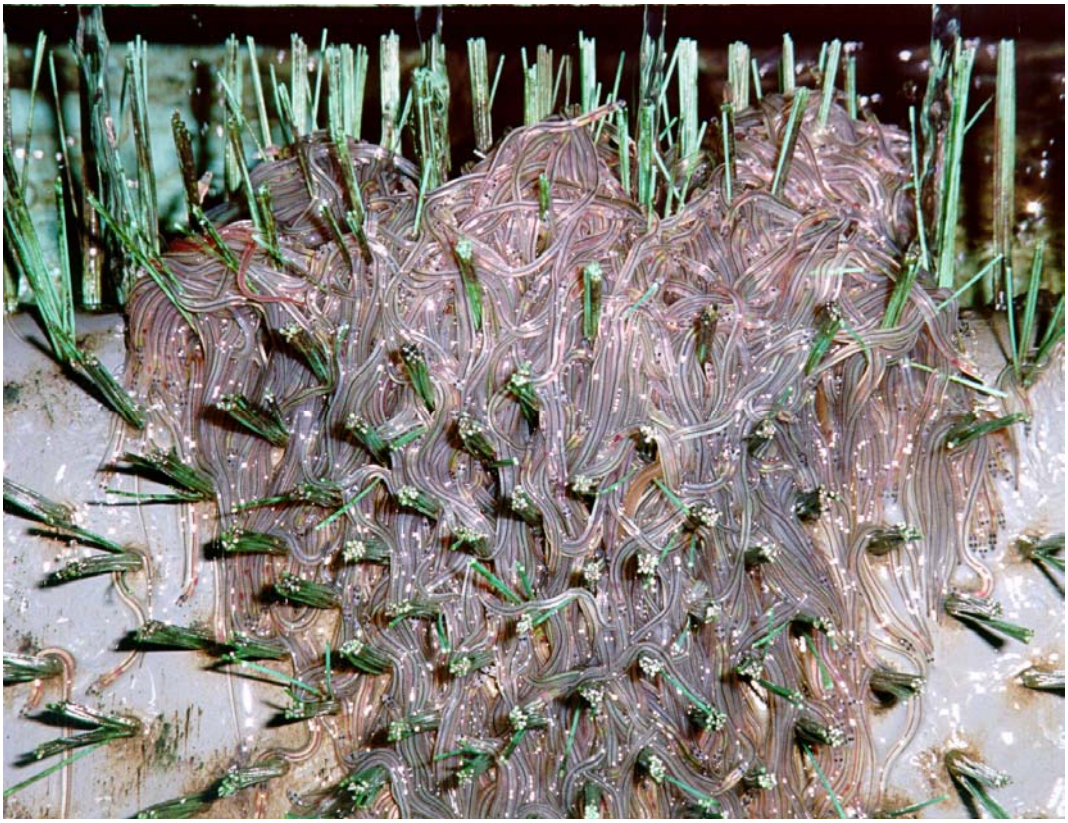
Recruitment and age dynamics of *Anguilla australis* and *A. reinhardtii* glass eels in the estuaries of New South Wales



Picture by C. Briand

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Department of Environmental Sciences
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Certificate of Authorship

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I also certify that this thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of candidate

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Table of contents

List of Figures

List of Tables

Abstract

Shortfin eels (*Anguilla australis*) and longfin eels (*A. reinhardtii*) are true freshwater eels of the genus *Anguilla*. There are many mysteries still unsolved for the freshwater eel lifecycle, such as location of the spawning grounds, conditions that promote metamorphosis from the leptocephalid to glass eel phase, and the mechanisms that affect glass eel recruitment. In Australia, little is also known about the estuarine habitats of glass eels as they migrate towards freshwater, and the age at which these eels enter estuaries. Both species are of commercial importance in the estuary fishery where they are caught in eel traps for export. There is also a small, but potentially lucrative, aquaculture industry for ongrowing glass eels to market demand size. This thesis investigates the spatial and temporal recruitment of both species of glass eels to estuaries within NSW, the habitats that may be of importance to them as they continue their upstream migration, and the age at which these eels entered the estuaries.

Firstly, a new sampling device needed to be developed since conventional methods to catch glass eels often required constant observation of gear, multiple operators, specific physical site characteristics, and/or were expensive. The artificial habitat collectors that were developed were then used to sample six estuaries in NSW monthly within one week of the new moon. Shortfins showed a more consistent and defined recruitment across all sites than longfins, where the peak shortfin recruitment season was from April – August. Longfins recruited primarily from January – May but often recruited outside of this period. Five year collections at one of these sites provided important recruitment information. It appeared that longfins failed to recruit to this site during 2000/01, which could affect commercial catches of this species when they enter the fishery. The East Australian Current (EAC) probably transports glass eels from spawning sites in the Coral Sea southward to the east coast of Australia but there was no predicted lag time in the recruitment of eels from northern to southern estuaries. Therefore, it was not possible to predict the timing of recruitment of glass eels in one estuary based on the timing of recruitment in another more northern estuary.

When glass eels enter estuaries their upstream migration is assisted by the night flood tide. During the ebb tide, glass eels burrow into the substrate and resurface at the next night flood tide. The eels do not select particular habitats at this time, rather, their location is dictated by the tide. However, once glass eels reach the estuarine/freshwater interface, they may prefer more complex habitats such as seagrass/macrophytes or rocks/cobbles in which to hide during the day. At this interface, glass eels undergo a physiological change to adapt to a freshwater existence and this change may take up to a few weeks. During this time, glass eels commonly enter the water column during the night flood tide and may be able to locate more suitable habitats in which to hide during the day.

The ages of shortfin and longfin glass eels caught in estuaries were examined both spatially and temporally. As the EAC travels north to south, glass eels recruiting to the southern sites were expected to be older. However, shortfins that recruited to the northern-most site in this thesis were older than at all other sites while there was no difference in the ages of longfins. Also, when the ages of longfins that recruited during the main recruitment period were compared to the ages of longfins that recruited outside of this period, there was no difference in ages. Therefore, the hypothesis that these later recruiting eels may have been caught in an eddy prior to their estuarine arrival has been disproved. The ages of shortfins that recruited in two separate years were significantly different from each other and may be due to shortfins' ability to detrain more easily from the weaker currents that exist at these recruitment periods. Conversely, there was no difference in the ages of longfins that recruited in the same month during three separate years. The estimated hatch dates for shortfins was estimated at October to January, while for longfins, estimated hatch time was July to September for eels that recruited during the peak recruitment period. For longfins that recruited outside of the main recruitment period, estimated hatch times were from December to February. It is unknown, however, whether longfins have an extended spawning period, or whether silver eels arrived at the spawning grounds later and thus produced later arriving longfins. Continuous monitoring of glass eel recruitment to estuaries is necessary to determine whether there are long term declines in the recruitment of Australian eels similar to the declines recently observed for eels in Europe and Asia.

Chapter 1: General Introduction

1.1 *Life History of Anguilla*

There are approximately 500 species of true eels which may be found in fresh, brackish or saltwater environments. Within the true eels, the major groups are freshwater eels, marine worm eels, moray eels and conger eels. True freshwater eels belong to the genus *Anguilla* (hereafter, 'eels' refers only to species of this genus) in which there are 15 species that are currently recognised (Beumer 1996, Watanabe 2001). These species occur throughout the world, both in the Atlantic and Indo-Pacific regions. Apart from Europe, the distribution of freshwater eels is on the eastern coastal regions of Africa, India, Australia, South-East Asia, Canada and the USA. This distribution reflects the southwards or northwards movement of tropical current systems from the Equator.

Within Australia, four species of *Anguilla* have been recorded. Three are classified as shortfinned (or shortfin) and the other as longfinned (or longfin). The shortfin eels are *Anguilla bicolor* (McClelland) which occurs in the north-western tropical areas of Australia, *A. obscura* (Günther) of which only one specimen was recorded at the time of Schmidt's (1928) descriptions and *A. australis* (Richardson). The longfin eel is *A. reinhardtii* (Steindachner). Both shortfin (*A. australis*) and longfin (*A. reinhardtii*) eels, as they will hereafter be termed, inhabit Australia's east coast and are the subjects of this thesis. Shortfins are classified as a temperate species with a distribution ranging from the Caboolture R. in Queensland to the rivers of Tasmania. This species also occurs in Norfolk and Lord Howe islands and New Zealand (Ege 1939, Beumer 1996, Jellyman et al. 1996) as well as New Caledonia (Marquet 1996). The more tropical longfin eel is distributed along the entire east coast of Australia from Cape York to Tasmania (Beumer 1996). It also occurs in New Guinea, Solomon Islands, New Caledonia, Lord Howe Island and New Zealand (Schmidt 1928, Ege 1939, Allen 1991, Jellyman et al. 1996). Longfins can be taxonomically distinguished from shortfins in that the dorsal fin originates well in front of the anal fin. When pigmented, longfins are a distinct olive green with black mottled spots

compared to shortfins which have a more uniform olive green to brown appearance along the dorsal surface.

There has been much speculation, discussion and controversy over where eels spawn and how their life cycle is actually completed. Historically, eels were considered to be "brides of snakes" and it was suggested that eels were the result of spontaneous generation, that an eel rubbed its body against rocks and young eels formed from the slime. Other theories held were that they were parasitic nematode worms or live bearers (Roughley 1955). Also, the leptocephalus larvae of the European eel (*A. anguilla*) were initially described as a separate species (*Leptocephalus brevirostris*) because their shape is vastly different from the cylindrical eel-shape. Grassi (1896) was the first to discover that this 'species' was the marine larval stage of Anguillid eels (Tesch 2003) and research into the spawning ground of the European eel began in earnest. In the early 1900's Schmidt, a Danish researcher, succeeded in locating this spawning ground after intensive trawling in the Atlantic Ocean yielded leptocephali of hatching size (5 mm; Tesch 2003). These discoveries led to the basic understanding of the eel lifecycle.

For all Anguillid eel species, the life-cycle, although complex, is essentially the same with a number of defined stages (Fig. 1.1). Eels are catadromous and the sexually mature adults, or 'silver' eels, spawn at sea at depths of 300 m or more at specific temperature, pressure and salinity gradients (Beumer 1983). For the east coast Australian species, spawning is thought to occur in the south-western Pacific Ocean north of New Caledonia (Aoyama et al. 2001). The fertilised eggs are planktonic and develop into leaf-shaped larvae called leptocephali. These transparent, laterally compressed, leaf-shaped larvae are carried passively from the spawning grounds (McCleave et al. 1998) to Australia via the South Equatorial Current (SEC) and then by the East Australian Current (EAC) (Jellyman 1987, Aoyama et al. 1999, Shiao et al. 2002, Tesch 2003). On approaching the continental shelf, the leptocephali change in body shape through a reduction in both depth and length to the familiar eel shape (Deelder 1970). This metamorphosis also involves the loss of teeth and a cessation in feeding for a brief period (Beumer & Harrington 1980, Tesch 2003). Now termed

glass eels, there is no body pigment and the skeleton and internal organs are clearly visible.

