estimates of the mean (±1SD) percentage of 2, 4 and 5 year old snapper derived from 0-age recruitment in Port Phillip Bay. Numbers above each bar indicate sample size with standard deviations of the mean estimates in parentheses.

4.3.2 Maximum likelihood analysis - 2001 cohort

Broad-scale comparisons

Age 1: Over 90% of the 1 year old snapper sampled in eastern Victorian waters were estimated to be derived from 0-age recruitment areas outside of Port Phillip Bay (Fig. 20). For Port Phillip Bay 80% of the 1 year old snapper were attributed to 0-age recruitment within Port Phillip Bay (Fig. 20). Unlike the 2 year old fish of 2000 cohort, only 40% of the 1 year old samples from western Victorian coastal waters and Western Port, were attributed to 0-age recruitment in Port Phillip Bay (Fig. 20). Standard deviations of the mean percentage contributions ranged from 2 to 9%, indicating a high level of precision in the estimations of the mixed sample compositions (Fig. 20).

Age 3: It was estimated that 66 % of the 3 year old samples from eastern Victoria were derived from 0-age recruitment areas outside of Port Phillip Bay (Fig. 20). This indicated a substantial increase, from < 5% at age 1 to 34% at age 3, in the proportion of fish in eastern Victoria attributed to 0-age recruitment in PPB (Fig. 20). For Port Phillip Bay there was a slight reduction in the estimated percentage of samples attributed to 0-age recruitment in Port Phillip Bay, from 80% at age 1 to 70% at age 3 (Fig. 20). In contrast to Port Phillip Bay, for the samples from western Victorian coastal waters and Western Port there was an increase in the percentage of samples attributed to 0-age recruitment in Port Phillip Bay from 40% at age 1 to 64% at age 3 (Fig. 20). Standard deviations of the mean percentage contributions ranged from 9 to 10%, indicating a high level of precision in the estimations of the mixed sample compositions (Fig. 20).

Age 4: By age 4 it was estimated that over 50% of samples from eastern Victoria were derived from 0-age recruitment areas outside of Port Phillip Bay (Fig. 20). This indicated a further increase in the estimated contribution to eastern Victoria of 0-age recruitment in Port Phillip Bay from 34% at age 3 to 49% at age 4 (Fig. 20). For Port Phillip Bay the percentage of 4 year old snapper attributed to 0-age recruitment in Port Phillip Bay remained high at 80%, similar to the younger ages (Fig. 20). For the samples from western Victorian coastal waters and Western Port there was a further increase, from 64% at age 3 to 82% at age 4, in the percentage of samples attributed to 0-age recruitment in Port Phillip Bay (Fig. 20). By age 4 the results for Port Phillip Bay and western Victorian waters outside of Port Phillip Bay had converged with just over 80% of samples attributed to 0-age recruitment in Port Phillip Bay (Fig. 20). Standard deviations of the mean percentage contributions remained low at 7% for Port Phillip Bay and western Victoria, however, increased slightly to 13% for eastern Victoria indicating greater uncertainty of the estimate for samples from this region (Fig. 20).

Finer-scale comparisons

Age 1: The percentage of 1 year old fish attributed to 0-age recruitment in Port Phillip Bay was lower with distance from the Bay (Fig. 21a). To the west of Port Phillip Bay, it was estimated that 52% of the Torquay to Apollo Bay, and 27% of the Warrnambool to Portland region samples were derived from 0-age recruitment in Port Phillip Bay (Fig. 21a). To the east, 20% of the Kilcunda to Waratah Bay, 12% of the Western Port, and less than 5% of the eastern Victorian samples were attributed to 0-age recruitment in Port Phillip Bay (Fig. 21a). For Port Phillip Bay the percentage of 1 year old samples attributed to 0-age recruitment in the Bay ranged from 76% in the northern to 87% in the southern region (Fig. 21b). The standard deviations of the mean estimates were slightly higher for the regions sampled outside of Port Phillip Bay, possibly related to the smaller sample sizes from these sub-regions (Fig. 21a).

Age 3: At age 3 the percentage of samples attributed to 0-age recruitment in Port Phillip Bay increased significantly for the eastern Victorian samples from 1% at age 1 to 34% at age 3 (Fig. 21a). Similar increases also occurred for Western Port; 12% at age 1 to 81% at age 3, and the Warrnambool to Portland region; 27% at age 1 to 71% at age 3 (Fig. 21a). For the other regions there were only minor differences between the results for 1 and 3 year old fish (Fig. 21a). For Port Phillip Bay the percentage of 3 year olds attributed to 0-age recruitment in the Bay ranged from 70% in the north to 67% in the southern regions (Fig. 21b). Standard deviations of the mean estimates ranged for 9 to 18%, and similar to the age 1 samples, were generally higher for the regions outside of Port Phillip Bay (Fig. 21a).

Age 4: At age 4 the percentage of samples attributed to 0-age recruitment in Port Phillip Bay increased significantly for the Kilcunda to Waratah Bay region from 35–45% at ages 1–3 to 90% at age 4 (Fig. 21a). A similar increase occurred for samples from the Torquay to Apollo Bay regions from 50–60% at ages 1–3 to

88% at age 4 (Fig. 21a). For the Warrnambool to Portland and Port Phillip Bay regions the estimated percentage of samples attributed to Port Phillip Bay was similar to age 3 (80%) (Fig. 21a). For eastern Victoria there was a further increase in the estimated percentage of samples derived from Port Phillip Bay from 34% at age 3 to 50% at age 4 (Fig. 21a). For Port Phillip Bay 70% of samples from the north and 94% of samples from the south were attributed to 0-age recruitment in the Bay (Fig. 21b). Standard deviations of the estimates ranged from 9 to 18%, and similar to the age 1 and 3 samples, were higher for the regions outside of Port Phillip Bay (Fig. 21a).

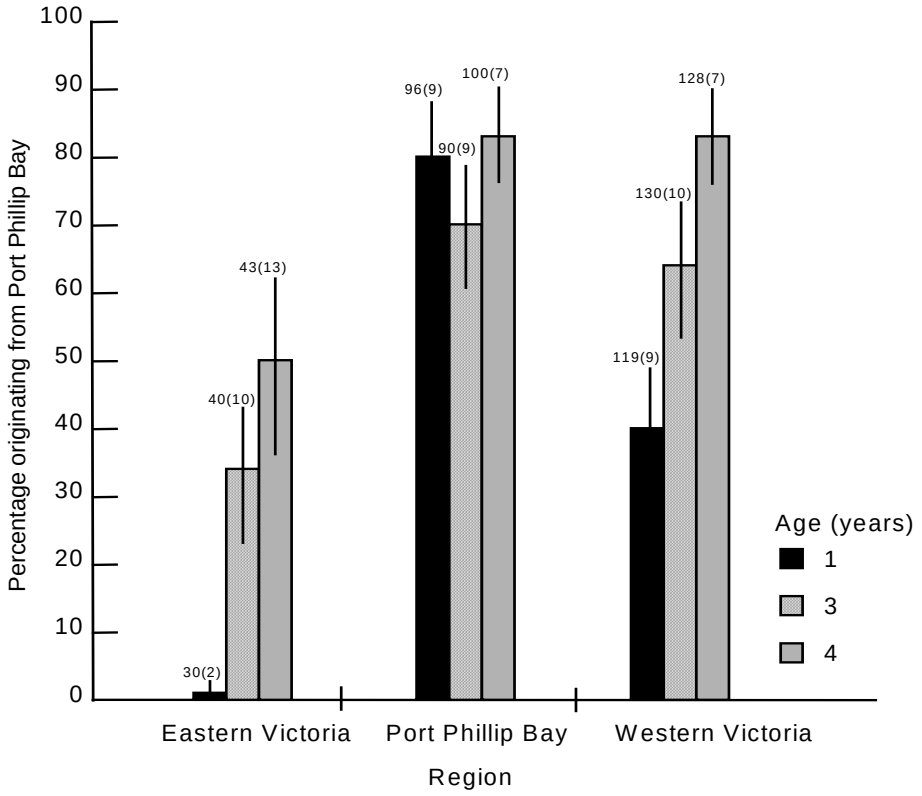

Figure 20. 2001 cohort broad-scale comparisons - Maximum likelihood estimates of the mean (±1SD) percentage of 1, 3 and 4 year old snapper derived from 0-age recruitment in Port Phillip Bay. Numbers above each bar indicate sample size with standard deviations of the mean estimates in parentheses.

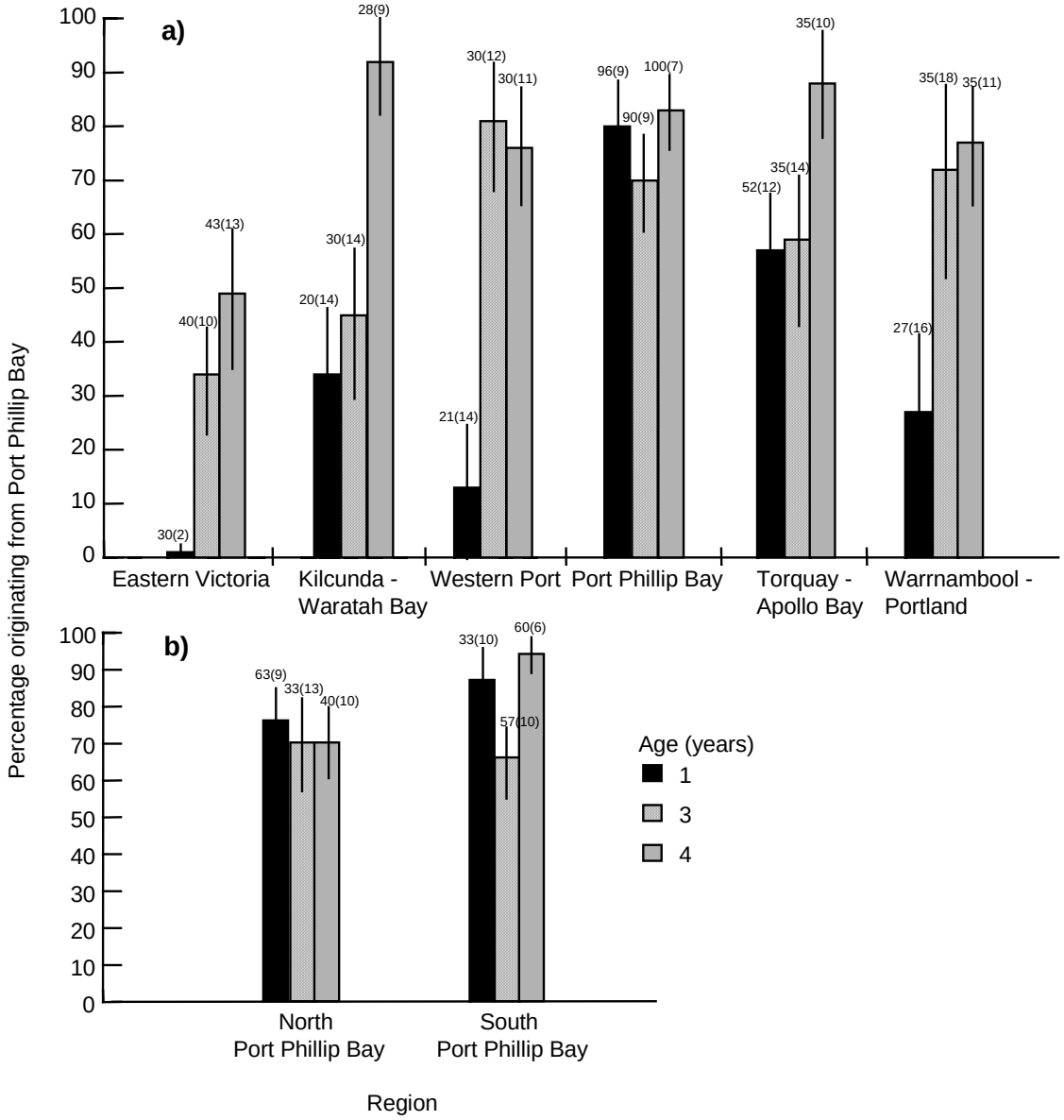

Figure 21. 2001 cohort finer scale comparison - Maximum likelihood estimates of the mean (±1SD) percentage of 1, 3 and 4 year old snapper derived from 0-age recruitment in Port Phillip Bay; a) compared among 6 regions of the Victorian fishery, and b) compared between north and south Port Phillip Bay. Numbers above each bar indicate sample size with standard deviation of the mean estimates in parentheses.

4.3.3 Summary of maximum likelihood analyses

In summary, the results of the MLA estimations indicated that samples of 1–5 year old snapper from Port Phillip Bay, irrespective of age, cohort or north/south regions, were dominated by fish that had originated in Port Phillip Bay. For the other regions the composition of samples differed both spatially and with age. For all the regions sampled outside of Port Phillip Bay, however, there was a trend for the percentage of samples attributed to Port Phillip Bay to increase with age. By age 4–5 years, fish that had originated from 0-age recruitment in Port Phillip Bay dominated the samples from western Victorian waters outside of the Bay, including Western Port.

4.3.4 Comparison of 0-age baseline data with 0-age otolith compostions of older fish - 2000 cohort

The 0-age otolith compositions of 2–4 year old fish from Port Phillip Bay and western Victorian coastal regions generally overlapped the data for the 0-age fish known to have originated in Port Phillip Bay (Fig. 22). Similarly, the 0-age composition of the 2–4 year old samples from eastern Victoria mostly overlapped the data for the 0-age fish known to have originated from recruitment areas outside of Port Phillip Bay, that were dominated be eastern Victorian estuaries (Fig. 22). These comparisons indicated that the 0-age otolith chemistry data from the older fish of the 2000 cohort were well matched to the 0-age baseline data.

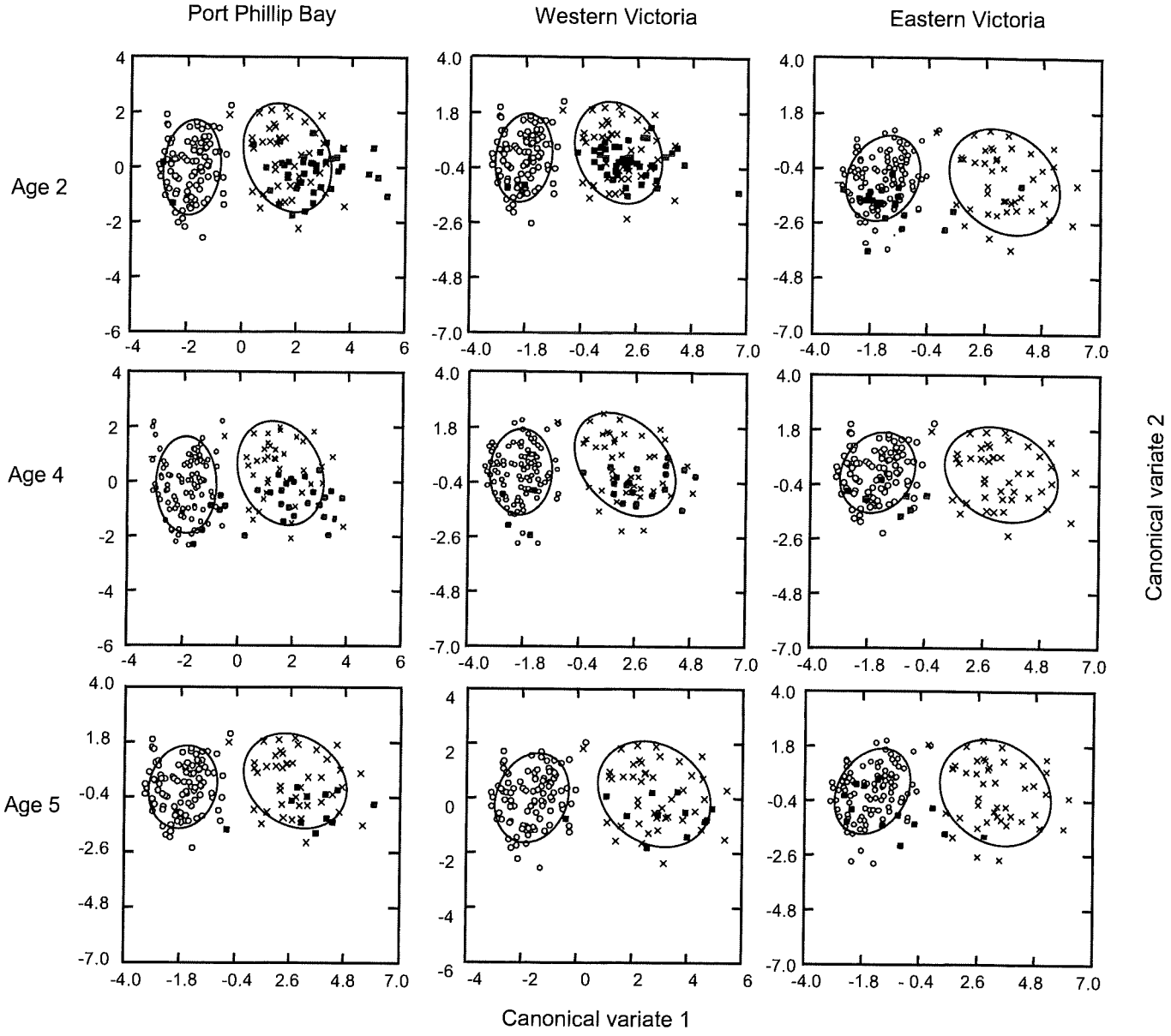

Figure 22. 2000 cohort: Canonical variate plots comparing multi-elemental compositions (Mn, Sr, Ba) of the 0-age regions of otoliths from 2, 4 and 5 year old snapper with the 0-age baseline data of known origin. Ellipses are 95 % confidence ellipses around the data for the two baseline groups; O = 0-age from areas outside of Port Phillip Bay, x = 0-age from Port Phillip Bay, ● = older fish of unknown origin. Data points represent individual fish. Collection regions for older fish indicated at top.

4.3.5 Comparison of 0-age baseline data with 0-age otolith compostions of older fish - 2001 cohort

The 0-age otolith composition data for the older fish sampled from Port Phillip Bay generally overlapped the data from 0-age fish known to have originated in Port Phillip Bay, although some data, particularly for the 1 and 3 year olds, were distributed outside, or in the region of overlap between, the 0-age baseline groups (Fig. 23). A similar result was obtained for the older fish sampled from western Victorian coastal waters, although for age 1 and 2 samples, a greater proportion of the data was distributed outside of, or in the region of overlap between, the 0-age baseline groups (Fig. 23). For the samples from Western Port, a large proportion of the data for age 1 fish was distributed outside both of the 0-age baseline groups (Fig. 23). However for ages 3 to 4 years, most of the samples from Western Port were distributed within the baseline group representing 0-age fish originating from Port Phillip Bay (Fig. 23). Data for the age 1 samples from eastern Victoria were mostly distributed within the baseline group representing 0-age fish from areas outside Port Phillip Bay (Fig. 23). This pattern also changed with age, with greater proportions of the age 3 and 4 data from eastern Victoria being distributed within the baseline group representing 0-age fish from Port Phillip Bay (Fig. 23). Samples of older fish from western Victoria and Western Port with 0-age otolith compositions that did not closely match either baseline groups would partly explain the higher standard deviations of MLA estimates for these areas, particularly for the age 1 and 3 samples (Fig. 21a). Further, although the proportion of outlying data did decrease with age (Fig. 23), the outlying data may be indicative of another 0-age recruitment area that was not included in the 0-age baseline data.

4.4 Discussion

The discovery that geographic variation in otolith chemistry can produce natural tags in otoliths has provided a new method for quantifying how different juvenile sources contribute to replenishment of fish populations (Gillanders and Kingsford 1996; Campana 1999; Thorrold et al. 2001). Although a number of studies have reported natural chemical tags in otoliths of juvenile fish, few studies have used this type of data to quantify the contributions of different juvenile sources to adult recruitment (reviewed in Gillanders 2005). We sampled sub-adult (1–2 years age, pre-fishery recruitment) and adult (3–5 years age, recruited to the fishery) snapper from two cohorts (2000, 2001) at various regions of the Victorian fishery (across ~ 700 km of the Victorian coast). We used previously characterised otolith chemical tags from 0-age fish to estimate the proportions of these older age classes that were derived from different 0-age recruitment areas. Specifically, we assessed the relative importance of Victoria's largest bay/estuary, Port Phillip Bay, as a source of population replenishment for the Victorian snapper fishery.

For both cohorts, the majority (>80%) of 1–5 year old snapper recruiting to the major Victorian fishery, Port Phillip Bay, originated from within the Bay. For both cohorts, the majority (>80%) of adult (> 3 years age) snapper recruiting to the fishery in western Victorian coastal waters (up to 350 km west and 150km east of Port Phillip Bay), including Western Port, also appeared to have originated from 0-age recruitment in Port Phillip Bay. Although most of the older snapper sampled from eastern Victoria were attributed to recruitment areas other than Port Phillip Bay, most likely east Victorian estuaries, there was a strong indication for the 2001 cohort, that by age 3, a significant proportion of snapper in eastern Victoria were in fact derived from Port Phillip Bay. For all the regions sampled outside of Port Phillip Bay there was a trend for the proportion of older fish attributed to 0-age recruitment in Port Phillip Bay to increase with age. These results indicate both a high level of self-recruitment for the Port Phillip Bay fishery, but also a high level of emigration out of the Bay to populate coastal waters over 300 km distant from the Bay.

Our results are consistent with conclusions on spatial population connectivity for snapper in South Australia based on a different otolith chemistry approach (Fowler et al. 2005). Fowler at al. (2005) compared chemical variation across otoliths and showed that while adults collected in different regions of the fishery showed significant variations in chemistry towards the margins of otoliths, the 0-age otolith chemistry was similar among regions. This was suggested to indicate that adults, regardless of capture region, originated from a one or two nursery areas in the north of two large sheltered bays (Spencer Gulf and Gulf St. Vincent) (Fowler et al. 2005). Another study in New South Wales involving the use of otolith chemical tags from 0-age fish indicated that recruitment of 2 year old snapper to one particular coastal region was derived predominantly from the nearest estuarine nursery area (Gillanders 2002a). Although the conclusions of these studies were based on one cohort only, they clearly support our conclusions for multiple cohorts that juvenile recruitment in sheltered bays and estuaries can be of considerable importance for replenishment of snapper populations in coastal waters.

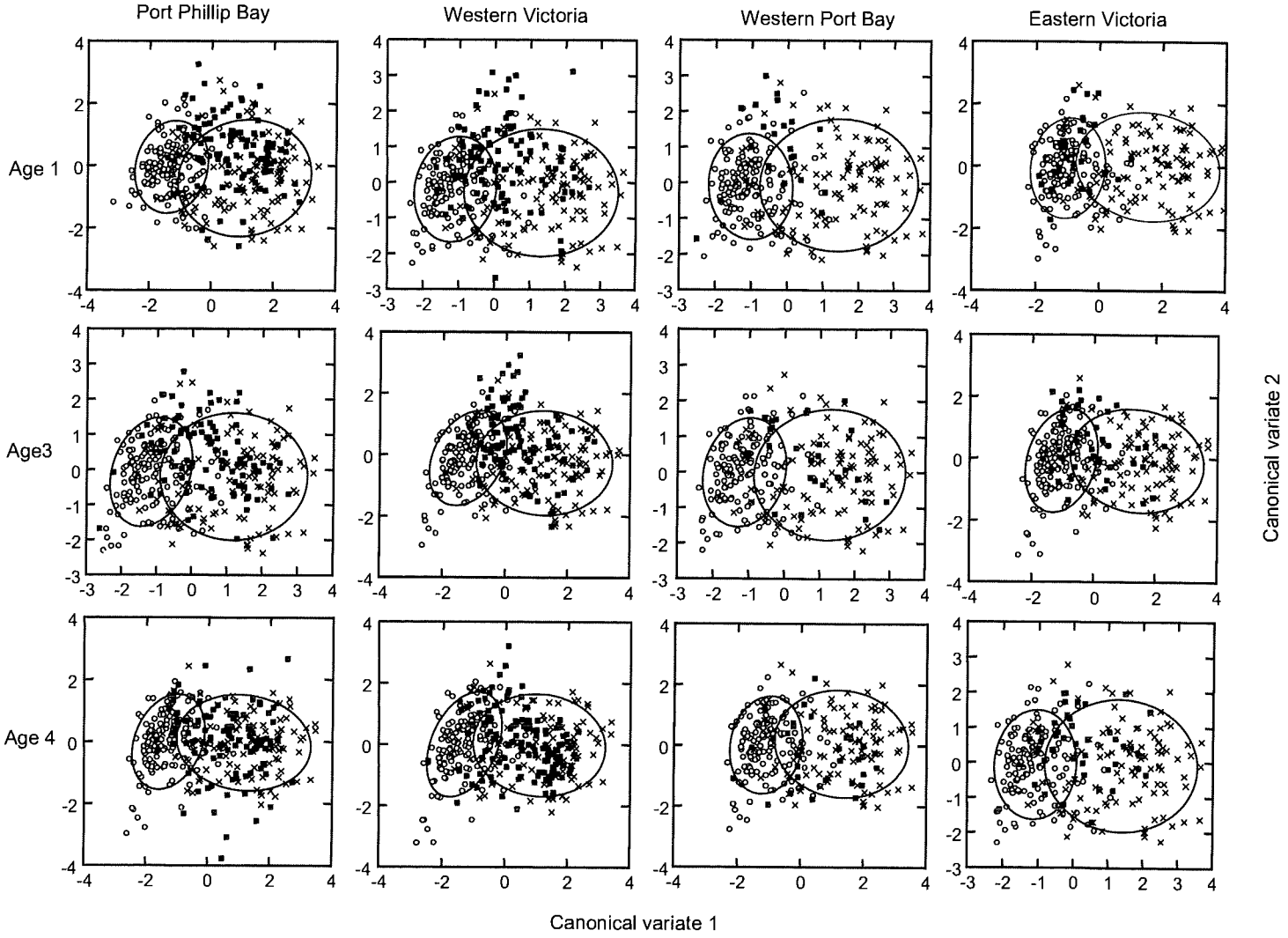

Figure 23. 2001 cohort: Canonical variate plots comparing multi-elemental compositions (Mn, Sr, Ba) of the 0-age regions of otoliths from 1, 3 and 4 year old snapper with the 0-age baseline data of known origin. Ellipses are 95 % confidence ellipses around the data for the two baseline groups; O = 0-age from areas outside of Port Phillip Bay, x = 0-age from Port Phillip Bay, ● = older fish of unknown origin. Data points represent individual fish. Collection regions for older fish indicated at top.

The conclusion from the otolith chemistry studies discussed above are, however, in contrast to the results from tag/recapture studies in Hauraki Gulf, New Zealand (Crossland 1982), Shark Bay, Western Australia (Moran et al. 2003), and Moreton Bay, Queensland (Sumpton et al. 2003). These studies have shown limited movement of snapper away from release locations within these large bays, leading to the conclusion that juvenile recruitment in the bays was not an important source of replenishment of coastal populations. In the case of Shark Bay, both otolith chemistry (Edmonds et al. 1989, 1999) and tag/recapture studies (Moran et al. 2003) have indicated a lack of connectivity among bay and coastal snapper populations. It therefore appears that the dependence of coastal snapper populations on juvenile recruitment sources within sheltered bays and estuaries varies geographically and from stock to stock.

We were fortunate to be in a position where we could compare models of population connectivity based on otolith chemistry with previous studies involving tag/recapture techniques (Sanders 1974; Coutin et al. 2003). Both methods indicated movement of young snapper from Port Phillip Bay to coastal waters, east

and west of Port Phillip Bay, and also that displacement distance away from Port Phillip Bay increases with age over the first 5 years of life (Coutin et al. 2003). Although the general patterns of movement reported for snapper tagged in Port Phillip Bay were similar to the indications from otolith chemistry, over 80% of the snapper that were recaptured from those tagged in Port Phillip Bay, were recaptured within Port Phillip Bay (Coutin et al. 2003). The other 20% were recaptured in Western Port and west Victorian coastal waters. Therefore, based on tag/recapture, movement of snapper out of Port Phillip Bay would appear significantly lower than indicated from the otolith chemistry approach.

When comparing population connectivity rates between conventional tag/recapture studies and otolith chemistry approaches it is important to recognise the differences between the approaches. Firstly, the age at which tags are manually implanted in fish or naturally incorporated into otoliths are likely to differ. The great advantage of otolith-based tags is that they can be characterised at a very early age (i.e. potentially the larval stage) and are permanent, whereas manual tagging is generally applied to older juveniles or adults due to mortality and tag shedding issues. Secondly, the otolith chemistry approach compares proportions of samples that have moved from the region of tag incorporation (i.e. nursery area) to particular sampling regions that can be specified *a priori*. Sampling can therefore be structured to answer specific questions at a population level. Tag/recapture compares recapture rates of individuals tagged and released at specific locations, and movement estimates depend on spatial distributions of tagging and recapture effort that most often are difficult to control *a priori* and are often subject to spatial biases.

We used otolith chemical tags incorporated during the first three to four months of life, whereas in previous tag/recapture studies most fish were not tagged and released until at least 2 years of age (Sanders 1974; Coutin et al. 2003). Our results indicated significant movement of young juvenile snapper (<1 years of age) out of Port Phillip Bay. This movement may have been largely missed by previous tag/recapture studies. Juveniles snapper that were tagged and released in coastal waters may have actually originated in Port Phillip Bay, further complicating interpretations of connectivity from tag/recapture data. Fishing pressure in Port Phillip Bay is considerably higher than in coastal waters (Coutin et al. 2003). Therefore recapture rates for Port Phillip Bay are likely to have been biased towards higher levels than coastal waters. Both these issues would lead to tag/recapture approaches underestimating the level of connectivity between Port Phillip Bay and the coastal population. This may also explain the significant proportion of eastern Victorian snapper (particularly for the 2001 cohort) that appeared to be derived from Port Phillip Bay, when very few (2 fish at the time of this study) snapper tagged in Port Phillip Bay have been recaptured in eastern Victorian waters (Sanders 1974; Coutin et al. 2003). Although we still suggest that the fisheries in eastern and western Victoria should be treated as separate stocks for fisheries management purposes, our results for the 2001 cohort indicate that movement of snapper from western to eastern Victoria is probably more common than indicated from previous tag/recapture studies.

The use of natural otolith tags to determine contributions of different juvenile sources to adult recruitment ideally requires that all sources that could contribute significantly to adult recruitment have been included in the juvenile (baseline) tag data. The canonical variate plots appeared to indicate that some 1–2 year old fish from the 2001 cohort had 0-age otolith compositions that were not closely matched to the baseline tag data. This was particularly the case for samples from west Victorian coastal waters and Western Port. Although the proportion of three to five year old adults with outlying otolith chemistry was negligible, the data for the 1–2 year olds may indicate that an unidentified 0-age recruitment area contributed to recruitment of the 2001 cohort in western Victorian coastal waters and Western Port.

Recruitment of 0-age snapper in Western Port has been shown, over four years of sampling, to be very low (section 2). It is also unlikely that significant numbers of sub-adult snapper less than 2 years age were migrating from estuaries in eastern Victoria to far western Victoria (i.e. Portland) (Sanders 1974; Coutin et al. 2003). Although it is quite likely that some sub-adult snapper in Western Port originated there, it is unlikely that either of these sources would have contributed significantly to recruitment of sub-adults in western Victorian coastal waters. Snapper are thought to spawn both in coastal waters and Port Phillip Bay (Coutin et al. 2003, Neira et al. 2000). Although our attempts to sample 0-age snapper from western Victorian coastal waters failed, this may have been due to low sampling effort relative to their naturally low density (Appendix 3). It is possible that during the strong recruitment year of 2001 (section 2) there was significant recruitment of 0-age snapper to west Victorian coastal habitats, but that this did not occur, or was incredibly low, in the poor recruitment year of 2000. However, irrespective of the occurrence of

coastal recruitment sources, there was no evidence that such sources contributed greatly to adult recruitment (i.e. ages > 3 years) to the fishery in western Victoria.

If 0-age snapper were recruiting to oceanic habitats along the western Victorian coast it is highly unlikely that they would have biased the MLA estimates of the contributions of the Port Phillip Bay nursery area to adult recruitment. This is because analyses of water samples taken during the period of tag incorporation in 2001 showed that water chemistry varied strongly between Port Phillip Bay and western Victorian coastal waters (section 5). In particular, levels of barium (the most important element for discriminating Port Phillip Bay 0-age) in coastal waters were half those determined for Port Phillip Bay (i.e. 5 $\mu g\ L^{-1}$ in coastal waters, 12 $\mu g\ L^{-1}$ in Port Phillip Bay). The incorporation of barium into otoliths is positively correlated with ambient levels for 0-age snapper and a range of other species (section 5) (Bath et al. 2000; Milton and Chenery 2001; Elsdon and Gillanders 2003). Ba in the water, as opposed to food, is also considered the major source of Ba to the otolith (Milton and Cheney 2001; Walther and Thorrold 2006). Therefore, based on the water chemistry data and the relationship between otolith and ambient Ba in section 5 of this report, if 0-age snapper had recruited in ocean waters they would have displayed otolith Ba levels similar to the 0-age fish from the recruitment areas sampled outside of Port Phillip Bay.