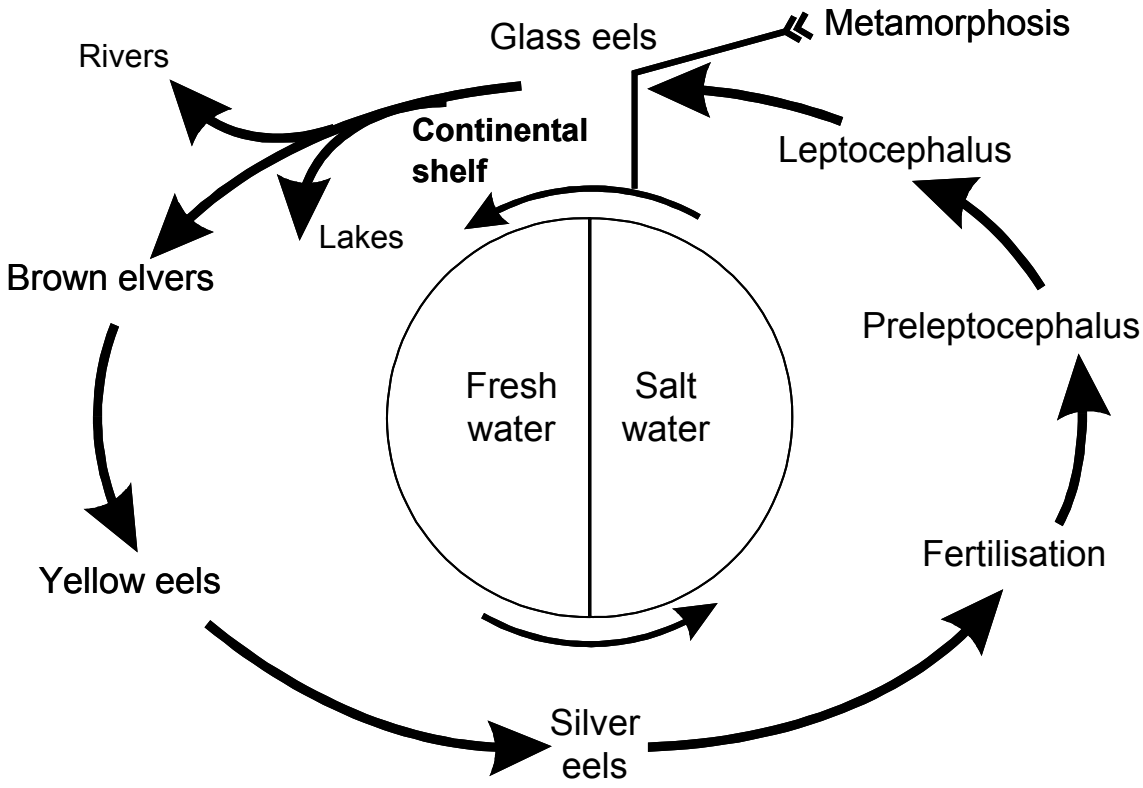

Fig. 1.1 – Life-cycle of Anguillid eels

Glass eels migrate from the continental shelf to the estuaries following environmental stimuli with the assistance of tidal currents. Glass eels may remain in brackish waters within the estuary for some time and this pause is to allow for physiological changes to occur in order to make the transition from the marine to freshwater environment. While the estuarine migration occurs, glass eels become increasingly more pigmented and, when the body pigmentation is complete, the young eels are known as elvers. This stage of the life-cycle may remain in the estuary or may migrate further upstream into rivers, lakes and swamps where the eels may have to pass natural or artificial barriers. Elvers feed and grow into sexually immature yellow eels that may inhabit rivers and lakes for many years with a well defined home range (Bozeman et al. 1985, Chisnall & Kalish 1993). The diet of these freshwater eels can be described as opportunistic as it includes insects, crustaceans, molluscs, fish and frogs as their prey.

Yellow eels develop into sexually mature silver eels after a trigger that alters the slow development of ovaries and testes into rapid maturity and silver eel metamorphosis. Externally, silver eels develop a grey to black upper body colouration and a white to silver lower body. Pectoral fins darken, an increase in eye size also occurs along with the formation of a coppery sheen around the pupil of each eye. The lateral line also becomes more pronounced in silver eels. This system assists in the detection of vibrations and in maintaining the positions of individuals while migrating to the spawning grounds. It is uncertain how the eels find the exact location of the spawning grounds but the use of the Earth's magnetic field as an orientation mechanism has been suggested Tesch et al. (1992). To date, no spawning adult eels or fertilised eggs have been observed or collected in or near the spawning grounds. However, silver eels have been collected in the ocean on their supposed migration to the spawning grounds (Sasai et al. 2001).

1.2 *Importance and justification for study of eels*

Eels have long occupied a significant place within human society. In ancient Egypt, eels were considered sacred and raised to the status of gods while the Romans kept eels as pets in inland saltwater ponds (Beumer 1983). In New Zealand, the Maori version of the creation story has an eel rather than a snake in the Garden of Eden while, in Australia, Aborigines use eels as the 'creators' (in the story of creation) of the Clarence River, the longest river in New South Wales (NSW) (Beumer 1983). The Aborigines captured eels by a variety of methods including by hand, traps woven from bullrushes or by an elaborate system of channels, weirs and fish-traps (Pollard 1969). Their importance as a food item is further highlighted by the current demand for glass eels as seedstock for the high value international aquaculture industry which, in 2002, produced 232 000 tonnes of eels at a value of over US$927 million (FAO 2004).

Presently, the ability to successfully obtain glass eels from artificially induced adult eels in captivity has proven impossible and there has been limited success in producing leptocephali from preleptocephali (Tanaka et al. 2001). Consequently, aquaculturists must harvest glass eels from the wild as the basis for their grow-out

operations. Glass eels are the preferred life history stage to use as seedstock for aquaculture as they are the first life stage after completion of metamorphosis, are almost at feeding stage, and can be more easily caught in comparison to previous life stages. This is advantageous to aquaculturists since they are dealing with a more stable life stage, they are able to wean glass eels onto special diets for fast growth and costs of capture are reduced because they can catch the eels in the estuary rather than the open ocean. As a result of the wild harvest of glass eels and the heavy exploitation of eel populations, particularly in south-east Asia (Queensland Department of Primary Industries 1995) and Europe (Dekker 2000), eel aquaculture is limited by the availability of wild caught seedstock (Queensland Department of Primary Industries 1995). Thus, the present research is designed to provide more detailed information on the timing of glass eel recruitment to NSW which can assist aquaculture operations in determining when and where to utilise their resources to maximise returns.

Current research and monitoring knowledge suggests that stocks of the European (Dekker 2000, 2003, 2004), American (Castonguay et al. 1994, Atlantic States Marine Fisheries Commission. 2000) and Japanese eel (Tzeng 1997) are in decline. In fact, by 2003, juvenile abundance of the European and Japanese eel had declined by 99% and 80% respectively, while recruitment of the American eel near its northern limit has virtually ceased (Dekker et al. 2003). Despite the rigours of the life-cycle, freshwater eels now need to survive further man-made pressures such as overexploitation of the stock (fishing mortality), loss of habitat (land reclamation and pollution of waterways), and obstructions to riverine passage (erection of dams and weirs on streams) (Haro et al. 2000). Therefore, the research presented here aims to provide a useful method that can form the basis of a long term monitoring program, as well as providing spatial and temporal information on the recruitment of glass eels to NSW. This will go some way to providing data for future use in a glass eel monitoring program and may be able to identify whether declines in eel stocks witnessed elsewhere are occurring here.

1.3 *Eel research and management in New South Wales*

Beumer & Sloane (1990) list the reasons for commencement of research into juvenile anguillid eel stages in Australia as: satisfying scientific understanding, the need to stock certain waters, the construction of dams and weirs which impeded upstream migration, and the potential of eel culture in Australia and overseas. However, the occurrence of glass eels in Australia and minimal spatial and temporal distribution information was described earlier by Schmidt (1928). Since this time, few research studies have focussed on comprehensively studying the recruitment patterns of glass eels to the east-Australian coast with the exception of Gooley & Ingram (2002). Due to the vast coastline to be sampled, the sampling methods employed, and financial restrictions, consistent sampling in that study was limited to only one or two estuaries in each state with NSW focussing on the Hacking River. Hence, spatial and temporal recruitment patterns of glass eels along the NSW coast are not well understood.

In Australia, both shortfin and longfin eel populations straddle state borders, which makes comprehensive and co-operative research and management difficult to obtain. Currently, 300 kgs of glass eels may be collected in NSW annually by permit holders for use in the aquaculture industry but only 15 – 20 kgs have been collected in any one year for the last four years. Complications have arisen that have driven aquaculturists away from culturing freshwater eels. These complications include difficulty of capture, unreliable supply of glass eels, and difficulty in identifying and separating the desired species without the use of a microscope. Also, there is only limited information on the recruitment and timing of shortfin and longfin glass eels along the NSW coast so harvesters of wild stocks are unsure of the species they have caught and are unsure of when to best concentrate their fishing efforts to maximise capture.

1.4 *Aims of this study*

Consequently, the research presented here aims to:

- Develop a new sampling technique that enables glass eels to be easily and efficiently sampled over broad spatial and temporal scales (Chapter 2).
- Determine the spatial (approximately 900 km) and temporal (up to 5 years) recruitment patterns of shortfin and longfin glass eels over their distributional range within NSW, and any factors that drive this recruitment (Chapter 3).
- Develop a theoretical transport and recruitment model for eels from the proposed spawning site to the coast of NSW, and as glass eels enter the estuary and continue their migration to freshwater (Chapter 3).
- Determine the estuarine habitats of shortfin and longfin glass eels (Chapter 4).
- Validate the deposition of daily increments in glass eel otoliths for both species of eels (Chapter 5).
- Determine the daily age of glass eels and compare spatial and temporal ages of shortfin and longfin eels (Chapter 5). This chapter also estimates hatch times for these eels and tests their validity by comparing them to the timing of oceanic leptocephali catches of shortfins and longfins.
- Determine some of the factors that may have caused the variability in eel recruitment (from Chapter 3) by the spatial and temporal ageing of eels (Chapter 5).
- Compare these findings with information available for other anguillid eel species (Chapters 2-6).
- Synthesize the outcomes of this thesis and their implications on the successful management of the eel resource in NSW (Chapter 6).

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Chapter 2: A novel artificial habitat collection device for studying resettlement patterns in anguillid glass eels

Silberschneider, V., Pease, B.C. & Booth, D.J. 2001. A novel artificial habitat collection device for studying resettlement patterns in anguillid glass eels. *Journal of Fish Biology* **58**: 1359-1370.

2.1 *Abstract*

Artificial habitat collectors, made from a PVC base and polyethylene split rope fibres, were used in a series of experiments designed to assess spatial and temporal aspects of their performance in sampling glass eels of the shortfin eel, *Anguilla australis,* and the longfin eel, *A. reinhardtii*. The experiments were conducted near the Audley causeway in the Hacking River, to the south of Sydney, Australia. It was first shown that glass eels are retained in collectors and thus adequately sampled when the collectors were pulled out of the water. The number of glass eels caught per collector was related to the number of rope fibre tufts attached to each collector rather than collector area directly. Ageing of collectors *in situ* to promote algal growth enhanced the catch of glass eels. Glass eels entered the collectors at night primarily during the flood tide, and did not move into the collectors during daylight hours. Glass eel abundance increased with increasing distance from the freshwater drain located in the causeway. The artificial habitat collectors used in this study have been shown to be effective for assessing relative numbers of resettling glass eels. It is suggested that these collectors may also be useful for studying recruitment and settlement patterns of other anguillid eel species, as well as identifying areas and habitats within a catchment that provide important shelter for glass eels. Sampling glass eels can be carried out with maximum effect and minimum effort using compact, aged artificial habitat collectors on the night time flood tide when low tide coincides with dusk.

2.2 *Introduction*

Glass eels are the juvenile life history stage of catadromous anguillid eel species that recruit to estuaries after an extended oceanic larval phase. An understanding of their recruitment patterns is especially useful for management of the extensive fisheries that have developed for both juvenile and adult eels worldwide (FAO 1999a,b). Variability in glass eel recruitment has been related to many environmental cues such as salinity (for *Anguilla anguilla* and *A. australis*), water temperature (*A. anguilla* and *A. australis*), rainfall (*A. japonica*), water odour (*A. anguilla*) (McKinnon & Gooley 1998) and lunar phase/tidal range (*A. australis* and *A. dieffenbachii*, Jellyman 1979). Glass eel recruitment in both North America and Europe has declined in the last 20 years and these declines may be due to a number of factors such as habitat loss, environmental variability and overfishing (Moriarty 1996, Haro et al. 2000).

A number of sampling methods have been used to collect glass eels of *Anguilla* spp. in estuaries and freshwater tributaries including fyke nets, plankton nets, flow traps, electro-fishing, dip or scoop nets, and the more traditional method of branch bundles (Beumer & Harrington 1980, McCleave & Kleckner 1982, Tesch 2003). The method used has depended upon the available technology, the site fished, and the quantity of glass eels required. Most of these methods collect actively moving glass eels from the water column. The difficulties encountered using these methods include a requirement for constant observation of gear, multiple operators, specific physical site characteristics, and/or are expensive.

Artificial habitats have been used for centuries as a means of aggregating and collecting fish for recreational and commercial harvest and, more recently, artificial habitats have been used in environmental management (Bohnsack & Sutherland 1985, Seaman & Sprague 1991). The use of branch bundles is the only published habitat-based method for collecting sedentary glass eels (eg Beumer & Harrington 1980, Jellyman & Chisnall 1999) and they are inexpensive and easy to use. A disadvantage is that they have a wide variety of interstitial space sizes. This allows larger eels to enter the habitat and these larger eels may prey upon the smaller glass eels.

The objective of this study was to experimentally assess the effectiveness of low maintenance, low cost artificial habitat collectors to sample the relative abundances of resettling glass eels of the shortfin eel and the longfin eel. Resettlement is defined as the movement of glass eels into the collectors, during their migration upstream. The collectors trialed were based on the rock lobster puerulus collectors developed by Phillips (1972).

2.3 *Methods*

2.3.1 *Sampling Details*

All experiments were conducted at the base of the Audley causeway on the Hacking River, to the south of Sydney in New South Wales, Australia. The Audley causeway is the first physical barrier encountered by glass eels on their upstream migration from the mouth of the Port Hacking estuary. The causeway is 2.4 m high with a crest length of 90 m and is located 12 km upstream from the ocean. Salinity at the sampling site below the causeway ranged from 0-35 ppt, depending on the tide and freshwater flow from above the causeway. The dominant substrates at this site were mud and bare rock.

The collectors used in this study were based on a design to collect lobster larvae initially developed by Phillips (1972) and later modified by Montgomery & Craig (1997). Each collector consisted of one grey PVC panel (61 cm x 35 cm x 0.4 cm) to which 25 evenly spaced 'tufts' of polyethylene split rope fibre were attached. Each tuft comprised 50 cm lengths of fibre and was secured by the middle of the tuft to the panel by a plastic tie which was passed through 2 holes in the panel. For puerulus collectors, two panels are attached back to back and suspended from a float. In this study, the collectors of Montgomery & Craig (1997) were modified by attaching lead weights of approximately 200g to each corner of a single panel with seizing wire. A length of rope was attached to the panel so that the collectors could be easily removed from the water in a vertical position.

The collectors were placed flat on the rocky substrate near the base of the causeway wall between the freshwater drain and the southern end at a depth of approximately 0.5 m MLW (mean low water), with tufts facing upwards. To retrieve and process glass eels, each artificial habitat collector was quickly pulled out of the water using the attached rope and placed in a plastic tub. Each collector was shaken 30 times (tufts facing down) into the tub and then placed into a second tub. The contents of the tub were poured through a fine sieve and any glass eels were euthanased in 100 mg/L benzocaine and preserved in labelled jars with 95% ethanol. Each collector was then given a further 20-30 shakes into the second tub to ensure all glass eels had been retrieved from the collectors, and the contents treated as above. At regular intervals during this process, the area around the tubs was checked for escaping glass eels. Between each experiment, the collectors were stored on the shallow seabed (< 2 m) near the NSW Fisheries Research Institute wharf in Port Hacking, tufts facing up, in order to maintain algal growth on the fibres.

Glass eels were taken to the laboratory where they were identified to species by looking at the relationship between the origins of the dorsal and anal fins. They were then assigned a pigmentation stage according to the criteria of Strubberg (1913).

All experiments, except Experiment 4, were conducted between the new and first quarter lunar period since previous studies of other anguillid species have shown that glass eel recruitment into estuaries generally peaks during this period (e.g. Antunes 1994). Experiment 4 was conducted during a full moon period when glass eels were known to be present.

2.3.2 *Experiment 1: Retention of glass eels in collectors*

To determine how effectively glass eels were retained in the collectors when they were removed from the water, nets were constructed to surround the collectors from the time they were lifted from the substrate to the time they were checked for glass eels. The nets were made of 1.5 mm stretched mesh material and the rectangular base of the netting was 122 cm x 102 cm. Material was sewn around this netting base so that the completed net was 122 cm in height. Fencing wire was threaded around the top of the net to form a rectangular frame.

Ten full-size collectors were placed at approximately two metre intervals along the base of the causeway at low tide just prior to sunset on 19 May 1999 and five collectors were randomly selected to have nets positioned under them. When positioning the nets under collectors, some excess material was tucked under the collectors and the wire frame weighted down the rest of the material. The frame sat at a height that was at approximately the same level as the collector base. There was no netting higher than the collector tufts.

Glass eels were collected the following morning, shortly after sunrise. All un-netted collectors were processed first as described above. To sample netted collectors, one person waded into the water and quickly lifted all sides of the net out of the water, using the wire frame. The collector and surrounding net were then lifted out of the water and the net was thoroughly checked for glass eels before processing each collector as described above.

The null hypothesis, that there was no significant difference in the number of glass eels collected between netted and un-netted collectors, was tested using a one factor ANCOVA, with distance of the collector from the freshwater drain in the causeway as the covariate (Zar 1974).

2.3.3 *Experiment 2: Effects of collector area on glass eel catches*

To test the sampling efficiency of smaller collectors, which were easier to handle, I compared glass eel catches of full-size collectors (61 cm x 35 cm) with catches of half-size collectors (30 cm x 35 cm). Full-size collectors (25 tufts) were cut in half to make two half-size collectors, each with 12 tufts. Half-size collectors were also fitted with lead weights in each corner and a length of rope.

Six full and six half-size collectors were placed in alternate order of size along the causeway as random allocation of collectors may have biased results if there was a positive relationship between distance from the drain and numbers of glass eels. They were placed at low tide, just prior to dusk, on the evening of the 17 June 1999, and processed on the following morning shortly after sunrise.

ANCOVA was used to test for a difference between the slopes of the regressions of the numbers of glass eels per tuft (log transformed) against distance from the causeway drain for the two types of collectors (full-size and half-size). This was followed by a t-test to determine if there was a difference between the intercepts of the two regression lines.

2.3.4 *Experiment 3: Effect of collector tuft density on glass eel catches*

Since full-size collectors in Experiment 2 had more tufts (25) than half-size ones (12), full-size collectors may have attracted more glass eels because of greater tuft numbers rather than greater collector area *per se*. An orthogonal experiment was conducted to simultaneously test the effect of collector tuft density and collector area on glass eel resettlement.

Six full and six half-size collectors were placed along the causeway wall just prior to sunset at low tide on 18 June 1999. On three of the full and three of the half-size collectors, every second tuft was covered with a plastic bag which was fastened to the base of the tuft with an elastic band. Small holes were punched into the bags to allow air to escape but without letting glass eels enter. This resulted in four treatments: 1) three full collectors with no tufts bagged, ie 25 exposed tufts per collector; 2) three full-size collectors with 13 tufts bagged, ie 12 exposed tufts per collector; 3) three half collectors with no tufts bagged, ie 12 exposed tufts per collector; and 4) three half collectors with six tufts bagged ie 6 exposed tufts per collector. In order to control for spatial location, one replicate from each treatment was then randomly allocated to one of three spatial blocks (based on distance from the causeway drain) and randomly positioned within each block. Collectors were processed on the following morning, shortly after sunrise.

The null hypothesis that there was no significant difference in the number of glass eels (log transformed) between treatments was analysed using a one-factor ANOVA followed by the Student-Newman-Keuls test (SNK) when a significant difference was found (Zar 1974).

2.3.5 *Experiment 4: Effect of collector ageing on glass eel catches*

Montgomery & Craig (1997) found that ageing collectors in seawater significantly increased their attractiveness to rock lobster pueruli. The collectors used here were soaked off the NSW Fisheries Research Institute wharf near the mouth of the Port Hacking estuary for approximately two months until algal growth was observed on the tufts of each collector. Collectors were then placed flat on the seabed in shallow water, with tufts facing upwards, and were considered to be 'aged' and ready for use when algal growth was observed on most of the tufts.

To determine whether ageing of collectors affected the number of glass eels caught, four un-aged and four aged half-size collectors were placed alternately along the causeway at low tide shortly before sunset on 23 August 1999. Glass eels were retrieved and collectors reset on the following two mornings, with collectors finally processed on 26 August, 1999.

A repeated measures ANOVA (SAS statistical software) was used to test the null hypothesis that there was no significant difference in the number of glass eels caught between aged and un-aged collectors between days.

2.3.6 *Experiment 5: Diel patterns of glass eel resettlement and influence of collection interval on estimates of resettlement*

This experiment was carried out by placing aged, half-size collectors along the causeway over two 24 hour periods from low tide at dusk on 18 July to dusk on 19 July 1999, and from high tide at dusk on 22 July to dusk on 23 July 1999. Two days were used in order to investigate the effect of tide. During each day, twelve collectors were allocated to one of three sample interval treatments (each with four collectors) and were sampled every three, six and 12 hours respectively. In order to control for location, replicates from each treatment were randomly allocated to one of four spatial blocks and randomly positioned within each block. At the allocated sampling time, all four replicates of a treatment were quickly lifted, processed, then placed back in the water in their respective positions. Diel patterns were determined by plotting glass eel numbers against time and tide height.

The null hypothesis tested was that there was no difference in the number of glass eels caught in collectors, with respect to time of day or tide conditions, throughout a 24 hour period. A chi-square test was used to analyse the data.

2.3.7 *Experiment 6: Abundance of glass eels in habitat collectors as an index of the relative abundance of glass eels moving through the estuary*

From May 1998 to April 2000, habitat collectors were used to sample glass eels at Audley causeway concurrently with Japanese net samples collected at a site 5 km downstream from the causeway in the main channel. These samples were taken during new and full moon periods. The sites, sampling gear and sampling protocol are described in Pease et al. (2003). Data were log transformed and a correlation analysis was performed on the two year data set for each species to determine if nightly catches of each species in the habitat collectors at the causeway were correlated with catches downstream in the Japanese net.

2.4 *Results*

2.4.1 *Glass eel samples*

A total of 788 glass eels were caught in the artificial habitat collectors at Audley causeway during the five experiments at a time when glass eels were abundant. Whilst sampling was conducted during the shortfin glass eel migration season, some longfin glass eels were also caught (Table 2.1). Data analyses were performed on the pooled numbers for both species as sample sizes of longfins were too small for analyses to be performed for each species separately. Pigmentation of glass eels caught ranged from V_B1 (only head and tail pigment present) to $VI_{A1V}2$ (developed mediolateral pigment and advancing ventrolateral pigment). By-catch in the habitat collectors consisted of small crustacean and mollusc species, with no other fish species observed.

Table 2.1: Species composition of glass eels caught in each experiment

Experiment	1	2	3	4	5	6 collector	Japanese net
Shortfins	157	181	172	31	184	309	3438
Longfins	19	9	7	20	8	240	1239

2.4.2 *Experiment 1: Retention of glass eels in collectors*

There was no significant difference in the number of glass eels caught in artificial habitat collectors with nets (mean $\pm$ se, 15.6 $\pm$ 2.46, n = 4 collectors) compared to collectors without nets (mean $\pm$ se, 19.6 $\pm$ 2.01, n = 4, $F_{1,7} = 0.007$, $p > 0.05$). The ANCOVA showed that there was a significant increase in glass eel catch with increasing distance from the causeway drain ($r^2 = 0.55$; $F_{1,7} = 6.040$, $p < 0.05$; Table 2.2).