5.4.1 Conclusions

In summary, 0-age recruitment in Port Phillip Bay is the major source of recruitment to the local fishery. Furthermore, young snapper can migrate large distances (at least 300 km) from Port Phillip Bay to replenish populations in coastal waters west of Wilsons Promontory and in Western Port bay. Movement of young snapper out of Port Phillip Bay appears to increase with age, such that by age 4 years, when fully recruited to the fishery, the entire west Victorian fishery appears dominated by fish that originated from Port Phillip Bay. Monitoring of juvenile (0-age) recruitment in Port Phillip Bay will therefore provide a valuable indicator of recruitment variation relevant to the entire west Victorian stock. The long-term future of the west Victorian snapper fishery will depend largely on careful management of the habitats/process required for sustaining 0-age recruitment in Port Phillip Bay. Given the high dependence of this fishery on one major spawning/nursery area in Port Phillip Bay, a conservative approach to management of the exploitation of juveniles and the spawning adults that aggregate within Port Phillip Bay during spring/summer is recommended.

5. Barium variation in *Pagrus auratus* otoliths: a potential indicator of migration between Port Phillip Bay and coastal waters

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5.1 Introduction

Understanding the migration behaviour of fish is fundamental to determining spatial population structure, local population dynamics, and habitat use. Knowledge of migration behaviour has, however, been limited by the lack of methods for studying movement of fish over their entire lives. Recent work has demonstrated that fish otoliths, calcified structures found in the inner ear, have the potential to store information on both the environmental and movement histories of fish (Campana 1999; Campana and Thorrold 2001). This is possible because the incorporation of certain chemical elements into otoliths can be influenced by variation in environmental factors such as temperature (Townsend et al. 1992; Elsdon and Gillanders 2002), salinity (Secor et al. 1995; Secor and Rooker 2000), and the concentration of specific elements in the water (Gallahar and Kingsford 1996; Bath et al. 2000; Elsdon and Gillanders 2003, 2004). Further, because otoliths grow throughout life and are metabolically inert, they can permanently record environmental variations experienced over the entire lifespan (Campana 1999). Otoliths also exhibit microstructure features such as daily and annual growth increments (Pannella 1971; Campana and Thorrold 2001) that allow chemical variations within otoliths to be placed into a chronological context. Individual migration histories can therefore be reconstructed from otoliths by coupling chronologies of otolith chemistry with knowledge of spatial variation in environmental parameters known to influence otolith chemistry.

Previous studies of diadromous fish have used chronologies of otolith strontium:calcium (Sr:Ca) ratios, and the Sr isotope ratio (^{87} Sr / ^{86}Sr) to indicate movement patterns of fish between marine, brackish and freshwater environments (Secor et al. 1995; Kennedy et al. 1995; Tzeng et al. 1997). However, the use of otolith Sr to indicate migration history can be ambiguous, particularly where movement is across smaller salinity gradients (Kraus and Secor 2004), and the ^{87} Sr / ^{86}Sr ratio is generally constant in marine waters (Hodell et al. 1989). The use of otolith chemistry to indicate movement histories of fish among marine dominated water bodies (i.e. constant salinity) may therefore depend on interpreting variations of other trace elements in otoliths. Trace elements found in otoliths, such as manganese (Mn), and barium (Ba), have nutrient type profiles in marine waters and potential to be enriched (above oceanic waters) in inshore coastal waters and marine dominated bays and estuaries (i.e. Port Phillip Bay) (Bruland 1983; Elsdon and Gillanders 2005a). For Ba in particular, there is also growing evidence that incorporation into otoliths is primarily influenced by ambient concentration (Bath et al. 2000; Elsdon and Gillanders 2003, 2004).

Assessing the potential for trace elements in otoliths to be used as indicators of migration between water bodies will initially require knowledge of variation in ambient chemistry between the water bodies of interest and over time (i.e. bay/estuaries versus ocean). If consistent spatial differences in ambient water chemistry are demonstrated, it will then be necessary to validate relationships between levels of specific elements in the water and incorporation into otoliths of the species of interest. Furthermore, it will also be important to demonstrate that variations in elemental incorporation into otoliths are not significantly influenced by factors such as ontogeny, stress, and seasonal variations in temperature and/or growth (Kalish 1991, 1992; Sadovy and Severin 1992, 1994). Significant influences of these factors on incorporation rates could result in confounding of interpretations of migration history from otolith chemistry.

In south-eastern Australia the major snapper fisheries are localised in several bays and gulfs (Coutin et al. 2003; Fowler et al. 2003). One of the most important fisheries occurs within Port Phillip Bay, Victoria (Fig. 24). During the spring/summer (October–February) adult snapper migrate into Port Phillip Bay from

ocean waters to spawn, and are thought to return to the ocean in late summer to autumn (February–May), although longer term residency in both coastal and Bay waters is also suspected (Coutin et al. 2003). The fishery is concentrated on migratory adults within Port Phillip Bay over the summer months (Coutin et al. 2003). Consequently, understanding the dynamics of these migrations is critical to understanding yearly and longer-term fluctuations in the fishery. Water temperature and salinity differences are negligible (i.e. < 2°C and 2 ‰) between Port Phillip Bay and Victorian coastal waters (Longmore et al. 1996). Port Phillip Bay, however, receives inputs from a catchment that includes approximately 4,000,000 people, considerable industrial development, one major and several minor rivers, and a sewerage treatment facility (Longmore et al. 1999; Murray and Parslow 1999) (Fig. 24). These inputs coupled with Port Phillip Bay's narrow entrance and long flushing time (approximately 300 days) (Walker 1999) offer strong potential for enrichment of its waters, above ocean levels, with trace elements that could be incorporated into otoliths.

We were interested in investigating whether otolith chemistry could provide information on movement histories of snapper between Port Phillip Bay and coastal waters. We initially compared ambient water chemistry (magnesium - Mg, calcium – Ca, Mn, Ba, Sr) among Port Phillip Bay, two other large bay/estuaries where adult snapper occur, and Victorian coastal waters. Based on these comparisons, we identified Ba as a potential migration indicator. To determine if otolith Ba could provide a reliable proxy for ambient Ba, we investigated relationships between ambient Ba levels and levels in otolith margins of wild caught juvenile and adult snapper. We also used otoliths from adult snapper maintained in tanks for three years to investigate whether seasonal temperature and/or growth cycles might influence variation in otolith Ba. To further assess longer-term stability of coastal water Ba levels we used Ba levels across otoliths from ocean resident and tag/recaptured snapper as a proxy for ambient oceanic levels. Based on these preliminary investigations we attempted to interpret chronological Ba variation in adult snapper otoliths in terms of residence periods in Port Phillip Bay.

5.2 Methods

5.2.1 Water chemistry

Initially we investigated spatial variation in water chemistry (Mg, Ca, Mn, Sr, Ba) across Victoria's three major bay/estuaries where adult snapper occur; Port Phillip, Western Port, Corner Inlet, and inshore coastal waters (Fig. 24, Table 8). This spatial comparison was conducted during summer (February–March) 2001. Secondly, we investigated temporal variation in ambient water chemistry. This involved comparing ambient levels of elements in water samples collected in Port Phillip Bay during summer (February–March) and winter (July–August) of 2001 and 2002, and from coastal waters during summer (February–March) 2001 and 2002 (Fig. 24, Table 8).

Snapper generally occur close to the bottom; therefore all water samples were taken within 1 to 2 m of the bottom with a niskin sampler. Samples were transferred to acid leached (10% HNO_3) and washed (4 x rinsed with Milli-Q water) high-density polyethylene (HDPE) bottles and stored on ice until return from the field. Immediately on return from the field water samples were filtered through 0.45 µm membrane syringe filters (Millipore) into new (acid leached/washed) HDPE bottles, acidified with 3 ml L^{-1} of ultrapure HNO_3 (Aristar) and refrigerated until analysis. Samples were diluted (1:10) with Milli-Q water and analysed for Mg, Ca and Sr by ICP-AES (inductively coupled plasma atomic emission spectrometry), and for Mn and Ba by ICP-MS (inductively coupled plasma mass spectrometry). Internal standards of lutetium (2 µg L^{-1}) and indium (5 µg L^{-1}) were used to correct for instrument drift. Detection limits in µg L^{-1} were; 5.0 (Mg), 5.0 (Ca), 1.0 (Mn), 1.0 (Sr), 1.0 (Ba). Blanks were prepared and analysed in the same way as seawater samples (including exposure in the field) except using Milli-Q rather than seawater. All elements were below detection limits in blanks. Average analytical accuracy (% recovery) and reproducibility (relative % difference) for each element were; 100, 1.3 % (Mg); 93, 2.7 % (Ca); 106, 3.3 % (Mn); 91, 1.3 % (Sr); 103, 2.9 % (Ba).

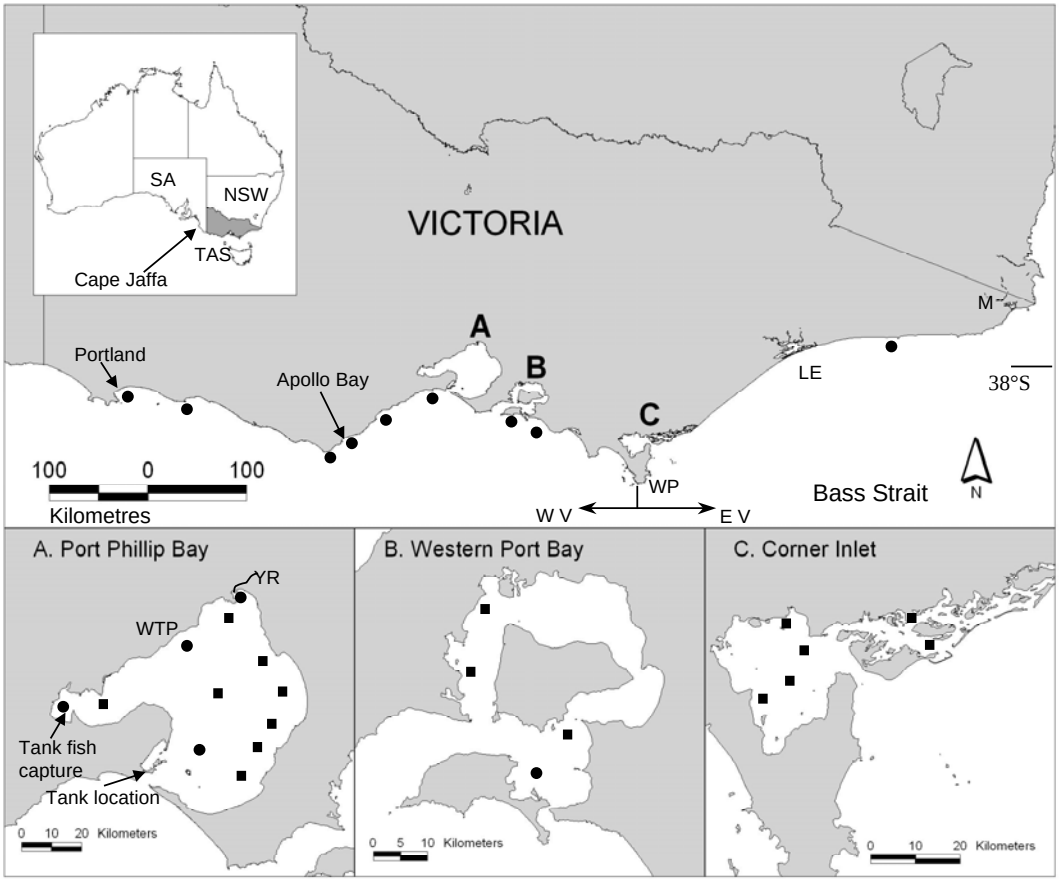

Figure 24. Map of Australia (top left) and the Victorian coast with insets for the different bays and estuaries where samples were collected. Dots indicate water sample collection sites, squares indicate sites where both water samples and 0-age *Pagrus auratus* were collected; WP – Wilsons Promontory, SA – South Australia, NSW – New South Wales, TAS – Tasmania, WV – western Victoria, EV – eastern Victoria, LE – Lakes Entrance, M – Mallacoota, WTP – western treatment plant, YR – Yarra River.

5.2.2 Otolith preparation and laser ablation ICP-MS

Transverse sections (~300 μm thickness) of otoliths were prepared using the methods described in section 3.2.2. Mounting resin was doped with indium (In) at ~30 μg g^{-1} as a resin indicator for sampling near otolith margins. Otoliths were analysed with a Merchantek 266 nm Nd:YAG laser microprobe operated in Q switched mode in conjunction with a Finnigan MAT ELEMENT 1 high resolution inductively coupled plasma mass spectrometer (Earth Sciences Department of Monash University, Melbourne, Australia). For the comparisons of water and otolith chemistry we used laser ablation and the methods described in section 3.2.3 to sample otolith material near the proximal margin (see Fig. 12). To determine chronological variations in otolith chemistry the laser was programmed to ablate continuously while the sample stage moved at 2 μm sec^{-1} along a predefined transect path. Laser transects were conducted from the core to the proximal margin in the region just ventral of the sulcus groove (Fig. 25). This sampling axis generally provided clear annual opaque increment zones and lower sampling time relative to other possible sampling axes. We chose this sampling axis after initial comparisons of different sampling axes and different otoliths from the same fish revealed similar chronological patterns of Ba variation (Appendix 4). The ICP-MS continuously scanned the selected isotopes during the course of each laser transect. For all transects we used a laser beam diameter of ~30μm (minimum possible spot size), energy 0.15–0. 20 mJ, and pulse rate 6.00 Hz. Ablation occurred in helium and the helium/ablation products were mixed with argon prior to introduction to the torch. Data was acquired for the isotopes ^{24}Mg, ^{55}Mn, ^{88}Sr, ^{115}In, ^{138}Ba, and ^{44}Ca, although only Ba data is presented in detail herein. The average counts from 30 scans of these

isotopes in blank sample gas were subtracted from the sample counts for each ablation. Calibration was achieved with the National Institute of Standards (NIST) 612 glass standard, and ^{44}Ca was used as an internal standard to control for variation in ablation yield. Otolith Ca concentration was taken as 388,000 $\mu g\ g^{-1}$ following the results from (Yoshinaga et al. 2000), and similar results from a previous study of *Pagrus auratus* otolith chemistry (Edmonds et al. 1989). The NIST 612 was analysed by continuous transects of 300 isotope scans at the beginning, middle and end of each analysis day, and to further minimise any possible influences of instrument drift, samples were analysed in a random order. The average detection limit for ^{138}Ba based on three standard deviations (SD) of the blank gas and adjusted for ablation yield, was 0.3 $\mu g\ g^{-1}$. Average recovery from 10 analyses of the NIST 612 as an unknown was 99%. Average RSD (relative standard deviation) (±SD) of raw counts from 10 laser transects, of 300 isotope scans each, on the NIST 612 were, 17.9 % (±4.9) ^{44}Ca and 17.4 % (±3.4) ^{138}Ba.

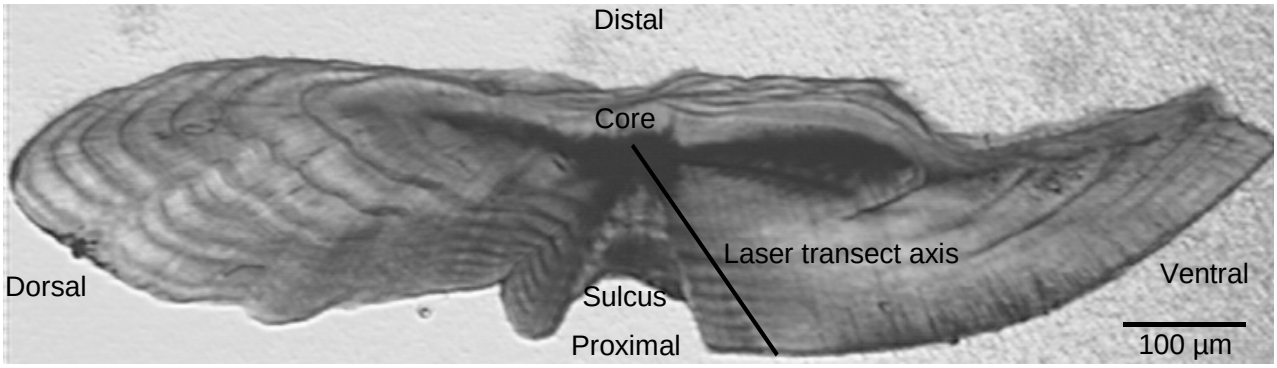

Figure 25. Transverse section of a sagitta from an eight year old snapper of approximately 50 cm fork length showing the region of the otolith where laser transects were conducted.

5.2.3 Relationships between otolith and water chemistry

To examine relationships between water chemistry and otolith chemistry we compared the marginal otolith chemistry of juvenile snapper (0-age, less that 4 months old, 20–90 mm standard length - SL) with water chemistry at 17 sites spread across Port Phillip Bay, Western Port and Corner Inlet (Fig. 24). For this comparison we used the average of three 100 µm diameter ablation spots around the proximal margin of each otolith, which represented the last 15–20 days of otolith growth. Otoliths were analysed from two to six fish from each site. Water chemistry data were averaged across two spatially random samples at each site, one sample was taken at the time when the fish samples were collected in March 2001 and the other approximately 20–30 days prior. We used small juveniles for these comparisons because small juveniles were likely to have spent the period just prior to capture in the general area of capture (Hartill et al. 2003), and therefore the water chemistry data should be relevant to the marginal otolith chemistry. Juvenile snapper were collected with a small beam trawl (see 2.2.2). We also compared water chemistry with marginal otolith chemistry of adult snapper (27–40 cm fork length – FL, 3 to 6 years of age) collected from Port Phillip Bay (n = 10) and west Victorian ocean waters (n = 10) during March–April 2001. For the adults we used a laser spot diameter of 40 µm to account for their slower otolith extension rate, and averaged across four ablations around the proximal margin. Although we were unsure of the time period this ablation size represented we assumed fish were either in ocean waters or Port Phillip Bay during this period of otolith deposition. Water chemistry data for this comparison were the averages of all samples taken in Port Phillip Bay and coastal waters during summer 2001.

5.2.4 The influence of seasonal temperature/growth cycles on chronological Ba variation

To investigate whether seasonal cycles in temperature and/or growth might influence chronological variation in otolith Ba we examined otoliths of adult snapper maintained in 2 large tanks (5000 L) for ~3.5 years. These fish were from the 1996 year-class (i.e. birthdate January 1996) captured in Port Phillip Bay in November 1998 at approximately three years of age. They were initially placed in a seacage before introduction to the tanks in late December 1998, and were sacrificed in May 2002 at just over 6 years of age. To confirm the age at release into the tanks a number of fish were tagged and injected with

oxytetracycline. The tanks were situated in an outdoor covered area and received continuous flow through water sourced from the entrance region of Port Phillip Bay (ocean dominated) (Fig. 24) and natural temperature and light/dark regimes. The fish were fed approximately daily on a mixture of squid (*Nototodarus gouldi*) and pilchards (*Sardinops neopilchardus*). Based on water temperature and salinity variations near the entrance to Port Phillip Bay, temperature in the tanks would have ranged from approximately 11°C in winter (July–August) to 21°C in summer (February), and salinity variation would have been negligible (i.e. < 1 ‰) (Longmore et al 1996; A.R. Longmore unpublished data). We used laser transects to compare Ba variation from the third to the sixth annual increments (opaque zones) among 10 tank fish that survived the entire three and half year period. Although water chemistry was not monitored in the tanks, if Ba levels in otoliths from tank fish varied little across years, and otolith growth appeared normal relative to wild fish, it would suggest that annual cycles of growth or temperature do not significantly influence chronological variations in Ba incorporation. Further, if there were noticeable differences in the chronological patterns, or levels, of Ba incorporation among otoliths from the tank fish it would suggest that otolith Ba incorporation is somehow dependent on the individual. Finally, to assess whether any variations we observed in otoliths of tank fish may have been peculiar to this cohort or time period, and also whether otolith growth rates of tank fish may have differed from wild fish, we compared Ba variations and otolith growth of the tank fish with 10 wild fish from the same genetic stock and year-class (captured in Port Phillip and Western Port Bay, Fig. 24).

5.2.5 Chronological Ba variation in otoliths of resident oceanic snapper

We further examined the potential for spatial and temporal variation in coastal water Ba levels by using Ba variation across the otoliths of snapper believed to have remained in coastal waters as a proxy for ambient Ba levels. These fish came from two sources. The first source was eastern Victorian waters (east of Wilsons Promontory, Fig. 24). Previous tagging studies suggest that snapper in this region remain oceanic from about 1–2 years of age and do not enter Port Phillip Bay (Sanders 1974; Coutin et al. 2003). We used laser transects to examine Ba variation across otoliths from 10 adult snapper (4 to 21 years of age) collected from two areas in eastern Victorian waters (Lakes Entrance, Corner Inlet), and from northern Tasmania (Fig. 22). The other source was snapper that were tagged and recaptured in ocean waters, both in eastern (n = 5) and western Victoria (n = 5). These fish ranged from three to six years of age and due to their tag and recapture locations and age we were confident that their time at liberty would have been spent in ocean waters.

5.2.6 Interpreting chronological Ba variation in otoliths – pilot study of suspected migratory snapper

We used laser transects to determine Ba variation across otoliths from eight adult snapper (7 years age) of the 1993 year-class captured in Port Phillip Bay during February–March 2000, and six adult snapper (5–7 years age) captured in western Victorian coastal waters off Apollo Bay (Fig. 24). Four of the fish from Apollo Bay were from the 1993 year-class but were captured in winter 1998 and 1999; the other two were from the 1991 and 1992 year-classes captured in winter 1998. Based on previous tag/recapture studies we expected that at least some of these fish would have moved between Port Phillip Bay and coastal waters (Coutin et al. 2003). We used the maximum Ba levels in the coastal water samples and otoliths from oceanic snapper to estimate maximum Ba levels that could be incorporated into otoliths when fish resided in coastal waters. Similarly we determined the minimum levels that could be incorporated when fish resided in Port Phillip Bay. Based on these estimates we attempted to interpret chronological variation of otolith Ba levels in terms of residence periods in Port Phillip Bay. Due to the narrowness of annual increments relative to the minimum laser spot diameter of ~30 μm we could not adequately resolve temporal Ba variation beyond age 10. For this reason we only included fish of less than 10 years of age in this component.

5.2.7 Data analysis

Water chemistry data were analysed by univariate analysis of variance (ANOVA). Year and region (i.e. the three bays and coastal waters) were fixed factors and sites were random within regions. Linear regression was used to describe the relationship between Ba concentrations in the water and the otolith margins of juveniles. For comparison, the relationship between water and otolith Ba levels for adult fish was superimposed on the plot of the juvenile data. For water chemistry/otolith chemistry comparisons, Ba data were compared as both ratios to Ca and absolute concentrations. The element/calcium ratio was

used to calculate partition coefficients for Ba (D_{Ba}) following Morse and Bender (1990) where D_{Ba} = (Ba : Ca otolith) / (Ba : Ca water).

Partition coefficients were compared between adult and juvenile snapper to compare the relationship between Ba incorporation into otoliths and ambient levels. Similar partition coefficients would suggest similar elemental discrimination or uptake across life-stages (Elsdon and Gillanders 2005b). We used T-tests to test for differences between the mean concentrations of elements at the margins of adult otoliths sampled from Port Phillip Bay and ocean waters. Box and residual plots were examined for deviations from normality and heterogeneity of variance assumptions. To meet these assumptions, Ba and Mn were transformed to $\log_{10}$ (x+1) for ANOVA of water chemistry data. We removed random noise from laser transect data by smoothing with an 11 point running median followed by an 11 point running mean (Sinclair et al. 1998). Data from laser transects were plotted as line graphs for qualitative comparison. For interpretive purpose, horizontal lines were included on transect graphs to delineate Ba levels suggested to indicate residency in Port Phillip Bay, unknown residency, and residency in coastal waters. Annual increment (opaque) zones were indicated on each transect graph based on records of the ICP-MS scan number when the laser beam was directly over the increment. Scan numbers were converted to distance from the otolith core based on the distance moved by the stage during each scan.

5.3 Results

5.3.1 Water chemistry

Spatial comparison of water chemistry in summer/autumn 2001 showed that Ba levels were significantly higher in Port Phillip Bay than the other areas sampled (Fig. 26), and Mn was significantly higher in Port Phillip Bay than ocean waters (Fig. 26). Mg, Ca, and Sr were significantly lower in Port Phillip Bay than Corner Inlet, although the differences were minor compared to overall levels (Fig. 26), and there were no differences among Port Phillip Bay, Western Port and ocean waters (Fig. 26).

Comparison across summers showed that Ba and Mn were significantly higher in Port Phillip Bay than ocean waters in 2001 and 2002 (ANOVA, $p < 0.001$, Fig. 27). Temporal variation of Port Phillip Bay water chemistry across two adjacent summers and winters was significant for Mg, Ca, Mn, and Sr (ANOVA, $p < 0.005$, Fig. 27). Although non-significant (ANOVA, $p > 0.05$), mean Ba levels in Port Phillip Bay appeared to decline over the two year period (Fig. 27).

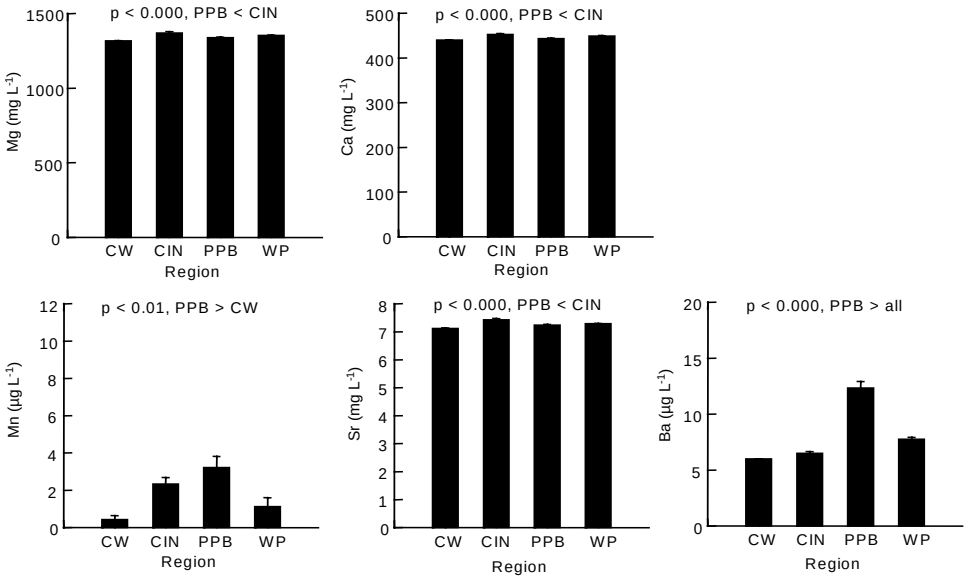

Figure 26. Comparisons of mean (±1SE) concentrations of elements from water samples collected in Victoria's major bay/estuaries and inshore coastal waters during February–March 2001. Significant ($p < 0.05$) differences among areas (Tukey's pairwise comparisons after ANOVA) are indicated above each graph; CW – coastal waters, CIN – Corner Inlet, PPB – Port Phillip Bay, WP - Western Port.

Table 8. Summary of elemental concentrations of water samples from the three major Victorian bays and inshore coastal waters. Sampling times refer to periods when samples were collected; s – summer (February–March), w – winter (July–August), 01- year 2001, 02 - year 2002. Numbers of sites and water samples analysed for each time period are indicated in parenthesis. Mean concentrations, standard deviation (SD) and ranges are for all water samples collected at each region.

Region	Sampling Times (N sites, N samples)	Mean concentration (SD, range) Mg (mg L^{-1})	Ca (mg L^{-1})	Mn (μg L^{-1})	Sr (mg L^{-1})	Ba (μg L^{-1})
Port Phillip	s01 (10, 19), w01 (8, 16)	1296	413	2.9	7.2	11.0
	s02 (8, 16), w02 (7, 9)	(38, 1210–1390)	(23, 380–460)	(2.1, <1–11)	(0.1, 6.8–7.5)	(2.5, 8–21)
Western Port	s01 (4, 8)	1354	449	1.1	7.3	7.8
		(11, 1340–1370)	(4, 440–450)	(1.2, <1–3)	(0.04, 7.2–7.3)	(0.5, 7–8)
Corner Inlet	s01 (6, 12)	1370	453	2.3	7.4	6.5
		(31, 1330–1420)	(6.2, 440–460)	(1.2, 1–5)	(0.2, 7.3–7.6)	(0.5, 6–7)
Coastal water	s01 (5, 7), s02 (6, 6)	1300	420	0.23	7.1	5.7
		(21, 1260–1320)	(23, 390–440)	(0.43, <1–1)	(0.1, 7.0–7.1)	(0.4, 4.5–6)

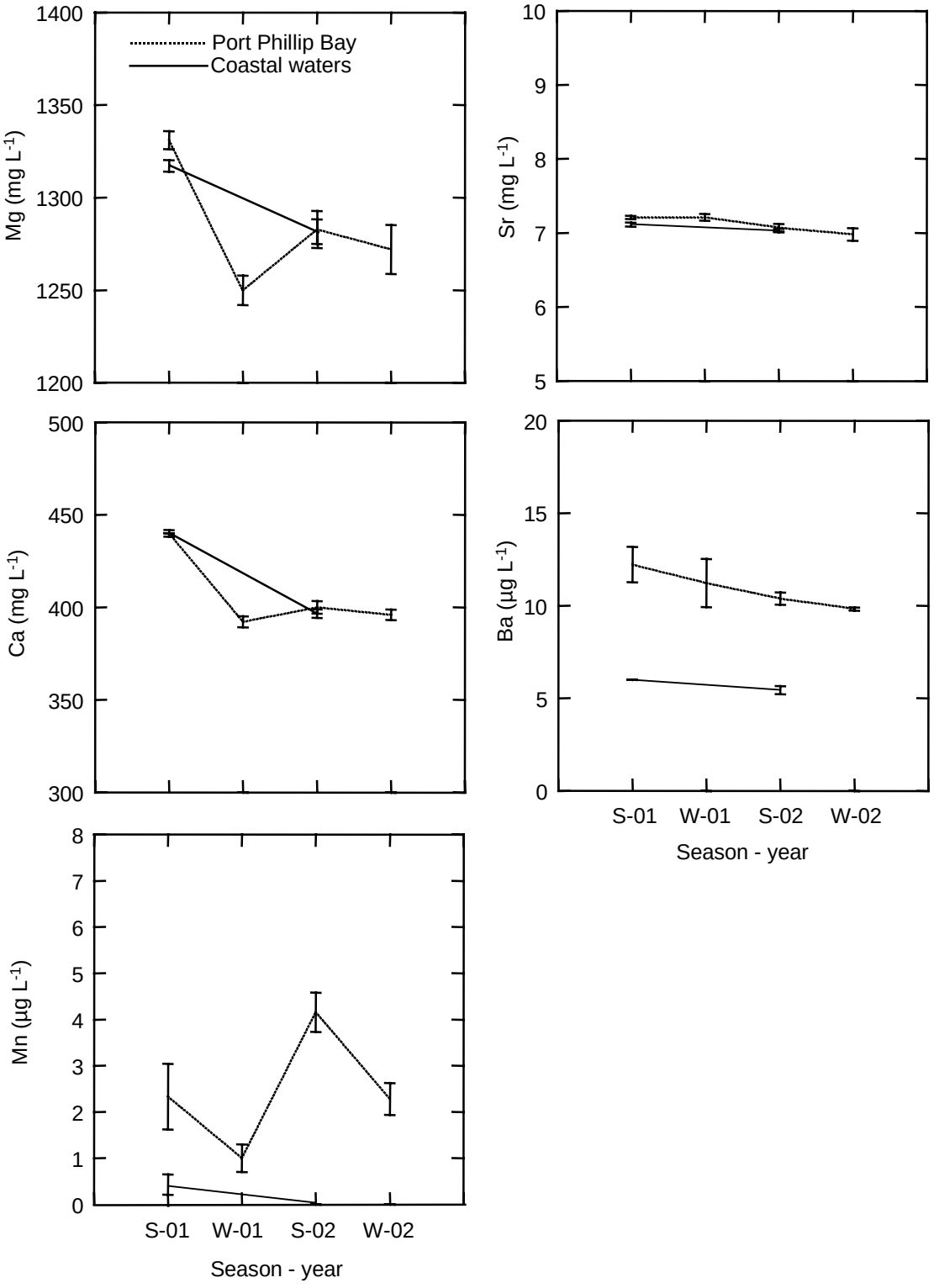

Figure 27. Temporal comparisons of mean (± 1SE) ambient water chemistry between Port Phillip Bay and Victorian coastal waters from 2001 to 2002; S-01 – summer 2001, W-01 – winter 2001, S-02 – summer 2002, W-02 – winter 2002.