Table 2.2: Spatial distribution of glass eels in each collector, numbered one to 10, in Experiment 1 from the causeway drain to the estuary bank

Drain	1	2	3	4	5	6	7	8	9	10	Bank
Number of glass eels	13	15	9	16	16	18	24	20	27	18	

2.4.3 *Experiment 2: Effects of collector area on glass eel catches*

There was no significant difference between the slopes of the number of glass eels per tuft between full and half-size collectors ($F_{2,8} = 4.36$, $p > 0.05$, n = 6 collectors per treatment). Based on the total catch of 121 glass eels in full-size collectors, the total catch of 69 glass eels in half-size collectors was very close to the predicted catch of 58 glass eels, based on the number of tufts. There was also no significant difference when comparing the y-intercepts of the two slopes ($t = 0.23$, $p > 0.05$, Fig. 2.1). Thus, it is assumed that there is no difference in catch when using half-size collectors compared to full-size collectors on a glass eel per tuft basis.

2.4.4 *Experiment 3: Effect of collector tuft density on glass eel catches*

The number of glass eels in a collector was directly related to the number of tufts attached to the collector, rather than absolute size of the collector. The one-factor ANOVA showed that there was a significant difference in the number of glass eels caught between collectors of different treatments. The SNK test confirmed that there

were significantly more glass eels in the full-size collectors with no tufts bagged (mean ± se, 1.48 ± 0.07, n = 3 collectors) compared to all other treatments. There was no significant difference between the mean number of glass eels found in full-size collectors with tufts bagged (mean ± se, 1.01 ± 0.16, n = 3) and half-size collectors with no tufts bagged (mean ± se, 1.10 ± 0.08, n = 3), but there was a significant difference between half-size collectors with tufts bagged (mean ± se, 0.60 ± 0.12, n = 3) and all other treatments.

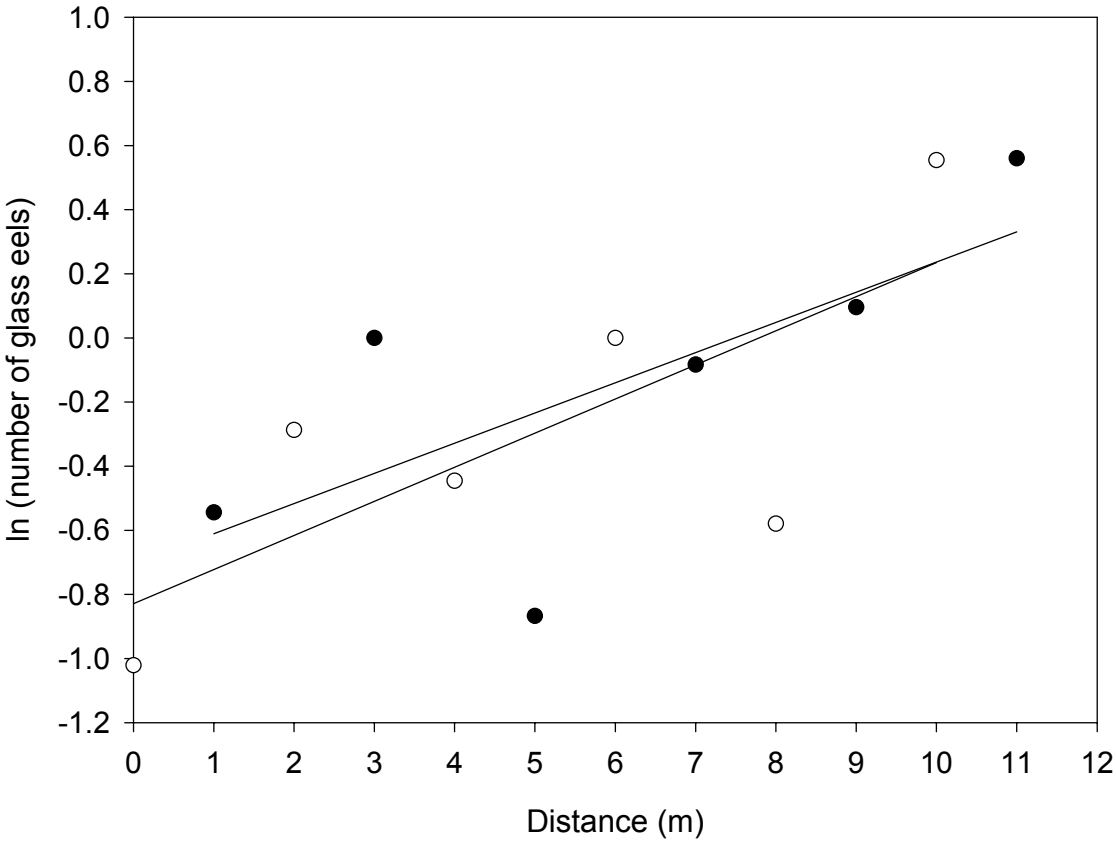

Fig. 2.1 – Number of glass eels per tuft (log transformed) caught in full-size collectors (open circles) and half-size collectors (solid circles) with distance from the causeway drain. n = 6 collectors per treatment.

2.4.5 *Experiment 4: Effect of collector ageing on glass eel catches*

A repeated measures ANOVA showed that there were significantly more glass eels caught in aged collectors (n = 40 glass eels, 4 collectors) than in un-aged collectors (n = 11 glass eels, 4 collectors, $F_{1,6} = 27.73$, $p < 0.05$). There was no significant difference in the number of glass eels caught between the three nights sampled ($F_{2,12}$

= 0.60, $p > 0.05$) and no night x aged/un-aged interaction ($F_{2,12} = 0.38$, $p > 0.05$). As there was no significant difference between nights, data for the three nights were pooled to give a mean of 3.33 (s.e = 0.3) glass eels per collector per night for aged collectors and 0.92 (s.e = 0.2) glass eels per collector per night for un-aged collectors.

2.4.6 *Experiment 5: Diel pattern of glass eel resettlement and influence of collection interval on estimates of resettlement*

The majority of glass eels entered the collectors at night during the flood tide, with only two glass eels entering the three hourly sampled collectors on the ebb tide (Fig. 2.2). In the first 24 hour experiment when low tide was at dusk, no glass eels entered collectors in daylight hours. In the second 24 hour experiment, when high tide was at dusk, one glass eel entered a collector that was sampled during daylight hours (0830 hrs) on the ebb tide, but this individual may have entered the collector at sunrise. This pattern was supported by the six and 12 hour samples (Fig. 2.3), where glass eel numbers were greatest at night during the flood tide in both experiments.

The chi-square test showed that, when grouping the three hourly data into day/flood tide, day/ebb tide, night/flood and night/ebb for each experiment, there was a significant difference between the proportion of glass eels caught and the expected ratios of equal catches (the null hypothesis) in each time of day/tide condition (Expt 1 $\chi^2 = 157.14$, $p < 0.05$, Expt 2 $\chi^2 = 45.78$, $p < 0.05$). A subdividing chi-square analysis showed that this difference was due to the fact that catches during the night/flood conditions were significantly higher than catches during other time periods (Expt 1 $\chi^2 = 157.09$, $p < 0.05$, Expt 2 $\chi^2 = 27.52$, $p < 0.05$).

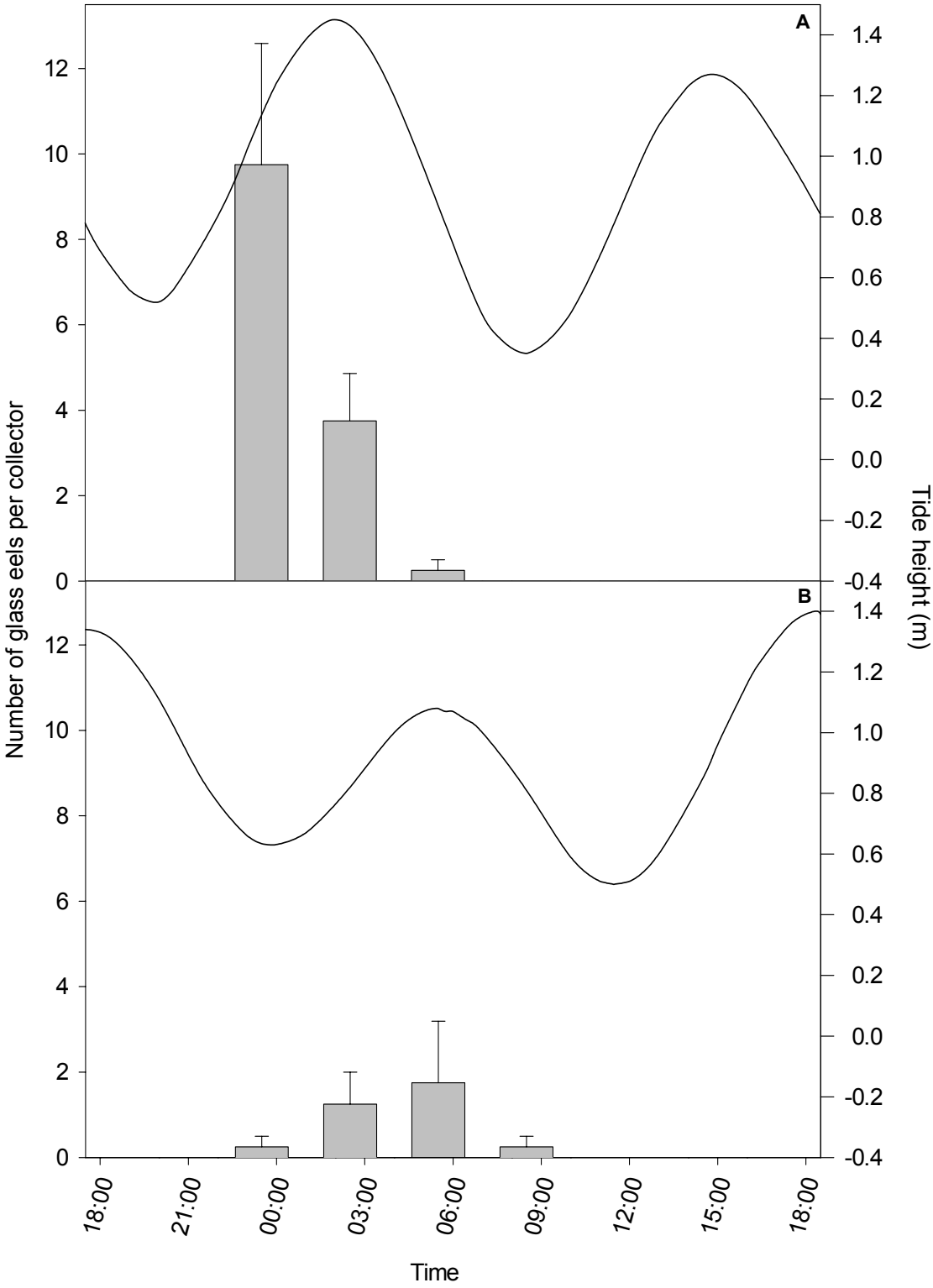

Fig. 2.2 – Average glass eel abundance using artificial habitat collectors sampled every three hours during the 24 hour period from A) 17:30 hrs on 18/7/99 to 17:30 hrs on 19/7/99 (mean ± s.e., n = 3) and B) 17:30 hrs on 22/7/99 to 17:30 hrs on 23/7/99 (mean ± s.e., n = 3).