Overall, the water chemistry results demonstrated close similarity of Sr levels and only minor differences in Ca and Mg levels among Victoria's major bay/estuaries and coastal waters (Table 8). Minimum Mn levels overlapped among all areas, although on average Mn was higher in the bays than in coastal waters (Table 8, Fig. 26). Ba levels showed the least overlap among areas, and average levels found in Port Phillip Bay were higher than all other areas although the minimum for Port Phillip Bay overlapped with the maximum for Western Port (Table 8, Fig. 26). Variation in Ba levels among samples was higher for Port Phillip Bay, than the other estuaries and coastal waters (Table 8).

5.3.2 Relationship between otolith chemistry and water chemistry

Mean Ba levels and Ba/Ca ratios at the margins of juvenile otoliths were highly positively correlated with mean levels in water samples from the sites of capture (Fig. 28 ab). This was the only significant relationship we detected between otolith and ambient water chemistry and therefore we have not included regressions or scatter-plots for the other elements. Similar to the water chemistry differences (Fig. 26), levels of Ba near the margins of adult otoliths were significantly higher in samples collected from Port Phillip Bay than those collected in coastal waters (t-test, $p < 0.05$) (Fig. 29). Although there was no relationship between ambient Mn and otolith Mn for the small juveniles, Mn levels were significantly higher near the margins of adult otoliths collected in Port Phillip Bay than coastal waters (t-test, $p < 0.05$) (Fig. 29), which reflected water chemistry differences (Fig. 26). The relationship between the mean marginal otolith Ba levels of adults and mean ambient Ba measured in Port Phillip Bay and west Victorian ocean waters was consistent with the positive relationship found for the 0-age juveniles (Fig. 28 ab). The mean partition coefficients for Ba were also similar between the juvenile and adult otoliths suggesting similar levels of Ba discrimination across life stages:

D_{Ba} juveniles = 0.43 ± 0.18 (mean ± 95% CI), range 0.06–1.04

D_{Ba} adults = 0.46 ± 0.10 (mean ± 95% CI), range 0.12–1.02

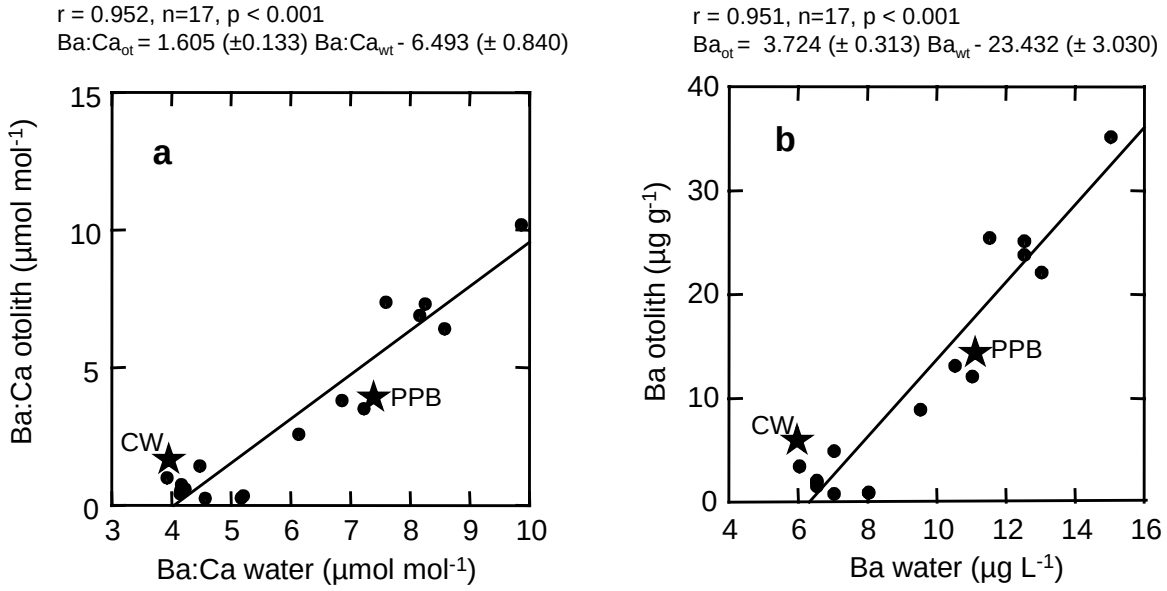

Figure 28. Relationship between mean ambient Ba and mean otolith Ba for snapper collected in Victorian waters. Circles indicate data for small juveniles (0-age) collected at 17 sites. Stars indicate data from adults (3–6 years age) collected in coastal waters (CW) and Port Phillip Bay (PPB). Ba data are presented as a) ratios to calcium, and b) absolute concentrations. The linear regression equations at the top of each graph are based on the juvenile data, and values in parentheses are standard errors.

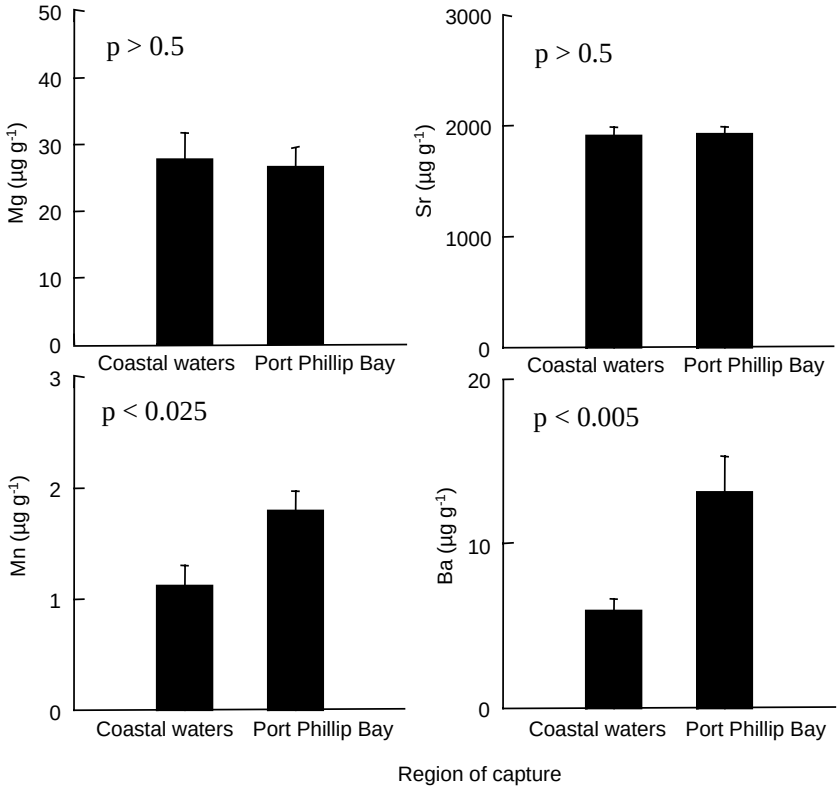

Figure 29. Comparisons of mean (± 1SE) element levels in the margins of adult (3–6 years age) snapper otoliths from Port Phillip Bay and adjacent ocean waters. Probability values of t-tests are indicated at top left of each graph.

5.3.3 The influence of seasonal temperature/growth cycles on chronological Ba variation

Otoliths from adult snapper maintained in tanks showed similar patterns of chronological Ba variation, irrespective of sex, size at death, or rearing tank (Fig. 30). The pattern of variation was characterised by a small peak in Ba just prior to capture followed by a sharp decline on introduction to the tanks and relatively low levels (< 5 µg g^{-1}) until they were sacrificed (Fig. 30). There was some low-level annual variation with amplitudes less than 5 µg g^{-1} (Fig. 30). The tank fish were all captured from the same area in western Port Phillip Bay (Fig. 24) which appeared to be recorded by a common peak in Ba just prior to introduction into tanks (Fig. 30). The consistent and low Ba levels during the period the fish were in the tanks were in contrast to the strong variation prior to introduction (Fig. 30) and the variation seen in wild snapper otoliths over the same period (Figs. 31). Wild snapper otoliths showed a variety of patterns from the third to the sixth increments, with variation in Ba levels from ~30 to less than 10 µg g^{-1} (Fig. 30). Increment widths from the third to the sixth increments were not significantly different (t-test, $p > 0.05$) between tank reared and wild fish suggesting the otoliths grew at similar rates (Figs. 30, 31). Although we did not measure ambient Ba in the tank water, the consistent low levels (< 5 µg g^{-1}) across otoliths would indicate that Ba in the tank water remained low (i.e. oceanic levels, Fig. 28 b, Table 8) over the three years.

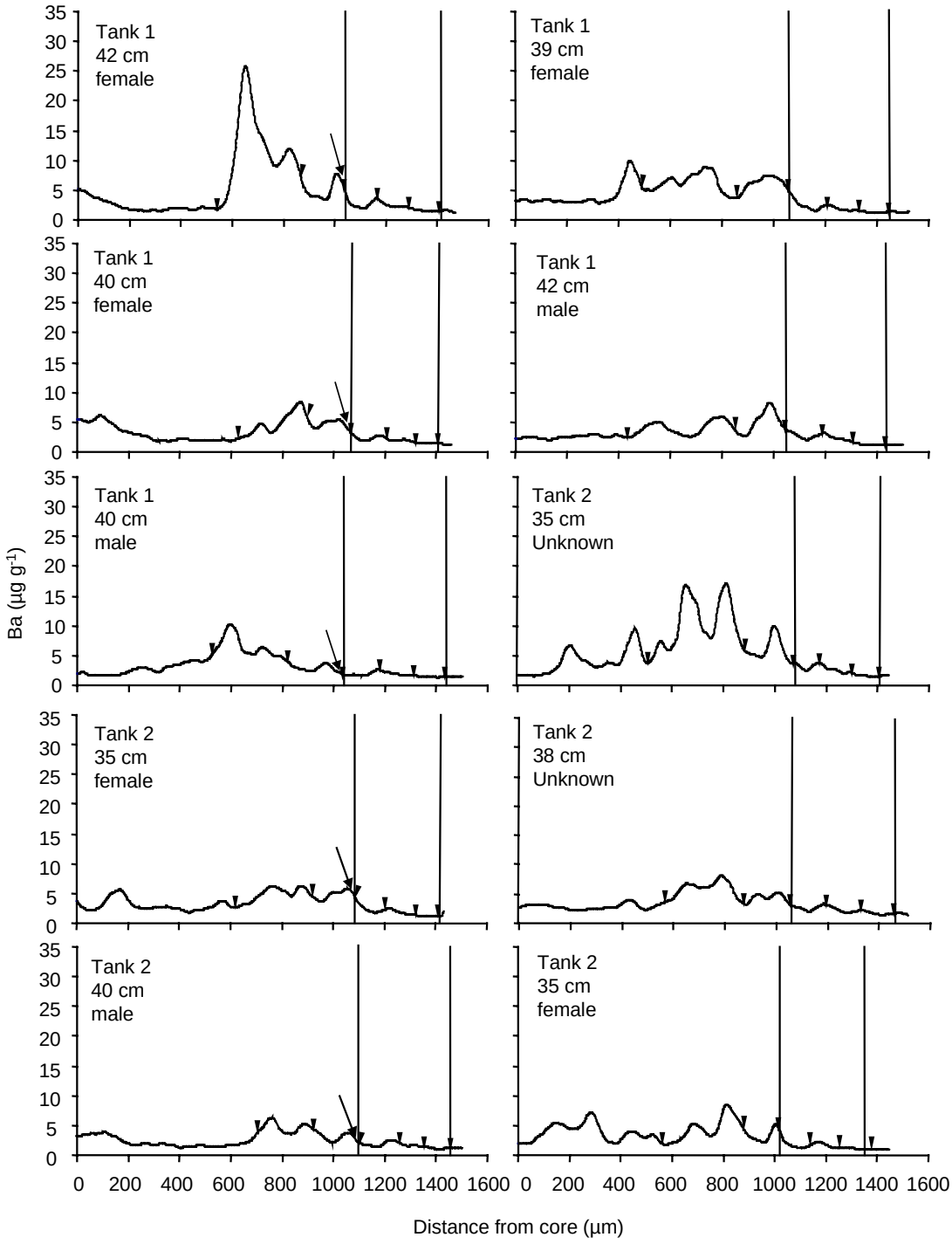

Figure 30. Comparisons of chronological Ba variation among otoliths of snapper maintained in tanks. Vertical lines demarcate the region of otolith representing the period when fish were maintained in the tanks (third to sixth increments from the left). Vertical triangles indicate annual opaque increment zones and large arrows indicate oxytetracycline (OTC) bands deposited on introduction to the tanks. Fork length, sex and rearing tank are indicated at top left of each graph.

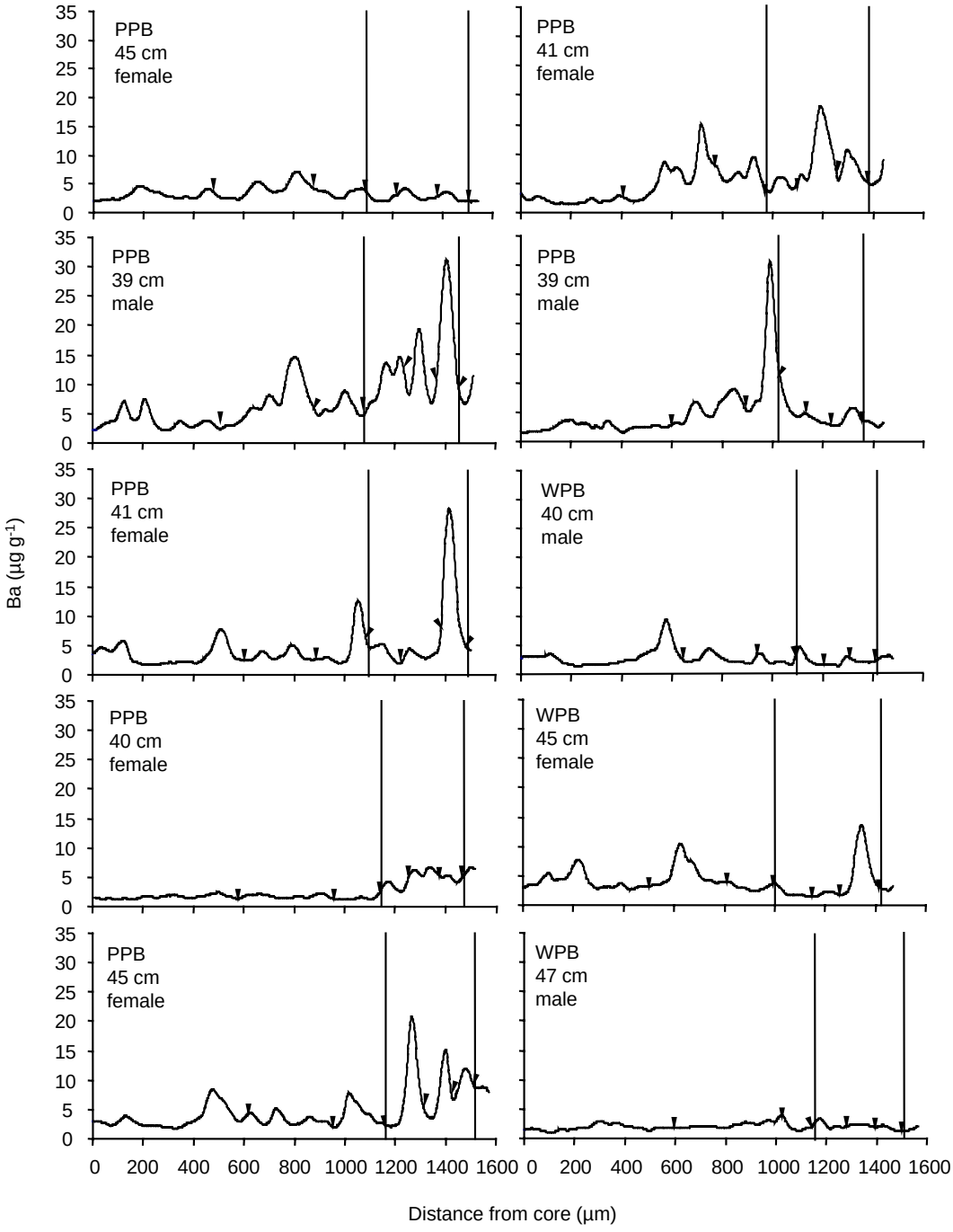

Figure 31. Chronological Ba variation in otoliths of wild snapper for comparison with tank maintained snapper (Fig. 7). Vertical lines demarcate the region of otolith equivalent to the maintenance period of the tank fish. Vertical triangles indicate annual opaque increment zones. Fork length, capture location (PPB - Port Phillip Bay, WP - Western Port) and sex are indicated at top left of each graph.

5.3.4 Chronological Ba variation in otoliths of resident oceanic snapper

Otoliths from east Victorian oceanic snapper showed low variation in Ba levels (Fig. 32). In all except one otolith, Ba levels remained less than 10 μg g^{-1} throughout life (Fig. 32). Ba levels above 10 μg g^{-1} occurred in the core region of one otolith when the fish may have been in an estuarine nursery area (Fig. 32). Some otoliths displayed periods of annual cycles with amplitudes below 10 μg g^{-1}, however, the level of Ba variation decreased towards the margins of otoliths from older fish (Fig. 32). This may have been due to the poor resolution of the laser spot size relative to the annual otolith growth increments at older ages. The Ba levels in otoliths of fish tagged and recaptured in ocean waters off eastern Victoria/New South Wales all remained below 10 μg g^{-1} over their time at liberty (Figs. 33 a). Similarly, Ba levels in otoliths from fish tagged and recaptured in ocean waters off western Victoria were below 10 μg g^{-1} over their time at liberty (Fig. 33 b).

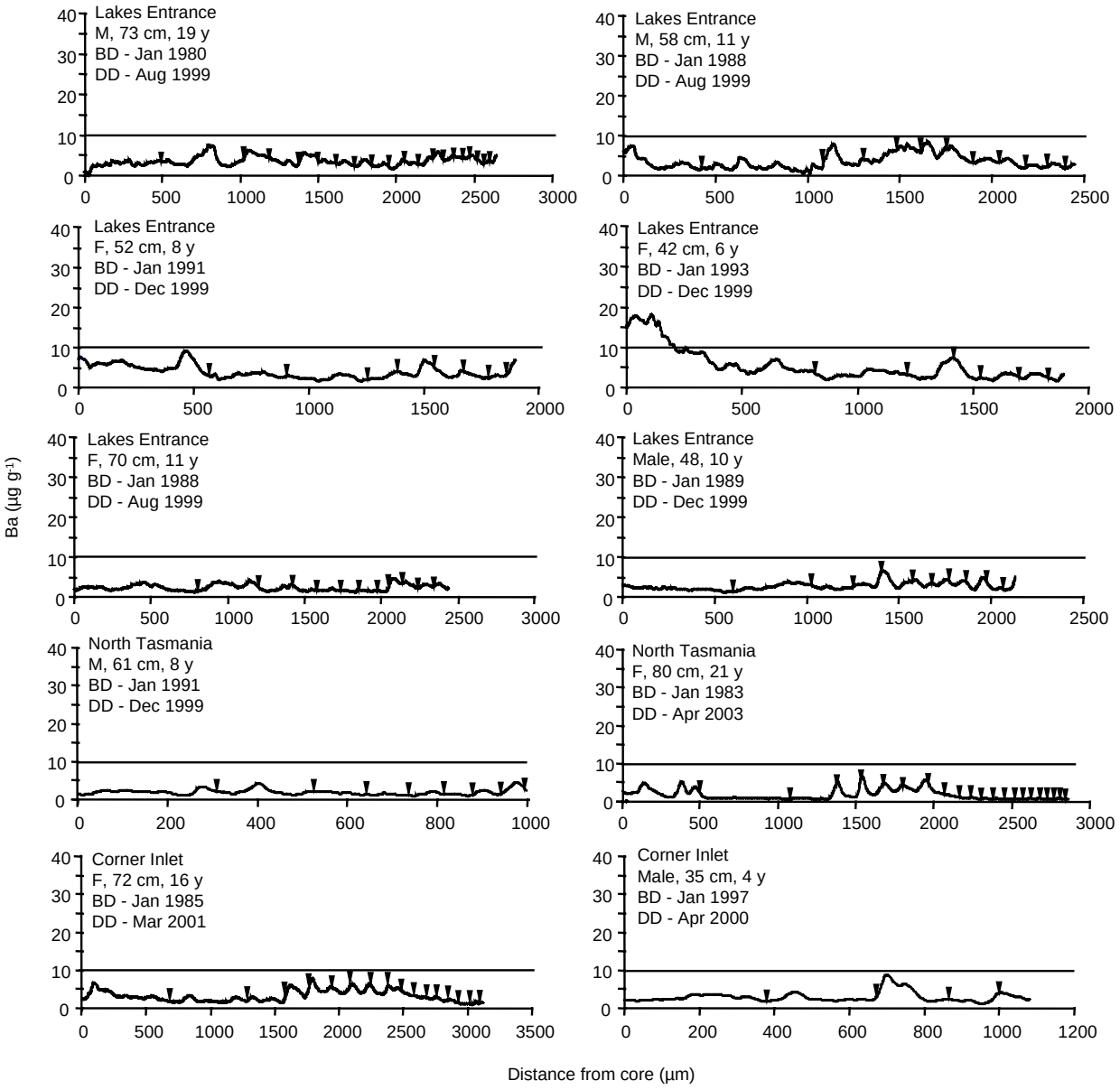

Figure 32. Chronological Ba variation in otoliths of oceanic snapper from east Victorian waters. Capture locations (see Fig. 1), sex (M-male, F-female), fork length (cm), age (y), birth date (BD) and capture date (DD) are indicated for each sample. Vertical triangles indicate annual opaque increment zones. The 10 μg g^{-1} line is indicated to highlight the maximum Ba level.

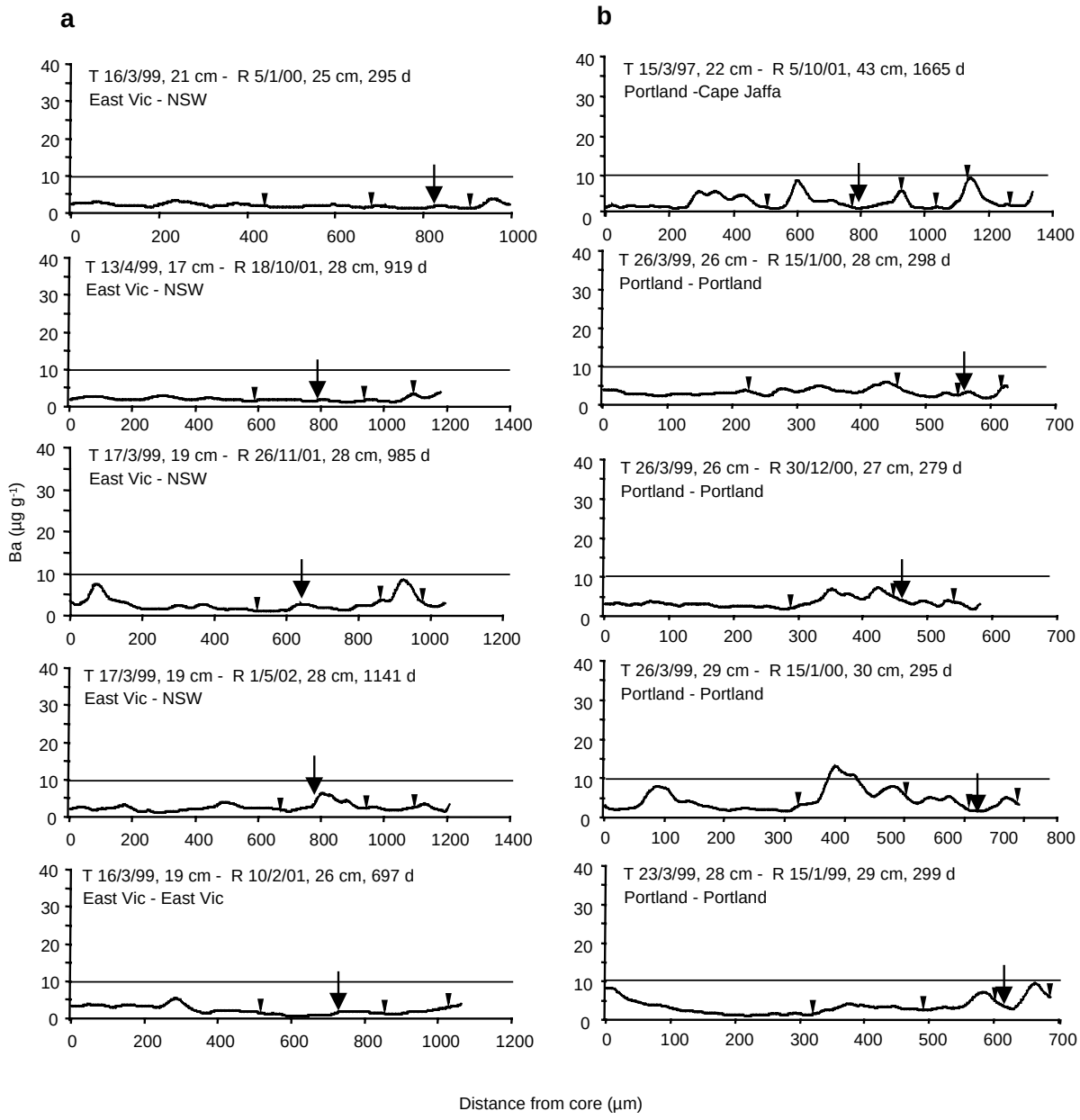

Figure 33. Chronological Ba variation in otoliths of snapper tagged and recaptured in ocean waters, a) eastern Victoria, b) western Victoria. All eastern Victorian fish were tagged at Mallacoota, and western Victorian fish, Portland (Fig. 1). Large arrows indicate the approximate point at tagging and vertical triangles indicate annual opaque increment zones. Dates of tagging (T) and recapture (R), fork length (cm) at tag and recapture, time at liberty in days (d) and region of movement are indicated at top left for each sample.

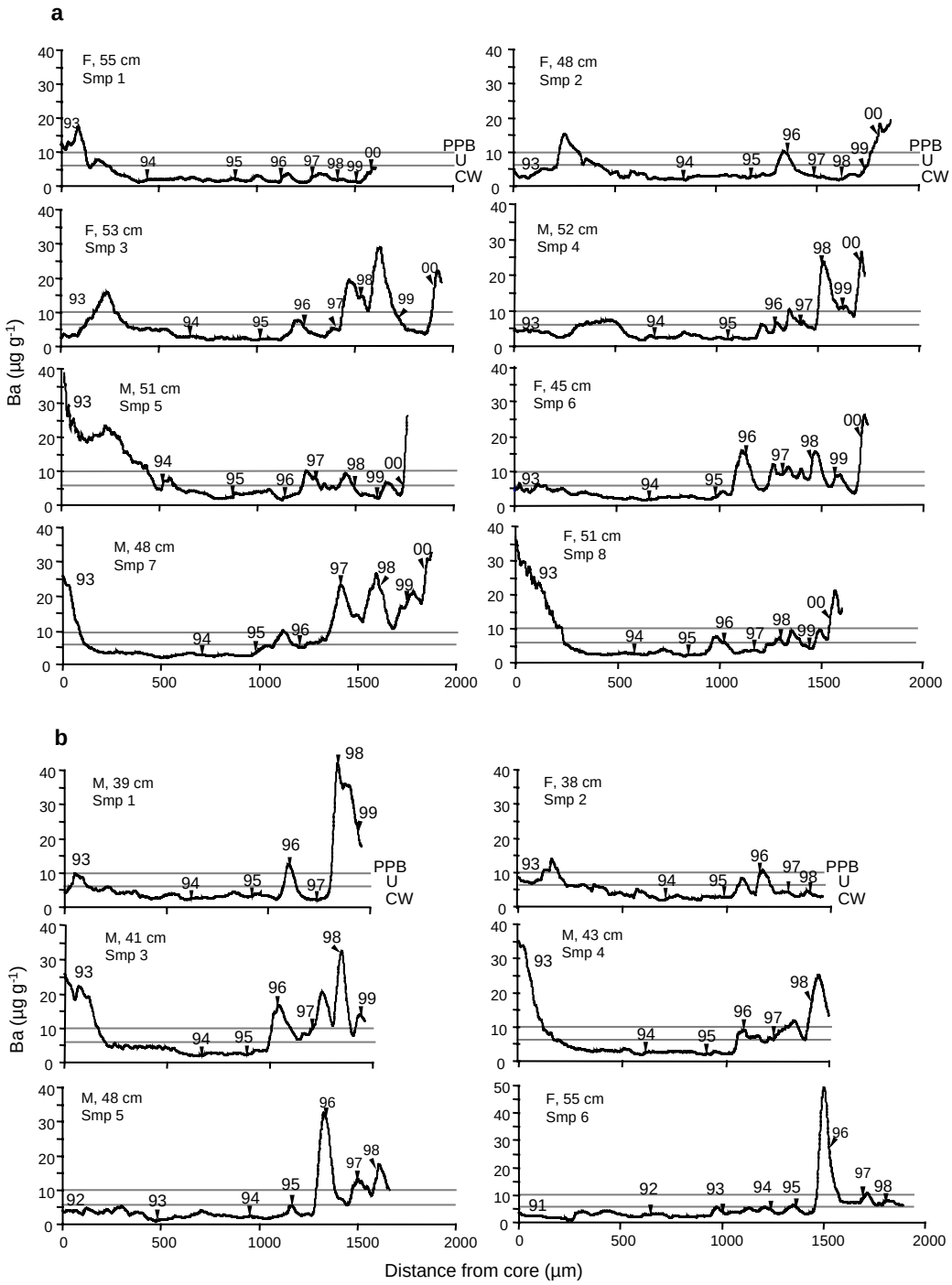

Figure 34. Chronological Ba variation in otoliths of western Victorian snapper suspected to have moved between Port Phillip Bay and coastal waters. a) 1993 year-class sampled in Port Phillip Bay, b) 1993 (smp 1–4), 1991 (smp 5) and 1992 (smp 6) year-classes sampled at Apollo Bay. Sex (M, F), and fork length (cm) are indicated at top left for each sample, vertical triangles indicate yearly opaque increment zones, numbers associated with increment zones are the year of increment formation based on a birth and increment formation date of January 1. Grey horizontal lines delineate Ba levels used to interpret whether fish were either in Port Phillip Bay (above line PPB), uninterpretable (U) location (between lines PPB and CW), or coastal waters (below line CW).

5.3.5 Interpreting chronological Ba variation in otoliths – pilot study of suspected migratory snapper

Based on the ranges of ambient Ba in water samples (Table 8) and the regression of ambient versus otolith Ba (Fig. 28 b), we estimated that Ba levels in otoliths could range from 6.4 to 54 μg g^{-1} in Port Phillip Bay, with an average level of 17.5 μg g^{-1}. For coastal waters and the other Victorian bays, the water chemistry data (Table 8) indicated a range of possible otolith Ba levels from ~1 to 6.4 μg g^{-1}. These estimates of maximum otolith Ba incorporation for waters outside Port Phillip Bay were slightly lower than those actually observed in otoliths of ocean residents, that ranged from ~1 to 9 μg g^{-1}, with an average of 3.2 μg g^{-1} (based on data in Fig. 31). Based on these estimates we made three assumptions:

1) Otolith Ba levels between 6 and 10 μg g^{-1} could indicate residence periods either in Port Phillip Bay or coastal waters (i.e. location uninterpretable)

2) Otolith Ba levels below 6 μg g^{-1} indicate residence periods in coastal waters

3) Otolith Ba exceeding 10 μg g^{-1} indicate residence periods in Port Phillip Bay

Consistent with these assumptions, Ba levels at the margins of otoliths from adults captured in Port Phillip Bay generally exceeded 10 μg g^{-1}, and for most, exceeded 15 μg g^{-1} (Fig. 34a, smp 2–8). This was also the case for several of the Apollo Bay captured fish, however, Ba levels appeared to be declining at the margin and most were below 15 μg g^{-1} at capture (Fig. 34b, smp 1, 3–5). These fish may have recently moved out of Port Phillip Bay. Following the three assumptions we would interpret that all fish, irrespective of capture location, had moved between Port Phillip Bay and ocean waters at some point in their lives, but that the periods and times of residency varied among fish (Fig. 33a,b). The largest peaks in Ba often occurred in association with the opaque annual increment zones that are formed in late spring/early summer (Fig. 34a, smp 4, 6, 7, Fig. 34b, smp 1, 3, 5).

Chronological Ba variation was, however, complex. Some otoliths displayed significant annual variations above 10 μg g^{-1} (i.e. Fig 34a, smp 3, 7), some displayed intense Ba peaks interspersed with regions of low Ba (Fig. 34b, smp 1, 6), and some displayed consistently low levels (Fig. 34a, smp 1, Fig. 34b, smp 2). Irrespective of capture location, most otoliths from the 1993 year-class displayed elevated Ba in the core region (first 6 months of life). There was also a trend for Ba levels to remain low from age one to age three after which Ba incorporation became highly variable (5 μg g^{-1} to greater than 30 μg g^{-1}) (Fig. 34a, b).