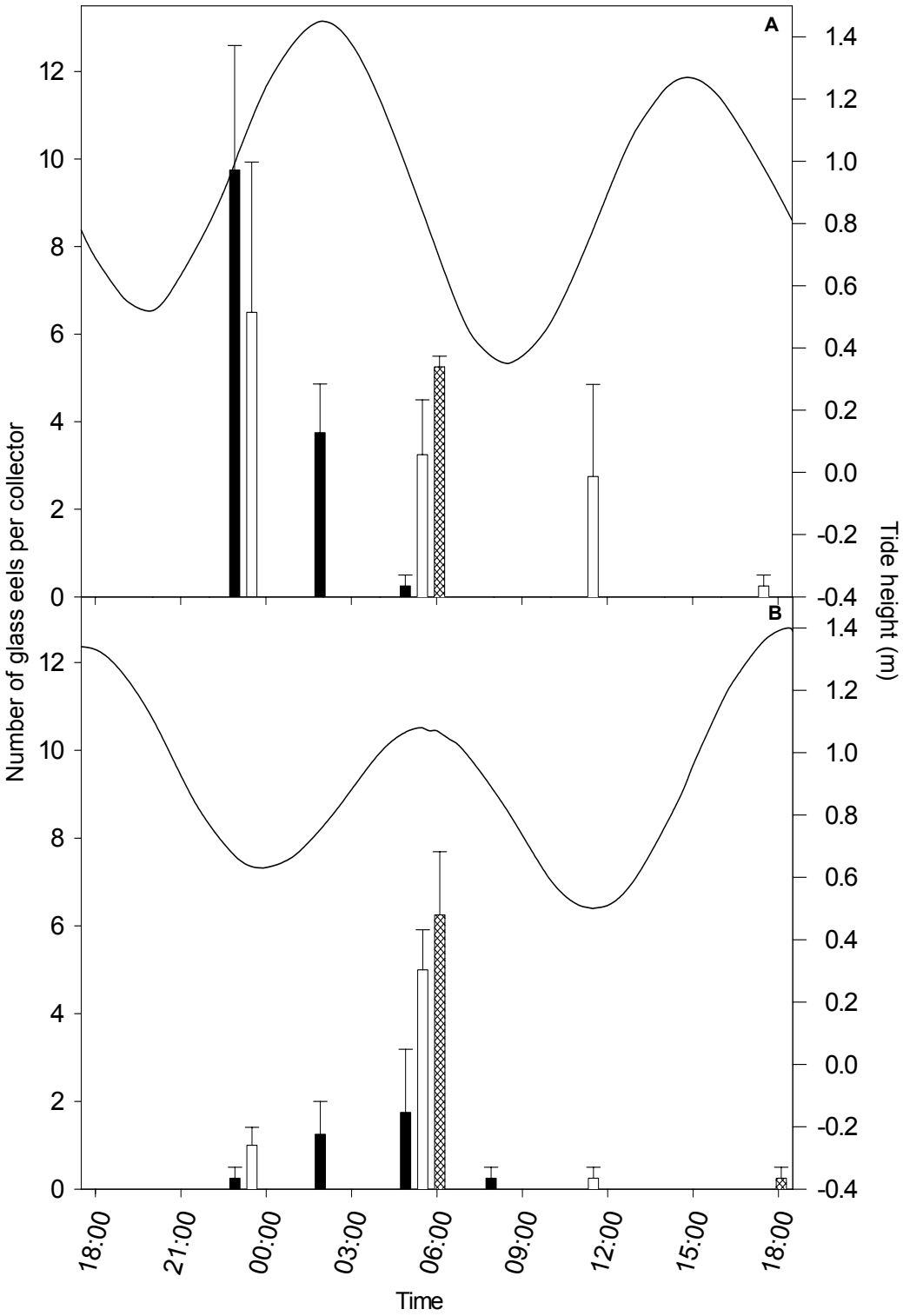

Fig. 2.3: Average glass eel abundance using artificial habitat collectors sampled every three (black bars), six (white bars) and twelve hours (hatched bars) during the 24 hour period from A) 17:30 hrs on 18/7/99 to 17:30 hrs on 19/7/99 (mean ± s.e., n = 3) and B) 17:30 hrs on 22/7/99 to 17:30 hrs on 23/7/99 (mean ± s.e., n = 3).

Combining the number of glass eels captured in all collectors used in the first 24 hour experiment, a total of 115 glass eels were caught at night compared to only 12 in daylight hours. During the second 24 hour experiment, a total of 62 glass eels were caught at night and only three were captured during daylight. The few glass eels collected during daylight hours (particularly in the six and 12 hour samples) may have entered collectors at night since some samples extended from night into daylight hours. Glass eel catches in artificial habitat collectors in the six and 12 hour samples after the night tide suggest that once glass eels enter the collectors they remain in them.

2.4.7 *Experiment 6: Abundance of glass eels in habitat collectors as an index of the relative abundance of glass eels at the sampling site*

The correlation analysis showed that there was a significant positive correlation between the numbers of both species of glass eels caught in the habitat collectors compared to what was caught in the Japanese net (for shortfins $r_{0.05(2),22} = 0.64$, $p < 0.05$ and for longfins $r_{0.05(2),22} = 0.68$, $p < 0.05$).

2.5 *Discussion*

The artificial habitat collectors developed in this study successfully attracted resettling glass eels, which remained in collectors as they were removed from the water. It has been widely reported that glass eels and elvers prefer complex natural habitats as refuges from predators. Jellyman & Chisnall (1999) reported that Maori used debris clusters to catch small eels in New Zealand, and eel fishers found small eels within macrophytes. Similarly, Sloane (1984) showed that glass eels were abundant in *Zostera* sp. seagrass beds. In controlled experiments, Glova (1999) showed that small eels, when given a choice of five habitats, preferred substrates with watercress, cobbles and, to a lesser extent, woody debris rather than choices which were less complex. The artificial habitat collectors used in this study are similar in complexity to *Zostera* seagrass, with many interstitial spaces, and possess tactile fibres (even more so with algal growth), which are vertically oriented when the collectors are placed horizontally on the substrate. They may attract glass eels by mimicking the complex natural habitat that glass eels seek in the wild. When collectors are disturbed, glass eels may burrow more deeply into the protective tufts, mimicking the protective behaviour they exhibit in natural habitats. In a review of artificial reef research, Bohnsack & Sutherland (1985) list increased complexity, surface roughness, and placement on gently sloping or flat areas (among others) as being crucial to the success of artificial reefs in attracting recruiting fish.

Ageing of collectors in seawater, which promoted algal growth, was shown to be an important factor in improving catches. This may be related to a number of factors. Placing the collectors in seawater may 'wash out' the synthetic odour of the fibres, which may be avoided by glass eels as they have well developed olfactory organs and are attracted or repulsed by particular odourants (Sorensen 1986, Tosi et al. 1990, Sola 1995, Sola & Tongiorgi 1996). The algal growth may provide positive visual, olfactory and tactile cues similar to the natural habitat where they seek refuge from predators. The algae and associated community on collectors may also provide glass eels with nutrition. The glass eel catch in collectors was also directly related to the number of tufts. Different size collectors may be used but tuft size and density

should be standardised. Number of tufts should be reported where the size of the collector is modified.

The results indicate that a peak in active movement and resettlement of Australian glass eels occurs during the night-time flood tide. Previous studies of glass eel migration into and through estuaries towards freshwater have also shown that movement occurs predominantly at night on the flood tide as the incoming tide assists glass eel migration upstream (Jellyman 1979, Sorensen & Bianchini 1986, Tesch 2003). Most of these studies were based on samples of actively moving glass eels from estuarine areas with tidal currents. Nearing the ebb tide, glass eels travelling in tidal currents either move downstream with the tide or seek shelter in the bottom substrate (Usui 1974, Beumer & Harrington 1980, McCleave & Kleckner 1982, McCleave & Wippelhauser 1987, Gascuel et al. 1995).

The sampling site for this study had no tidal current because it was located immediately below a tidal barrier. Glass eel activity during the flood tide may have been in response to cues other than tidal current, such as a tidally programmed biological clock, as suggested by McCleave & Wippelhauser (1987), or simply to moon phase or increasing tide height. Other studies in areas with no tidal current (Deelder 1952, Jellyman and Ryan 1983) found that peak glass eel and elver activity occurred during a specific period of time during the night and was unrelated to tidal height. Regardless of tidal cues, the artificial collectors effectively sample glass eels that actively move and subsequently resettle, if they are fished from sunset to sunrise. In areas where glass eel activity is known to peak during night-time flood tide periods, sampling may be optimised by deploying samplers only during nights when the flood tide occurs during one continuous night-time period.

This study provided evidence of a non-random spatial distribution, showing that glass eel densities increased with increasing distance from the drain in the centre of the causeway and were highest near the bank. A number of factors related to hydrography (Jellyman 1979, Domingos 1992) and habitat (Jellyman & Chisnall 1999) may cause such density gradients. Spatial location of replicate collectors must

be adequately accounted for in experimental designs employing this sampling technique.

Glass eel catches in artificial collectors at the tidal barrier were consistently lower than catches obtained with a Japanese net in the tidal current within the lower estuary (Table 2.1). However, monthly catches obtained with the two different methods were significantly correlated over a two year period, indicating that catches in the artificial collectors may provide a useful index of relative glass eel abundance.

The artificial habitat collectors developed in the present study may have great utility for sampling glass eels of all *Anguilla* spp. The collectors are easy to deploy, sample and transport, and many replicates can be easily deployed over a broad spatial and temporal scale in a wide variety of habitats, with little or no by-catch. The ability to sample a wide geographic range of sites simultaneously makes them potentially powerful tools for monitoring regional glass eel recruitment in multiple estuaries. Within a single estuary, collectors can be easily deployed in many different areas with potentially different habitat types in order to collect recruitment and resettlement data that can be used to address population modelling and conservation issues. In conjunction with samples of actively moving glass eels, information about sedentary glass eels from artificial habitat collectors will lead to a better understanding of anguillid glass eel recruitment and migration patterns.

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Chapter 3: Recruitment dynamics of *Anguilla australis* and *A. reinhardtii* glass eels in New South Wales

3.1 *Abstract*

Recruitment of glass eels to Australia's east coast is poorly understood with patchy information available for only a few estuaries over small spatial scales. There is certainly no information relating to the variability in recruitment of both shortfin and longfin glass eels to multiple New South Wales (NSW) estuaries or to a single estuary for more than two years. Spatial sampling of glass eels was done at six sites in NSW that are in the middle of the geographical distribution of these species. Temporal sampling at Audley provided five and four yearly recruitment seasons for shortfins and longfins respectively. This study shows that the recruitment season of shortfins is more easily defined than for longfins, and the main recruitment period for shortfins is from April to August, depending on site. The main recruitment season for longfins is from January to May and, again, this is site dependent, with recruitment pulses of longfins occurring well outside this period. The recruitment of these eels to NSW estuaries was not related to sea surface temperature or to downstream estuarine water temperature but a general trend shows that shortfins are caught during colder water months and longfins during warm water months of the year. There is seasonal overlap in recruitment of these species and shortfins become the dominant species in the catch from April/May while longfins become the dominant species from October/November. The relative abundance of longfin glass eels collected is not consistent with their adult distribution. They are classified as a tropical species yet longfin glass eels were more abundant at the Tweed R. (northernmost site), Mullet and Croobyar creeks (the third and second most southern sites, respectively).

The five year monthly collections of glass eels at Audley showed that the main shortfin season is April – September, depending on year, and the main longfin season is January – May, depending on year. The shortfin recruitment season at this site was more consistent and well defined than for longfins, with recruitment of longfins

often continuing outside of this season in relatively small numbers. However, the 2000/01 season showed an apparent failure of longfins to recruit to Audley. Again, there was no consistent relationship between sea surface temperature or downstream water temperature with the recruitment of either species at this site. The relative abundance of longfins at this site decreased every year from the 1998/99 season to 2000/01 season.

The condition of shortfins was significantly related to eel length so temporal declines in condition could not be assessed. However, shortfin lengths decreased with subsequent catches and progression of the recruitment season. Longfin condition was not related to length and there were significant temporal declines in the condition of eels at four of the six sampled sites. Also, there was a general temporal increase in the pigmentation of eels but the pigmentation frequencies were not consistent among sites. The temporal increase in pigmentation of eels was also found at Audley and the high variability in pigmentation frequencies between months was similar among years.

The lack of a lag time between recruitment of glass eels from the northern to most southern estuaries is discussed in relation to location of the spawning grounds and oceanic currents, with a theoretical glass eel transport and recruitment model proposed.