5.4 Discussion

5.4.1 Incorporation of Ba into snapper otoliths

Incorporation of Ba into snapper otoliths was positively correlated with ambient levels. Our results for snapper are consistent with previous studies of other species that have indicated ambient levels are a major influence on Ba incorporation into otoliths (Bath et al. 2000; Milton and Chenery 2001; Wells et al. 2003; Elsdon and Gillanders 2003, 2004). The Ba partition coefficients for snapper (0.43–0.46) were within the range reported at similar ambient levels for another temperate sparid, black bream, *Acanthopagrus butcheri* (~ 0.2–0.8) (Elsdon and Gillanders 2005b). Previous studies investigating the effects of water chemistry on otolith chemistry have generally involved laboratory trials on small juveniles (usually 0-age) (Bath et al. 2000; Elsdon and Gillanders 2002, 2004). It is unclear whether the results for small juveniles can be extrapolated to adults, however, this is essential if otolith chemistry is to be used as a proxy for environmental variation across the life-history. The mean partition coefficients for Ba incorporation in wild snapper otoliths were similar between juvenile and adult life-stages. Therefore, although we recommend more controlled investigations, our field comparisons suggest that the relationship between ambient Ba and incorporation into otoliths is similar across life-stages for snapper.

Low Ba variation across otoliths of tank maintained snapper suggested that seasonal cycles in temperature and/or growth do not produce strong cycles in otolith Ba. Further, fish exposed to the same environmental conditions in tanks showed similar low variation and levels of otolith Ba suggesting that incorporation rates were not dependent on the individual. The low variation that did occur in otoliths from tank fish could have been related to small effects of temperature, growth or ambient water chemistry variation. Although we cannot isolate these affects, the lack of strong variation in otolith Ba levels in the face of temperature variations that would have been at least 10°C provides sound evidence that temperature did not have a strong influence on chronological variation in otolith Ba. This is

consistent with previous studies of otolith Ba that have indicated weak or non-significant effects of temperature on the incorporation of Ba into otoliths (Bath et al. 2000; Elsdon and Gillanders 2004; Martin and Thorrold 2005). Similarly, the lack of variation in otolith Ba for the tank snapper, in the face of apparently normal otolith growth cycles, is consistent with previous studies that have suggested negligible effects of otolith growth on Ba incorporation (Bath et al. 2000; Martin and Thorrold 2005). The results for snapper are consistent with previous studies on other species (Bath et al. 2000; Milton and Chenery 2001; Wells et al. 2003; Elsdon and Gillanders 2004), and further validate the use of otolith Ba as a proxy for ambient levels throughout the life-history.

5.4.2 Consistency of ambient Ba levels in bay and ocean waters: a key for reconstructing migration histories from otolith chemistry

Understanding the temporal stability of geographic variation in ambient Ba is a key requirement for reconstructing migration histories from chronologies of otolith Ba. Recent work has suggested that consistent differences in ambient Ba levels between freshwater and marine environments can potentially be exploited to reconstruct migration/salinity histories from otolith chemistry (Elsdon and Gillanders 2005a). Similarly, although ambient Ba in estuaries is likely to vary over a range of temporal scales due to mixing of fresh and salt-waters (Elsdon and Gillanders 2006), ambient Ba in estuaries may remain on average higher than ocean waters. Differences in ambient Ba levels between more stable environments such as sheltered marine bays, saline estuaries, and ocean waters are less well understood, but are also likely to occur.

Potential sources of Ba to sheltered bays such as Port Phillip include; terrestrial runoff, groundwater, pollution/waste-water and remobilisation from sediments (Bruland 1983; Falkner et al. 1991; Carroll et al. 1993; McManus et al. 1994; Coffey et al. 1997; Shaw et al. 1998; Davidson et al. 2005). Removal of dissolved Ba could result from processes including flux with ocean waters, biological removal and precipitation (Dehairs et al. 1980; Fisher et al. 1991; Coffey et al. 1997; Hunter and Boyd 1999). Ambient Ba levels in Port Phillip Bay were on average approximately double inshore coastal waters over the two years we sampled. Elevated Ba levels in Port Phillip Bay could be related to significant inputs from the Yarra River and Western Treatment Plant (Fig. 24) (Longmore et al. 1999). Further, the basalt plains that form a large portion of the western catchment of Port Phillip Bay are enriched in Ba (Price et al. 1991). Although there are several potential sources of Ba to Port Phillip Bay, cycling or removal processes for Ba are unknown, and the long-term temporal stability of ambient Ba levels in Port Phillip Bay is unclear. Our earlier work on juvenile snapper otolith chemistry provides some indication that Ba levels in Port Phillip Bay may vary over longer time scales than this study (i.e. > 5 y) (section 3.3.2).

Ambient Ba levels in Victorian coastal waters would be expected to be more consistent over time due to the lack of significant rivers and generally low terrestrial runoff along this region of the coast (Holmes 1982). Localised upwelling events are, however, known to occur in far-western Victoria (Schahinger 1987; Neira et al. 2000). Upwelling in this region is driven by alongshore south-easterly winds that occur predominantly during summer (Schahinger 1987). As indicated from studies of coral aragonite, upwelling could potentially result in episodic Ba enrichment of surface waters that could influence variation in otolith Ba levels (i.e. Lea et al. 1989). Ba levels in surface waters of the Southern Ocean north and south of the polar front have, however, been reported to range from 4.2–5.5 $\mu g\ L^{-1}$ (Jeandel et al. 1996). This is similar to the values for inshore Victorian coastal waters found in the current study (i.e. 5–6 $\mu g\ L^{-1}$). We found negligible variation (< 2 $\mu g\ L^{-1}$) in ambient Ba levels among coastal water samples separated over hundreds of kilometres and across years. Although further spatio-temporal sampling is required, these data coupled with the consistent low Ba (< 10 $\mu g\ g^{-1}$) in otoliths from ocean resident snapper, provide some indication of temporal and spatial consistency of low ambient Ba levels in Victorian coastal waters.

Although ambient Ba in Port Phillip Bay may on average remain consistently higher than coastal waters, differences could vary over time, and there is poor understanding of spatio-temporal variability of ambient Ba within the bay. To fully understand the implications of variable water chemistry for reconstructions of migration history from otolith chemistry we will require more information on temporal and spatial variation of ambient Ba in Port Phillip Bay and coastal waters.

5.4.3 High otolith Ba as an indicator of residency in Port Phillip Bay

We estimated that Ba levels exceeding 10 μg g^{-1} in otoliths of western Victorian snapper indicated residence periods in Port Phillip Bay. In a concurrent study of snapper otolith chemistry in South Australia (Fig. 24), involving similar methods to the current study, of the 110 otoliths examined, only 12 displayed Ba levels above 10 μg g^{-1} at some point in life, and only one above 20 μg g^{-1} (Fowler et al. 2004). Importantly, the otoliths analysed in the study by Fowler et al. (2004) were from fish of a similar age and year-class to the fish in this study, therefore allowing comparisons to be made over the same time period. Further, they were collected from a range of locations including sheltered gulfs and locations in oceanic areas, including the upwelling zone previously mentioned (Schahinger 1987; Fowler et al. 2004). The generally lower Ba levels across snapper otoliths from South Australian waters would further support the suggestion that high Ba levels in western Victorian snapper (up to 50 μg g^{-1}) are a result of exposure to the Ba enriched waters of Port Phillip Bay.

Migration of snapper between Port Phillip Bay and ocean waters from late spring to autumn is well known (Coutin et al. 2003). Ba peaks in the region of otolith encompassing the annual opaque increments, deposited in late spring/early summer (Fowler et al. 2004), were therefore consistent with this known migration behaviour. Although the major Ba peaks appeared to occur on an annual basis they did vary in magnitude among years and were not present in all years. There was considerable variability among fish in the patterns of Ba variation, although some annual peaks were consistent across multiple fish (i.e. 1998, Fig. 34 ab). High Ba levels in the core region of otoliths was consistent with origins in Port Phillip Bay, suggested to be the major snapper nursery area in western Victoria (see sections 3 and 4). Further, in section four of this report there was evidence that juvenile snapper can migrate from Port Phillip Bay to coastal waters within the first year of life, which is consistent with the drop in otolith Ba during the first year of life observed in most otoliths examined from the 1993 cohort. Variable Ba levels from age three onwards could be related to the onset of maturity, when snapper are suggested to show more extensive migrations between Bay and coastal waters (Coutin et al. 2003).

Although otolith Ba peaks greater than 10 μg g^{-1} appear likely to indicate residence in Port Phillip Bay, low level variations above and below this threshold are likely to be difficult to interpret in terms of migration. Furthermore, regions of low otolith Ba could possibly reflect residence in Port Phillip Bay during periods of low ambient Ba rather than residence periods in coastal waters. For this reason, to fully exploit otolith Ba chronologies as a tool for studying migration in and out of Port Phillip Bay a long-term program of water sampling would be required so that otolith chemistry can be interpreted in the light of variable water chemistry.

5.4.4 Conclusions

The results for snapper support the use of otolith Ba as a proxy for ambient Ba levels across different life-stages. Knowledge of spatial and temporal variation in ambient Ba can therefore be used to interpret migration history from Ba chronologies in otoliths. Uncertainty over the longer-term consistency of Ba levels in Port Phillip Bay relative to coastal waters limited our ability to reconstruct individual chronologies of movement between Port Phillip Bay and coastal waters, and therefore to examine whether changes in migration patterns had occurred over recent time. Monitoring of Ba levels at a variety of spatial and temporal scales in Port Phillip Bay, Western Port and coastal waters is recommended to fully explore the potential to accurately reconstruct spatial migration histories of western stock snapper between Port Phillip Bay and coastal waters from otolith Ba chronologies. However, because strong chronological variation in otolith Ba levels appear largely influenced by exposure to variation in ambient levels, comparisons of Ba chronologies among fish of the same cohorts and ages could potentially be used to infer different environmental histories (i.e. Ba exposure history). Differences in Ba exposure histories among chronologically matched otoliths may be useful for exploring questions related to migratory contingents within the western Victorian snapper stock (i.e. proportions of ocean residents versus bay/ocean migrants).

6. Benefits and Adoption

The Port Phillip Bay snapper fishery is one of the most important inshore commercial fisheries, and arguably the most important recreational fishery in Victoria. This project has made a significant contribution to the understanding of population structure and dynamics of snapper in Victorian waters. In particular, the relationship between the Port Phillip Bay, coastal and Western Port fishery regions, has been clarified. Demonstration that spawning aggregations and juvenile recruitment in Port Phillip Bay are the major source of replenishment for the entire western Victorian snapper stock will increase the emphasis on conservative management of adult spawning aggregations, and juvenile/sub-adult snapper within Port Phillip Bay. Furthermore, the results clearly demonstrate the importance of the habitats and environmental requirements for successful spawning and juvenile recruitment in Port Phillip Bay. This new information will benefit both commercial and recreational fishers by leading to more informed and conservative management decisions regarding environmental protection and exploitation of snapper in Port Phillip Bay. This awareness is spreading to the fishing communities who are becoming increasingly proactive towards more conservative management of this fishery.

This project has provided the framework for using 0-age recruitment monitoring in Port Phillip Bay to model and predict changes in the west Victorian snapper fishery. Fisheries Victoria has continued to fund monitoring of 0-age recruitment in Port Phillip Bay following the methods developed in this project. This monitoring is providing valuable data for explaining and predicting significant variations in the western Victorian and Port Phillip Bay snapper fisheries, which is of considerable benefit to fisheries managers and the recreational and commercial fishing sectors. The results of this project have been utilised by managers to help explain variation in the Victorian snapper fishery to representatives of the recreational fishing community at a recent forum on the state of the snapper fishery in Victoria.

This project has highlighted the importance of understanding the processes that influencing high recruitment variation in Port Phillip Bay. Fisheries Victoria has provided a further three years of funding to initiate research into the area of larval dynamics. This recent work has indicated that water column processes that influence larval survival in Port Phillip Bay are critical in driving high recruitment variation. The recreational and commercial fishing sectors will continue to benefit from the new knowledge arising from more focussed research on causes of recruitment variation.

Further benefits from this project emanate from capacity building in the area of otolith chemistry. This project has led to skills being developed and transferred to other FRDC projects (i.e. blue-eye trevalla and warehou stock structure), and the establishment of a dedicated otolith chemistry research facility (high-resolution ICP-MS and laser ablation facility) at Marine and Freshwater Systems, Queenscliff. Fisheries Victoria is now funding a pilot study to investigate the potential of the methodology used in the current study to be applied to King George whiting. The methodology developed in this project for sampling 0-age snapper is now being employed in studies of snapper early life-history in South Australia and New Zealand, and is being considered in Queensland.

7. Further Development

Information on spatial distributions of 0-age snapper, along with the development of sampling methods during this project, have led to ongoing monitoring of 0-age recruitment in Port Phillip Bay (funded through Fisheries Victoria). The results of monitoring are incorporated into the State of Victorian Fisheries Report compiled by Fisheries Victoria. This work has also led to a new project funded by Fisheries Victoria which is providing valuable data on linkages between larval supply and 0-age recruitment, providing further insights into the processes that influence recruitment variation in Port Phillip Bay.

Our investigation of chronological Ba variation in otoliths has provided a basis for reconstructing migration histories from otoliths and investigating the occurrence of different migratory contingents within the western stock. We are further exploring the potential for otolith chemistry transect studies to

provide information on migration behaviour. This has involved a program of long-term monitoring of Ba levels in Port Phillip Bay and coastal waters, which began in May 2006. It is possible, that even without a time series of ambient Ba levels, future studies can investigate the occurrence and/or predominance of migratory contingents (i.e. residents versus migrants) in the western stock by comparing Ba chronologies among fish of the same cohorts and ages.

The finding that most of the snapper recruiting into the Port Phillip Bay fishery originated from within the Bay suggests that natal homing of reproductive adults could be occurring in Victorian snapper. Confirmation of this behaviour for spawning adults could be achieved by using the otolith chemical tags from the current project and sampling larger adults of the 2000 and 2001 cohorts during the spring/summer spawning season in Port Phillip Bay, Western Port and coastal waters. Furthermore, this type of analysis could provide important information on whether significant numbers of juvenile snapper that migrate out of Port Phillip Bay, in fact do not return as large adults to spawn, and are therefore lost from the Bay fishery.

The 0-age otolith samples collected during this project will provide the basis for post-graduate student projects. An honours project has recently begun that is utilising otolith samples collected during this project to investigate how spawning times and early life-history growth dynamics relate to recruitment variation in Port Phillip Bay.

The results of this work will play an important part in reviewing the status of the Victorian snapper fishery and refinement of population models during the next 'Victorian Snapper Workshop'.

8. Planned Outcomes

The planned outcomes for this project were based around improving understanding of population dynamics and structure of snapper in Victoria. The first planned outcome was to understand whether high annual variability in the abundance of pre-recruit (0-age) snapper in Port Phillip Bay is reflected by the entire stock, and therefore to focus future research and monitoring at the appropriate scale. The second, fourth and fifth planned outcomes were related to understanding the importance of spawning/0-age recruitment in Port Phillip Bay to fishery recruitment both within and outside the Bay. The third planned outcome was to improve knowledge of the movement patterns of snapper between Port Phillip Bay and coastal waters, and assess whether these may have changed over time.

This project has indicated that high annual variability in recruitment of 0-age snapper in Port Phillip Bay is not reflected at other Victorian nursery areas. Furthermore, comparisons with similar monitoring in South Australia (Spencer Gulf) suggest that, although 0-age recruitment variation is also high in South Australia (Dr A. Fowler, SARDI, pers. comm.), the patterns of variation in South Australia are not the same as in Port Phillip Bay. This means that recruitment processes need to be studied independently for different bay/estuary nursery areas and snapper stocks in southern Australia.

The importance of 0-age recruitment in Port Phillip Bay to replenishment of the western Victorian snapper stock was clearly demonstrated by using otolith chemical tags. Importantly, the high contribution of Port Phillip Bay to replenishment of western Victorian snapper populations was demonstrated for two cohorts of very different abundance. This indicated that the importance of spawning/0-age recruitment in Port Phillip Bay to replenishment of the western Victorian snapper stock is likely to be consistent over time. The results from this component of the study also yielded new information on movement of young snapper, by indicating that movement of young sub-adult snapper (from 1–3 years age) from Port Phillip Bay to coastal waters (including eastern Victoria) is higher than previously thought. The conclusion that replenishment of the western Victorian snapper stock is largely dependent on spawning/juvenile recruitment within Port Phillip Bay will allow pre-recruit monitoring in Port Phillip Bay to be used in predictive modelling of population dynamics for the entire western Victorian snapper stock.