3.2 *Introduction*

It is generally considered that most marine organisms come from open populations where replenishment of local populations is from elsewhere (Booth & Brosnan 1995). The recruits that replenish these open populations can vary inter-annually, and are regulated by settlement rates in suitable habitats, post-settlement movements and mortality rates (Connell 1985). In the case of fish larvae, survival is determined by food availability, predator abundance and physical conditions, and the interaction of these factors can lead to spatial and temporal variation in recruitment (Cowen 2002). Therefore, we must study the early life history stages of marine organisms. However, studying the survival and dispersal of eggs and larvae at sea are problematic since these objects are small, are massively diluted, have lengthy development periods, may have critical environmental requirements and may travel long distances (Doherty 1992). Accordingly, recruitment of individuals to the population can be measured at the earliest stage of the life cycle after hatching that is practical to sample. For eels, the earliest and most practical life stage to study is that of the glass eel. Glass eels offer an estimate of recruitment strength because they pass through a "funnel" from the sea to freshwater, and are relatively easy to catch and identify.

The definition of "recruitment" can differ depending on the field of biological study. In fisheries studies, recruitment refers to the age at which fish join the fishery but it can also mean the stage at which juvenile fish are caught in surveys or samples (Booth & Brosnan 1995). The former is usually employed by fisheries biologists since they are interested in only those fish that are able to be caught by commercial fishing practices. The latter is often used by ecologists when describing the arrival of fish to specific habitats. Estuarine fish biologists often use the term recruitment to describe the arrival of juvenile fish into the estuary and this forms the basis of the definition of recruitment here. In this study, glass eel recruitment corresponds to the arrival of glass eels in the estuary zone (Guerault et al. 1991) and, more specifically, to the estuarine sampling location. The term "settlement" is the boundary between larval and juvenile stages of development. After metamorphosing from leptocephali, glass eels are no longer larvae but are the juvenile stage of the adult eels. However,

glass eels have not yet reached their desired destination ie. they are continuing their migration towards freshwater, so are still considered to be recruiting.

Glass eel recruitment to estuarine and freshwater habitats has been extensively studied in countries with a rich history and culture of eels. Scientists in the Netherlands have gathered data for the European eel *(A. anguilla*) glass eels recruiting from the Waddensea to Lake IJsselmeer from 1938 onwards and have detailed length distributions from 1960 onwards (Dekker 1998). This detailed and lengthy time series of data enabled Dekker (1998) to report on the recruitment failure of European eels in the late 1940's – early 1950's and from 1987 – 1993. However, this extensive recruitment information is lacking for most regions and for most species of Anguillid eels.

The New Zealand Maori had a good understanding of the upstream migration of shortfin (*A. australis*) and New Zealand longfin (*A. dieffenbachii*) glass eels prior to European settlement (Jellyman 1977) and they constructed methods for catching eels since they were a valuable food source (Downes 1917). There have been a number of glass eel recruitment studies done in New Zealand (Jellyman 1977, 1979, Jellyman & Ryan 1983, Jellyman et al. 1999, Chisnall et al. 2000, 2002) and, while there are no long term data sets, medium term data sets (ie approx 7 years) are available. This research seems to suggest that there is no sign of recruitment failure over this time scale.

There has also been a lack of research into the recruitment of glass eels in other parts of the world despite the eel's historic and/or economic importance. Despite the Japanese eel (*A. japonica*) being important in China, Taiwan, Japan and Korea as a food source, and the export of which has been economically lucrative, there is relatively little information on the current status of the stock. It appears, however, that annual catches of glass eels of this species has declined in all the above countries, with Japan showing the greatest decrease in catch from 130 tonnes in 1979 to approximately 50 tonnes in 1995 (Tzeng 1997). This status has mainly been inferred from commercial landings and production statistics, however this can vary due to demand and fishing pressure and may provide little use in determining

accurate yearly glass eel recruitment indices. This apparent decline in wild stocks of glass eels of the Japanese eel was seen as the cause of increased demand on exports of American glass eels (*A. rostrata*) (Atlantic States Marine Fisheries Commission 2000) and increased interest in using Australian eels to supplement the Asian aquaculture market. In an attempt to kerb the decline of the Japanese eel to Asia, recent glass eel recruitment studies have been completed that document the recruitment season and daily ages of these recruits to Japanese waters as well as oceanographic processes that influence the recruitment (Cheng & Tzeng 1996, Kawakami et al. 1999, Yamamoto et al. 2001).

The American eel has historically been an important food source for native Americans, and the first systematic records of eel harvests in the United States of America (USA) were in 1887. Recording of commercial eel landings in the USA commenced in the 1950's yet the current status of American eel stocks is still poorly understood (Atlantic States Marine Fisheries Commission 2000). A continent-wide decline in the abundance of American eels in North America was documented by Richkus & Whalen (1999) however there were time series limitations in some of the data sets used such as unavailability of data from all locations over the same time period. The commencement of research into glass eel/elver recruitment in North America has only been recent (McCleave & Kleckner 1982, Sorensen & Bianchini 1986, Martin 1995, Jessop 2000, Powles & Warlen 2001) and there are no long term (> 20 years) recruitment data available. In fact, as seen from the above, even reliable and standardised five year recruitment datasets are absent or not readily available from many countries that harvest Anguillid eels.

Prior to the 1990's, relatively little research was done on Australian eels and these reports included data that was patchy for some areas of the eastern seaboard. The earliest descriptions of anguillid glass eel collections in Australia were Schmidt (1928) followed by Ege (1939). Both authors gave detailed accounts of the biological and geographical attributes of anguillids found in Australia, with Ege (1939) extending his comparisons to all species within the *Anguilla* genus. Beumer & Harrington (1980) then provided a general overview of the glass eel and elver life history stage that included methods for collection. Sloane (1984a) reported the first

of the more detailed studies into glass eel recruitment of shortfins (*A. australis*) and longfins (*A. reinhardtii*) at the southern end of their Australian distribution. He hand-netted and electrofished at the estuary-freshwater interface in six rivers on the east coast of Tasmania over a four year period. Beumer & Sloane (1990) presented the status of the knowledge of the distribution and abundance of glass eels in eastern Australia. This illustrated the lack of information from New South Wales (NSW) with less than 3% of the glass eel occurrence data (6 records) from this state but which comprised almost half of the coastline studied (Pease et al. 2003). In contrast, there were 33 records from Queensland, 67 records from Victoria, and 55 records from Tasmania.

The potential of Australian eels as seedstock for intensive aquaculture instigated a 1995 survey to investigate the distribution of glass eels in south east Queensland. Sampling was conducted at barriers at the estuary-freshwater interface or where a barrier restricted water flow, and shortfins were suggested as having potential for intensive stocking (Queensland Department of Primary Industry, 1995). McKinnon & Gooley (1998) investigated the invasion of shortfin glass eels in association with environmental cues in 17 estuaries in south-eastern Australia using glass eel nets (fyke nets constructed of 2 mm stretched mesh with 3.5 m wings) and stow nets (2 mm stretched mesh as described by Weber 1986 with a 6.9 m codend attached). However, each estuary was sampled over a few days only so no comparisons of spatial and temporal recruitment patterns can be made. Gooley et al. (1999) focussed their study on the assessment of the shortfin glass eel fishery in south-eastern Australia (predominantly Victoria) for intensive culture to satisfy both the domestic and export markets.

The continuing interest in Australian glass eels as seedstock for aquaculture, along with the paucity of data relating to the spatial and temporal recruitment of these eels over their Australian geographic range, provided a basis for a collaborative research program to assess the stocks of shortfin and longfin glass eels in 1997 by Australia's eastern states (ie Queensland, NSW, Victoria and Tasmania). While of a preliminary nature, information on glass eel recruitment, the wild fishery and aquaculture potential contained in the report by Gooley & Ingram (2002) filled some of the

knowledge gaps on the Australian eel species. Pease et al. (2003) documented the spatial and temporal characteristics of migration from estuarine to fresh waters of both species in one estuary in NSW over two migration seasons. However, while the Gooley & Ingram (2002) study, and subsequent associated research from that study (Pease et al. 2003), vastly improved our knowledge of recruitment timing of shortfin and longfin glass eels, it also highlighted the need for more localised research due to the high variation in glass eel recruitment temporally and over large spatial scales. Ultimately, the limitation of all these studies is that they lack the consistent and simultaneous sampling of estuaries over a wide geographic range, an attribute that has shown to be vital for identifying recruitment failure and for successful management of the resource.

As seen in Chapter 1, the anguillid eel life-cycle is complex. The main aspects of this life-cycle that are of interest here are the assumed passive transport of leptocephali (McCleave et al. 1998) via the South Equatorial Current (SEC) from their south-western Pacific Ocean spawning grounds, the transport of glass eels (after metamorphosis from leptocephali) by the East Australian Current (EAC), and the subsequent recruitment of these glass eels to the estuary. Since the EAC is the dominant oceanographic influence along the Australian east coast and, as it flows from north to south, this study examines the null hypothesis that the recruitment of both species of glass eels to the six sites along the NSW coast occurs sequentially from north to south, with recruitment at each site separated by a time lag period. Furthermore, this study also aims to identify the recruitment seasons, condition and pigment stages of shortfin and longfin glass eels at six sites along the NSW coastline and to determine the physical factors that affect that recruitment. Long term temporal patterns were examined using five and four year temporal recruitment information for shortfins and longfins, respectively, at one site in NSW and factors that may have affected this recruitment. Finally, a conceptual model of glass eel recruitment in NSW was developed in accordance with the results obtained.

Future studies may use this spatial and temporal recruitment information in conjunction with subsequent cohort analysis of yellow eels to determine mortality rates and assess yellow eel stocks. The identification of temporal and spatial

recruitment patterns in NSW is needed to establish sustainable management policies that fairly allocate glass and yellow eels to stakeholders.

3.3 *Methods*

3.3.1 *Site selection*

Estuaries within NSW can be divided into three bioregions based on environmental and fisheries attributes (Pease 1999). Estuaries in NSW found at latitudes below 32°S are in the northern region, estuaries between 32°S and 35°10'S are in the central region, and estuaries in NSW at latitudes above 35°10'S are in the southern region. To make possible comparisons of recruitment among bioregions, six sites were chosen along the NSW coast to monitor glass eel recruitment, two sites within each bioregion (Table 3.1, Fig. 3.1). These sites were chosen using the primary criteria that they were to be at the tidal interface in each estuary. This was so that the likelihood of catching glass eels was increased since glass eels accumulate at these locations to undertake a physiological change to survive in freshwater. Secondly, at this interface, a barrier was required, man-made or natural, that defined where that interface was. This was so that, irrespective of tidal conditions, the interface would always be at the same location. Also, each site had to be accessible by car so that one person could collect a monthly sample at all sites within a one week period. The sites, listed from north to south, are as follows:

1. Bray Park weir on the main channel of the Tweed River.
2. The Lansdowne weir on the Lansdowne River which flows into the Manning River before reaching the ocean.
3. Audley weir on the main channel of the Hacking River.
4. William Beach Park weir on Mullet Creek which flows into Lake Illawarra before reaching the ocean.
5. Avonlea weir on Croobyar Creek which flows into Narrawallee Inlet before reaching the ocean.
6. Boggy Creek which flows into Merimbula Lake. At this site a natural rocky barrier separates the salt and freshwater sides of the river, rather than a man-made weir.

Table 3.1 – Structural details of barriers where glass eels were sampled.

Barrier name	*Estuary*	*Length (m)*	*Height (m)*	*Distance upstream (km)*	*Latitude(E)*	*Longitude (S)*
Bray Park weir	Tweed R.	110	2.6	33	153°22'25.8960"	28°20'47.8428"
Lansdowne weir	Lansdowne R.	28.5	2.4	28.25	152°31'53.3388"	31°47'44.3688"
Audley weir	Hacking R.	90	2.4	12	151°03'19.4688"	34°04'29.0208"
William Beach Park weir	Mullet Ck.	15	1.0	4	150°48'25.3872"	34°28'52.9572"
Avonlea weir	Croobyar Ck.	9	2.0	6.6	150°24'52.326"	35°18'32.8428"
Natural barrier	Boggy Ck.	2	1.0	7.25	149°52'17.6304"	36°53'07.1736"

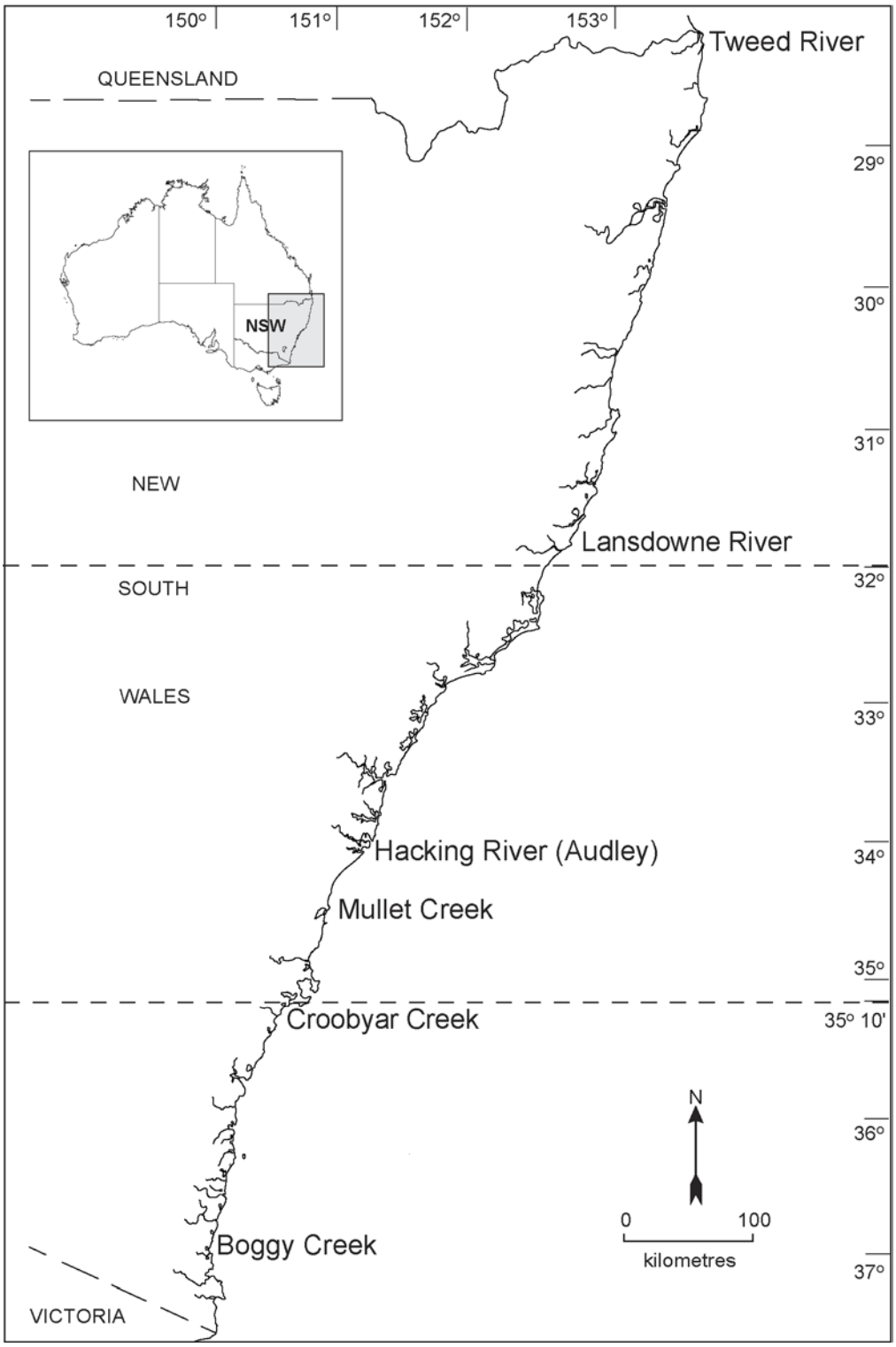

Fig. 3.1 – Location of sampled estuaries in NSW. Delineation of each bioregion is shown by dashed line.

3.3.2 *Sampling Protocol*

Sampling commenced at five of the six sites on 2nd July 2000 and commenced at Croobyar Ck on 30th Nov 2000. Samples were collected monthly at all sites during the new moon period until Dec 2001. All sites were sampled within seven days of the new moon in each month and the order in which sites were sampled differed between sampling trips to avoid any bias in catch quantities by always sampling the same sites at the beginning and end of the lunar sampling period.

The Audley site was sampled for an extended period from May 1998 until June 2002. Two full sized collectors (Chapter 2) were used at this site from 1998 to May 2000, after which 10 half sized collectors were used at all sites. Half sized collectors were implemented as standard due to ease of use as they are more manageable and Silberschneider et al. (2001) demonstrated that glass eel densities were linearly related to the number of fibres on collectors. Therefore, glass eels caught in full and half sized collectors are comparable.

The collectors were left in the water for one night so that all sites could be sampled within the one week new moon period. Glass eels were collected, identified and measured as described in Chapter 2. Length, weight and pigmentation stage were taken for all glass eels collected and were done using preserved samples. To ensure that the preservative did not bias measurements, eels were patted dry on absorbent paper prior to weighing. Peak glass eel recruitment periods at a site were defined as when catches were $\geq$ 9 glass eels/month, based on analysis of initial samples at Audley (Pease et al. 2003).

Newly arrived glass eels were defined as those eels that had reached a pigmentation stage of up to, and including, $VI_{AII}4$ (Strubberg 1913) which is equivalent to pigmentation stage 4 in Ege (1939). This was done to distinguish between those eels that had arrived during the sampled new moon period and those that had arrived during previous lunar periods (including full moon migrations). Pigment groupings, to separate new and previously arrived glass eels, were based on rates of pigment increase with increased water temperature (Jellyman 1977, Jellyman et al. 1999, pers.

com., Dou et al. 2003) extrapolated from the mean water temperature during the recruitment season for each species at each site.

To determine whether sea surface temperature, downstream estuarine water temperature or the difference between these two parameters were correlated with recruitment of newly arrived shortfin and longfin glass eels, correlation analyses (using the Pearson correlation co-efficient) was performed using Statistica version 6.0 (Statsoft 2001). Sea surface temperature data was accessed from Many Hydraulics Laboratory and from http://poet.jpl.nasa.gov and water temperature both above and below the barriers was recorded with a temperature datalogger.

The relative abundance of each species from the six sample sites was calculated from November 2000 – October 2001 as this incorporated the shortfin and longfin recruitment seasons for the same year. For the longer term Audley study, November – October was used to calculate relative abundance for those years where sampling of this entire period was completed. In this analysis, all eels that had not yet attained a Strubberg (1913) pigment stage of VI_B1, equivalent to Ege (1939) stage 8, were used since all eels arrived during the same recruitment year.

Condition factor (K) was used to determine the condition of newly arrived shortfin and longfin glass eels entering an estuary during their peak recruitment times. This was done since length and weight are autocorrelated, and condition is determined by both of these parameters by the equation:

$$K = 1000W/L^3$$

where W = weight (g) of each glass eel, and L = length (mm) of each glass eel. One-way ANOVA and correlation comparisons (using the Pearson correlation co-efficient) were performed for each site separately to test the hypothesis that glass eels entering the estuary later in the recruitment season, had reduced condition.

The percentage of each pigmentation stage (1 – 8; Ege 1939) from each collector during each monthly catch at each site was analysed using Primer for Windows version 5.2.9. Ege (1939) pigmentation stages were preferred over Strubberg (1913) pigmentation stages to gain more distinct categories. This removed bias from an

increased number of eels that could have a pigment stage on the border of any two stages in the Strubberg stages, and gave the added benefit of more robust analyses since there were more data within the 8 pigmentation stages. For shortfins, data used was from April – September 2001. For longfins, data used was from January – April 2001. These were the peak recruitment times for each species and were the only entire seasons sampled at all sites. The similarity matrices were constructed using Bray-Curtis similarity with no transformation since the data was already transformed to percentage composition of each Ege (1939) pigmentation stage. An MDS plot was then constructed and the adequacy of the two dimensional representations of the similarities among samples was assessed by examining the stress value. According to Clarke & Warwick (1994), stress values < 0.05 give an excellent representation with no prospect of misinterpretation, and stress values < 0.1 indicate a good ordination with no real prospect of a misleading interpretation. If the MDS solution was degenerative (one sample separated from the rest of the data points), the analysis was re-run (with the one sample excluded), and this data was used.

The MDS was followed by ANOSIM (analysis of similarity) to determine significant differences between sites and the critical value was set at the default of 0.001 (following Clarke & Warwick 1994). Differences between pairwise tests of sites were measured using the pairwise R values since they give a measure of how separated the groups are, from 0 (groups are indistinguishable from each other) to 1 (all similarities within groups are less than any similarity between groups). Groups are thought to be well separated when R > 0.75 and overlapping but still clearly different when R is approximately 0.5 (Clarke & Gorley 2001). SIMPER (analysis of similarity percentages) was then performed to test for the pigmentation stages that contributed most to the significant differences between sites.

Where applicable, the Kolmogorov-Smirnov (K-S) test was used to test for normality and Bartlett's test was used to test for homogeneity. While most analyses used are robust with respect to departures from normality and homogeneity (Zar 1999), the assumptions were met.

Salinity was recorded using a datalogger both above and below the barrier on the day sampled to check the validity that the barrier separated salt and freshwater areas.

Information gained from the recruitment data was then related to sea surface temperatures and ocean current maps for the east Australian coast to develop a theoretical transport model of glass eels to NSW estuaries. These maps were accessed from www.aodc.gov.au.

3.4 *Results*

Newly arrived shortfin glass eels accounted for 58.7% of all shortfins (newly and previously arrived) collected at the six sites sampled from June 1999 – December 2000 (n = 2161). Newly arrived longfin glass eels accounted for 78.0% of all longfins collected at the six sites sampled during the same period (n = 3645). For Audley, newly arrived shortfin glass eels accounted for 85.5 % of all shortfins collected from May 1998 – June 2002 (n = 1361). Newly arrived longfins at Audley accounted for 84.1 % of all longfins collected in this same period (n = 656).

3.4.1 *Spatial recruitment patterns*

Shortfin glass eels were caught at all sites throughout the sampling period (Table 3.2). There was an extended period of capture of newly arrived shortfin eels at more southerly sites (Fig. 3.2). This is indicated by the failure to catch newly arrived shortfins for 11 of the 19 months at the northern most sites (Tweed and Lansdowne rivers), for eight months at the mid region sites (Audley and Mullet Ck) and for five and ten months at the southern most sites (Croobyar and Boggy creeks respectively). There does not appear to be a lag period in shortfin recruitment from north to south in 2001 since peak recruitment occurred between May and July at all sites. The beginning of the recruitment season may have been missed in 2000. All shortfin glass eels can be found over an extended period at each site (Table 3.2), predominantly during the cold water months. They were found in the Tweed and Lansdowne rivers from April – Oct, in the Hacking R from March – Oct, in Mullet

Ck from March – Sept, in Croobyar Ck from Feb – Nov, and in Boggy Ck from March – Oct.

Table 3.2 – Total number of newly arrived (n) and previously arrived (p) shortfin glass eels collected at each site and time. na = not sampled.