Detailed investigation of water chemistry and relationships between water chemistry and otolith chemistry indicated the potential for chronological variation in otolith Ba levels to be used to investigate movement patterns between Port Phillip Bay and coastal waters. However, because of the possibility that

water chemistry may vary over time in Port Phillip Bay, we concluded that reconstructions of migration histories from otolith Ba levels would require validation against time series of Ba levels in Bay and coastal waters. Therefore, we could not confidently assess whether migration patterns had changed over time. The impetus for this component of the study was the suggestion that declining snapper catches in Port Phillip Bay during the 1980–1990s were the result of long-term changes in the number of snapper residing in Port Phillip Bay or migrating into Port Phillip Bay. The recent strong recovery of the snapper fishery in Port Phillip Bay over the last 10 years has allayed fears that long-term changes in migration behaviour were responsible for declining catches. We now suspect that the decline in the Port Phillip Bay fishery may have been related to a prolonged period of poor juvenile recruitment success. Although variable residency and migration rates will no doubt influence dynamics of the Port Phillip Bay fishery, this variability is likely to be difficult to predict, and its influence on fishery dynamics is likely to be considerably less than the influence of high variability in 0-age recruitment.

9. Conclusions

Objective 1: Determine whether high annual variability in the abundance of pre-recruit (0-age) snapper in Port Phillip Bay is reflected by the entire western stock. This objective is dealt with in section 2 of the report.

Recruitment of 0-age snapper to Victorian bays and estuaries is highly variable both in space and time. Annual recruitment variation appears dependent on the individual bay/estuary recruitment area. Patterns of annual variation were similar irrespective of sampling time within a two month period (February–March) after the spawning season. Strong recruitment variation (approximately 10 times among years) observed in Port Phillip Bay was not reflected in other Victorian estuaries. Ongoing monitoring also indicates that although annual recruitment variation in both Port Phillip Bay and Spencer Gulf (South Australia) is high, the patterns of variation are not closely matched. Therefore, we conclude that recruitment variation should be studied and monitored at the scale of individual bays and estuaries for southern Australian snapper stocks. Settlement of snapper larvae to Victorian estuaries is generally completed by March, therefore annual monitoring of 0-age recruitment in Victoria should occur in mid March. High and low recruitment years in Port Phillip Bay were obvious by February, indicating that high 0-age recruitment variation is related to processes occurring prior to February, and most likely prior to larval settlement. Future research into the causes of 0-age recruitment variation should therefore be concentrated around spawning and the first 1 to 2 months of life (i.e. November—January). Recruitment of 0-age snapper in Western Port appears very low compared to Port Phillip Bay, and we could not confirm that 0-age recruitment was occurring in coastal waters, although sampling effort was low (Appendix 3).

Objectives 2, 4, 5: 2) Determine the importance of spawning of snapper within Port Phillip Bay to the western stock as a whole. 4) To determine the proportion of the snapper population on the open coast that originates from Port Phillip Bay and how this changes with age. 5) To determine the proportion of the snapper population in Port Phillip Bay that originates from areas outside the bay and how this changes with age. These objectives are dealt with sections 3 and 4 of the report.

This project has demonstrated that the western Victorian snapper fishery (comprising ~500 km of coastline west of Wilsons Promontory to Portland) is highly dependent on 0-age recruitment in Port Phillip Bay. Our more recent work, funded by Fisheries Victoria, also confirms that 0-age recruitment in Port Phillip Bay is derived from spawning within the Bay rather than in coastal waters. Therefore, we conclude that spawning aggregations and 0-age recruitment in Port Phillip Bay are the primary source of population replenishment for the western Victorian snapper fishery. Pre-recruit (0-age) monitoring in Port Phillip Bay will therefore provide a valuable indicator of year class strength, not only for Port Phillip Bay, but for the entire western Victorian stock. Development of spatial management strategies for the fishery should focus on maintaining the habitats/areas important for spawning and 0-age recruitment in Port Phillip Bay, and the exploitation of spawning aggregations and juvenile/sub-adult fish within the Bay.

The proportion of adult snapper sampled in Port Phillip Bay that originated there was consistently high irrespective of age or cohort. There was also an accumulation of young snapper originating from Port Phillip Bay in coastal waters and Western Port over the first 1–3 years of life. By 4 years of age, when fully recruited to the fishery, the populations in these areas became dominated by snapper that originated from Port Phillip Bay. This indicates high rates of movement of juvenile snapper out of Port Phillip Bay. Although the fishery in western Victoria relies heavily on replenishment from spawning and juvenile recruitment occurring within Port Phillip Bay, the fishery in eastern Victoria is most likely replenished from recruitment sources in several eastern Victorian estuaries, where larval settlement is thought to be derived predominantly from spawning in coastal waters off eastern Victoria and New South Wales.

Objective 3: Determine the movement patterns of snapper between Port Phillip Bay and offshore waters and whether they are changing over time. This objective is dealt with in section 5 of the report.

We found that incorporation of Ba into snapper otoliths is directly related to ambient Ba concentrations, and that over a two-year period ambient Ba levels in Port Phillip Bay were consistently double those of inshore coastal waters. Furthermore, Ba variation across snapper otoliths was not significantly influenced by seasonal variation in temperature or growth. We therefore conclude that chronologies of otolith Ba have considerable potential to be used to reconstruct migration histories of individual snapper between Port Phillip Bay and coastal waters. Although there is clear potential for otolith Ba chronologies to yield information on migration patterns, unknown history of temporal variation in Ba water chemistry prevented us from investigating whether migration patterns between the Bay and coastal waters may have changed over time. To apply this methodology to the accurate reconstruction of migration histories, longer-term spatio-temporal monitoring of ambient Ba levels in Bay and coastal waters will be required. With this data interpretations of otolith chemistry can be validated against any water chemistry variation. However, in the absence of a time series of ambient Ba variation, we suggest there is potential to explore questions related to the occurrence of migratory contingents within the western stock by comparing otolith Ba chronologies among adult fish of the same cohorts and age classes.

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Appendix 1: Intellectual Property

There are no intellectual property issues associated with this project.

Appendix 2: Staff

Dr Greg Jenkins (Primary Industries Research Victoria)	Principle Investigator
Paul Hamer (Primary Industries Research Victoria)	Co-Investigator
Dr Bronwyn Gillanders (University of Adelaide)	Co-Investigator
David Hatton (Primary Industries Research Victoria)	Research Technician

Appendix 3: Beam trawl sampling in coastal waters

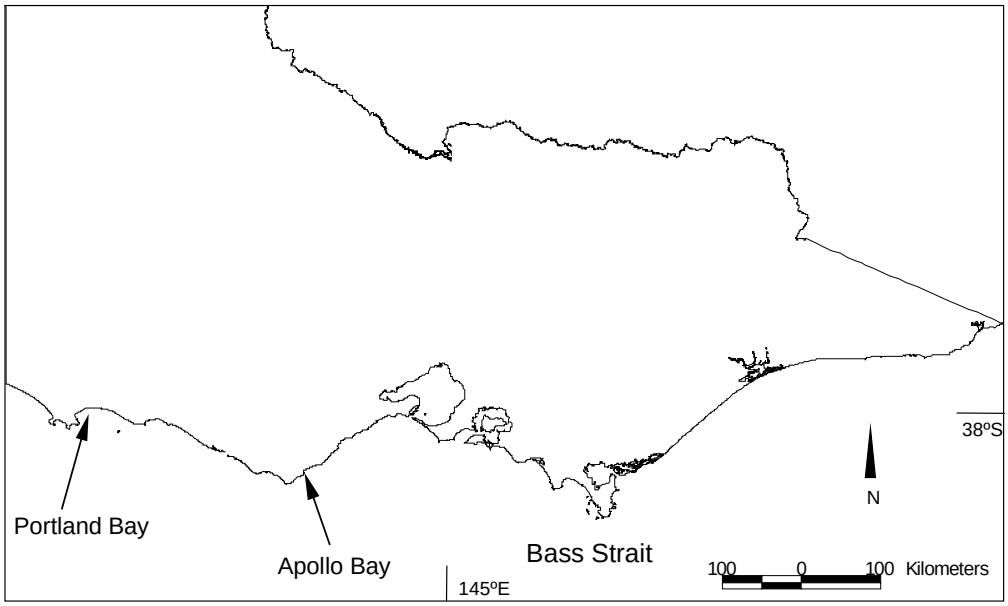

Figure 1. Map of Victorian coast showing location of Portland Bay and Apollo Bay.

Portland Bay

In March–April 2000 we made two pilot sampling trips to Portland Bay (Fig. 1) aimed at testing the small plumb-staff beam trawl for sampling 0-age snapper in coastal waters. Unfortunately, the beam trawl was not suited for sampling in this area due to the lack of continuous non-reef habitats. Further, the vessel we had access to was not entirely suitable for deploying and retrieving the beam trawl and could not maintain a slow enough towing speed (i.e. 1.5–2 knots). We attempted 20 trawl samples over 3 nights (1/3/00, 17/4/00, 18/4/00) in depths from 6 to 19 m. Of these trawls 4 were snagged on reef, and for several others due to vessel speed we were not convinced the net was fishing effectively. While a variety of other species were collected, no 0-age snapper were collected. Future attempts to sample 0-age snapper in this region should involve other methods such as fish traps.

Apollo Bay

Pilot sampling for 0-age snapper in coastal waters was also conducted at Apollo Bay (Fig. 1). In the strong recruitment year of 2001 we conducted one sampling trip to Apollo Bay (3–5/5/01). In this case we used the same vessel as for sampling in Port Phillip Bay. After a day of surveying the bottom habitats with a small dredge sampler, we determined that much of the area was suitable for the plumb-staff beam trawl. Over two nights we conducted 16 trawls from 17 to 60 m depth (Table 1). Approximately 300 teleosts and chondrichthyans were collected from 22 families and 30 species (Table 2). The most common species in samples were red cod, *Pseudophycus bachus*, school whiting, *Sillago flindersi*, and silver belly, *Parequula melbounensis* (Table 2). The number and variety of fish collected suggested that the trawl was fishing effectively, however, there were no 0-age snapper in any of the trawls. Although the plumb-staff beam trawl is suitable for sampling small demersal fish in deeper coastal areas, the spatial coverage of this small-scale sampling gear may have been insufficient to detect 0-age snapper if they were in low

densities. Due to the logistical problems (i.e. vessel size and cost) involved with sampling in ocean waters we discontinued coastal sampling after this trip. Confirming whether or not 0-age snapper recruit in open coastal waters will require more dedicated sampling from large vessels with larger trawls, and the addition of fish trap surveys for reef habitats.

Table 1. Positions (decimal minutes) and depths (metres) of trawl shots conducted off Apollo Bay. * indicates snagged shot

Depth	Latitude	Longitude
57	38° 45.973′	143°45.355′
55	38° 46.063′	143°45.869′
53	38° 43.371′	143°50.025′
34	38° 44.994′	143°44.147′
43	38° 45.608′	143°44.835′
43	38° 45.597′	143°44.838′
33	38° 44.945′	143°44.145′
25	38° 44.377′	143°43.415′
36	38° 44.015′	143°45.326′
60	38° 43.487′	143°50.032′
48	38° 43.133′	143°49.423′ *
26	38° 45.559′	143°45.769′
38	38° 44.487′	143°45.701′
17	38° 44.530′	143°42.219′
21	38° 44.732′	143°42.446′
60	38° 43.490′	143°50.199′

Table 2. List of fish species and total numbers collected during sampling with the plumb -staff beam trawl off Apollo Bay.

Family / Species	Common name	Number collected
Torpedinidae		
Narcine tasmaniensis	Tasmanian numbfish	2
Urolophidae		
Urolophus cruciatus	banded stingaree	2
Urolophus paucimaculatus	sparsely spotted stingaree	4
Ophichthidae		
Muraenichthys breviceps	longfinned worm eel	2
Gobiesocidae		
Unidentified gobiesocidae	unidentified clingfish	2
Moridae		
Pseudophycis barbata	bearded rock cod	1
Pseudophycis bachus	red cod	59
Atherinidae		
Unidentified Atherinidae	unidentified hardy head	5
Zeidae		
Cyttus australis	silver dory	3
Sygnathidae		
Unidentified sygnathidae	unidentified pipefish	3
Scorpaenidae		
Scorpaena papillosa	red rock cod	20
Triglidae		
Lepidotrigla modesta	minor gurnard	11
Pterygotrigla polyomata	latchet	3
Platycephalidae		
Neoplatycephalus richardsoni	tiger flathead	5
Platycephalus bassensis	sand flathead	9
Serranidae		
Caesioperca rasor	barber perch	2
Sillaginidae		
Sillago flindersi	school whiting	51
Gerreidae		
Parequula melbournensis	silverbelly	47
Cheilodactylidae		
Nemadactylus macropterus	jackass morwong	2
Callionymidae		
Foetorepus calauropomus	common stinkfish	7
Gobiidae		
Nesogobius hinsbyi	orange spotted gobie	18
Gempylidae		
Thyrsites atun	barracouta	4
Bothidae		
Lophonectes gallus	crested flounder	13
Pleuronectidae		
Ammotretis macrolepis	largescale flounder	8
Taratretis derwentensis	derwent flounder	3
Monacanthidae		
Acanthaluteres vittiger	toothbrush leatherjacket	2
Meuschenia scaber	velvet leatherjacket	7
Thamnaconus degeni	degens leatherjacket	4
Unidentified monacanthidae	unidentified leatherjackets	3
Tetraodontidea		
Omegophora armilla	ringed toadfish	2

Appendix 4: Chronological variation in Ba: compared between and within otoliths and different treatment methods

Chronological Ba variation was similar for different transect paths and left and right otoliths (Figs. 1cd). Treatment with hydrogen peroxide (H_2O_2) (12 - hour immersion) had negligible effect on chronological Ba variation but did reduce the clarity of annual increments (Figs. 1ef). Because we required clear annual increments we chose not to use the H_2O_2 treatment in routine preparations. To minimise transect length and maximise increment clarity we chose to conduct laser transects in the region along the ventral side of the sulcus (i.e. Figs. 1cf).

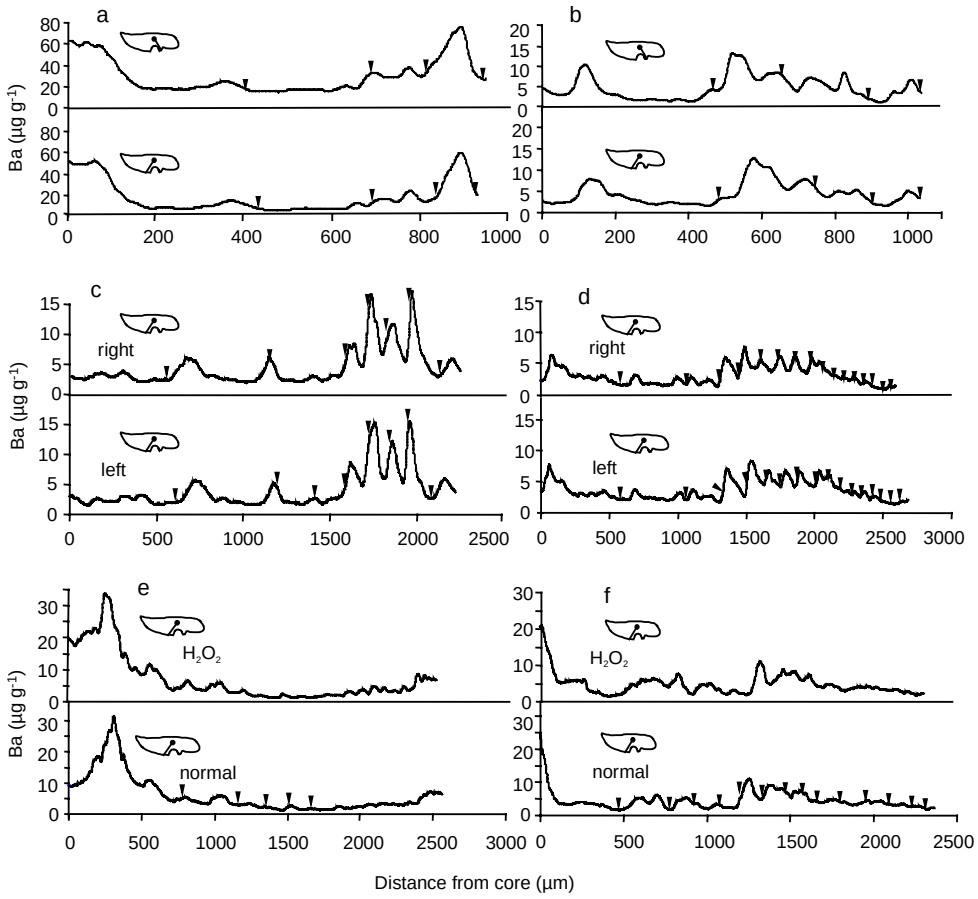

Figure 1. Comparisons of chronological Ba variation among; a, b) different laser transect axis; c, d) left and right otoliths of the same fish, and e, f) with and without H_2O_2 treatment. Transect paths are indicated on the diagrams of transverse otolith sections at the left of each chart. Vertical triangles indicate annual opaque increment zones.