	Tweed R.		***Lansdowne R.***		***Audley***		***Mullet Ck.***		***Croobyar Ck.***		***Boggy Ck.***	
Month	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**
June '00	1	4	12	17	393	61	27	10	na	na	0	5
July	8	11	13	17	25	9	17	19	na	na	0	51
Aug	0	3	0	32	9	2	4	5	na	na	6	43
Sept	1	8	0	9	1	8	2	5	na	na	0	4
Oct	0	3	0	0	1	2	0	0	na	na	0	0
Nov	0	0	0	0	0	0	0	0	11	2	0	1
Dec	0	0	0	0	0	0	0	0	0	0	0	0
Jan '01	0	0	0	0	0	0	0	0	0	0	0	0
Feb	0	0	0	0	0	0	0	0	12	0	0	0
Mar	0	0	0	0	0	1	3	2	0	1	2	0
Apr	0	1	14	10	2	2	23	1	2	7	1	0
May	0	1	21	4	25	3	15	8	1	5	10	5
June	40	9	1	19	268	24	82	17	0	16	8	19
July	7	2	1	22	36	36	37	66	42	18	5	10
Aug	2	2	2	38	3	3	23	21	3	16	2	23
Sept	5	2	1	112	3	1	3	6	11	8	0	9
Oct	0	3	0	1	0	2	0	0	5	6	1	8
Nov	0	0	0	0	0	0	0	0	5	0	0	0
Dec	0	0	0	0	0	0	0	0	0	0	0	0

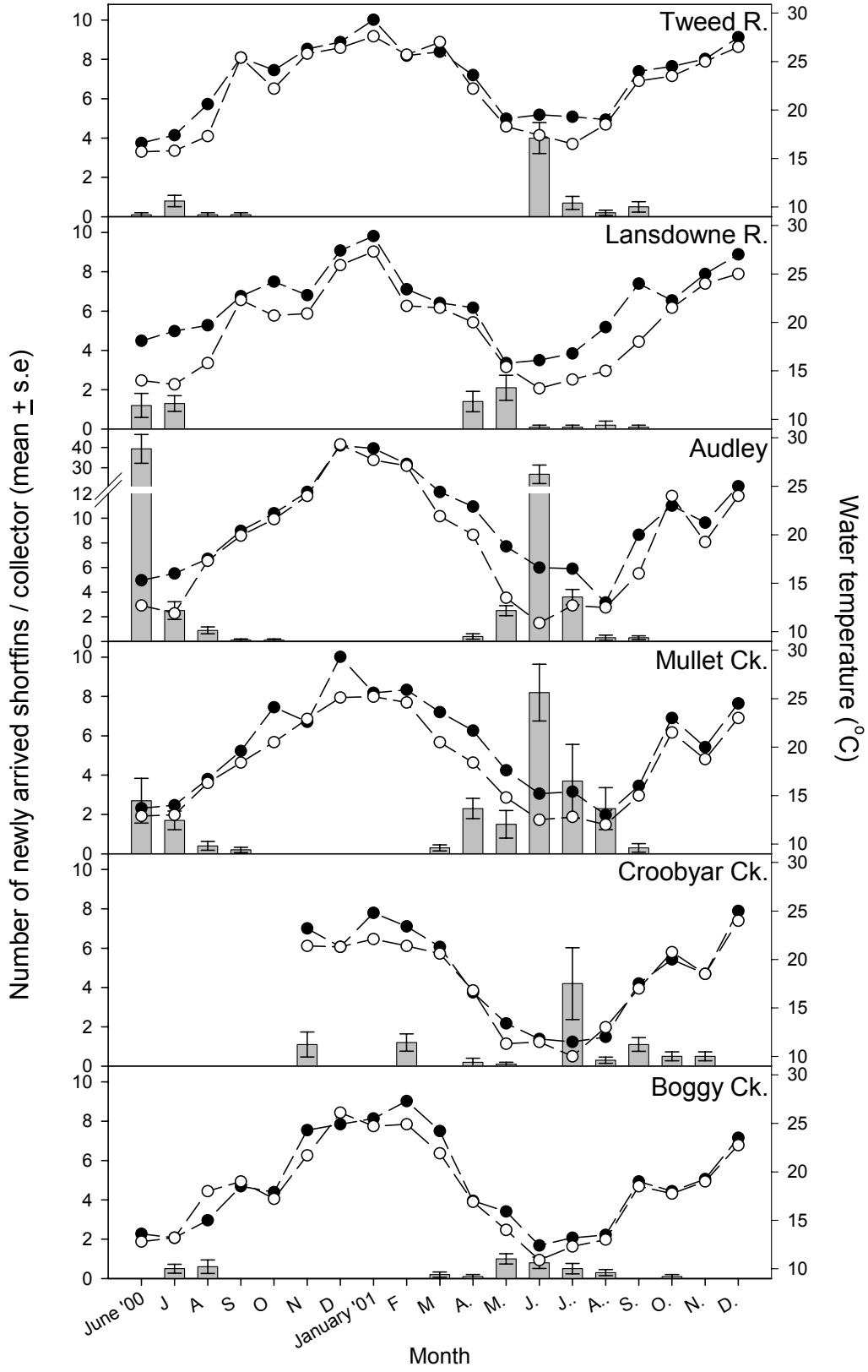

Fig. 3.2 – Catch per unit effort (CPUE) of newly arrived shortfin glass eels at each site. Filled circles indicate downstream temperature, open circles indicate upstream temperature.

Longfin glass eels were also caught at all sites during the sampling period and displayed a less distinctive seasonal recruitment pattern between sites than shortfins (Table 3.3, Fig. 3.3). The length of the recruitment period decreased with increasing latitude, resulting in more distinct seasons at the southern most sites. There appeared to be no progressive spatial recruitment pattern of longfins. January and February appear to be the months when highest catches of these glass eels were recorded, however this seems to be site-dependent eg. Tweed R and Mullet Ck had highest catches in January, Croobyar Ck had its highest catch in February while Audley and Boggy Ck had highest catches in March. There appears to be no lag period in catches from north to south, however the longfin season did not commence until March in Boggy Ck, well after that of most sites.

Table 3.3 – Total number of newly arrived (n) and previously arrived (p) longfin glass eels collected at each site and time. na = not sampled.

	Tweed R.		***Lansdowne R.***		***Audley***		***Mullet Ck.***		***Croobyar Ck.***		***Boggy Ck.***	
Month	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**
June '00	0	1	3	13	7	1	1	0	na	na	0	0
July	1	8	0	3	1	0	0	0	na	na	0	1
Aug	0	21	0	2	0	0	0	0	na	na	0	1
Sept	1	16	0	0	2	0	0	0	na	na	0	2
Oct	0	0	0	0	0	0	0	1	na	na	0	0
Nov	1	0	5	0	0	7	11	1	396	65	0	0
Dec	0	1	0	0	6	0	33	4	6	13	0	0
Jan '01	55	1	0	0	2	0	291	63	217	28	0	0
Feb	6	4	13	3	10	2	57	26	1487	307	0	3
Mar	1	0	0	0	17	5	60	37	5	2	2	11
Apr	1	8	0	6	14	1	17	8	2	44	0	18
May	0	3	0	0	0	2	0	3	0	1	0	3
June	2	3	0	1	1	3	1	0	0	0	0	0
July	0	1	0	3	1	1	0	1	0	3	0	0
Aug	1	1	0	6	0	0	1	0	1	0	0	1
Sept	4	1	00	5	6	1	13	5	41	10	0	1
Oct	0	0	0	1	0	0	1	0	2	0	0	1
Nov	4	0	0	0	0	0	0	0	4	0	0	0
Dec	2	0	21	3	0	0	0	0	6	3	0	0

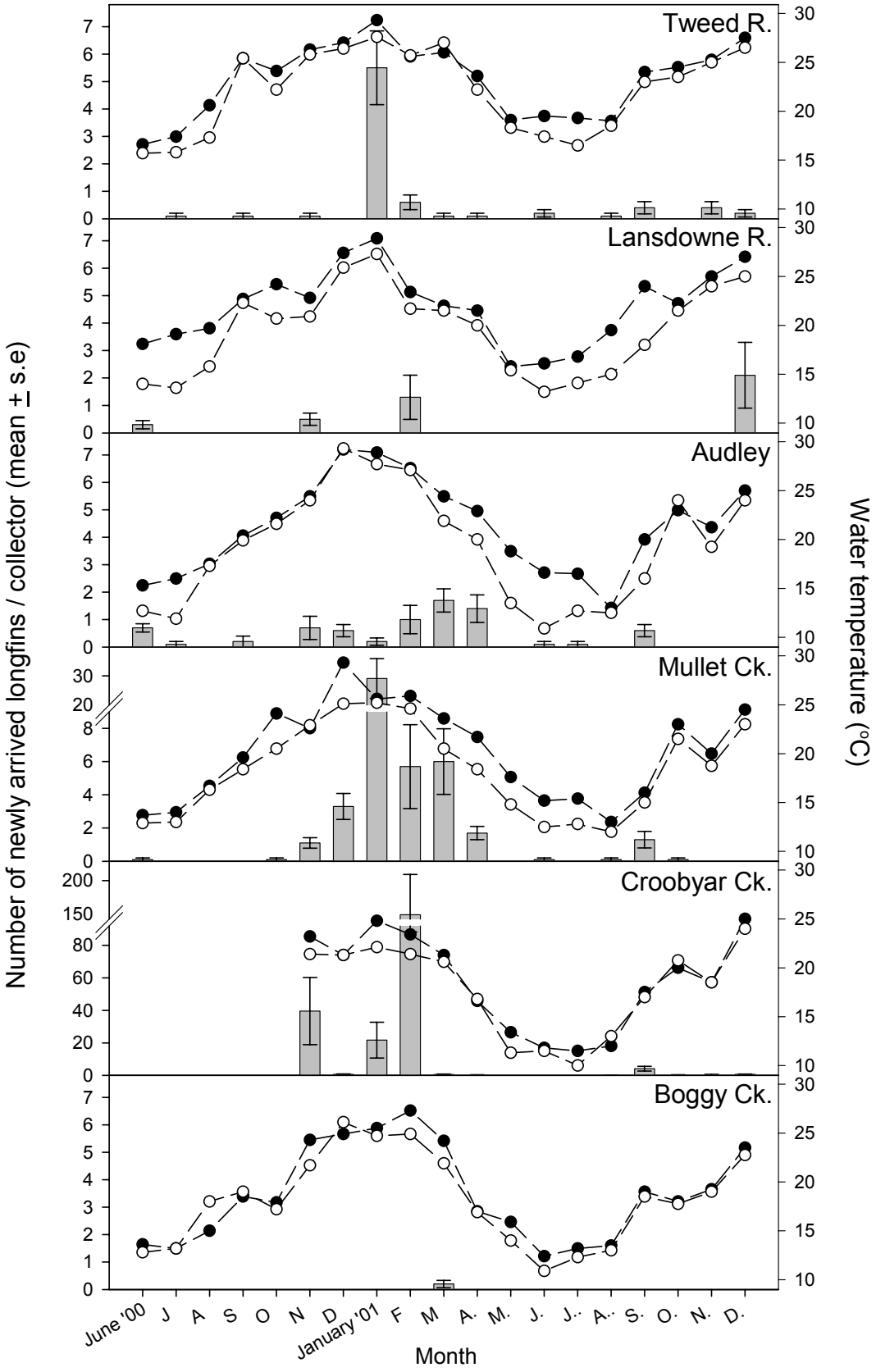

Fig. 3.3 – CPUE of newly arrived longfin glass eels at each site. Filled circles indicate downstream temperature, open circles indicate upstream temperature.

Based on the catches of newly recruited glass eels in 2000/01, the peak recruitment period for longfins appears to be January for the Tweed R., February for Lansdowne, February - April for Audley, November – April for Mullet Ck, November – February for Croobyar Ck, and March for Boggy Ck. However, based on $\geq$ 9 newly recruited glass eels, the main recruitment period could also include September for Mullet and Croobyar creeks while December 2001 at Lansdowne R could indicate the early onset of the longfin recruitment season for 2001/02.

Allowing for one complete recruitment season for each species at each site, the relative abundance of longfins was calculated (Table 3.4). Longfins were most abundant at Tweed R., Mullet and Croobyar creeks. Hence, shortfins were most abundant at Lansdowne R., Audley and Boggy Ck.

Table 3.4 – Relative abundance of longfins at each site from November 2000 – October 2001.

	Tweed R.	***Lansdowne R.***	***Audley***	***Mullet Ck.***	***Croobyar Ck.***	***Boggy Ck.***
Shortfins	74	246	409	307	166	104
Longfins	94	43	79	633	2630	38
% Longfins	56%	15%	16%	67%	94%	27%

3.4.2 *Temporal recruitment patterns*

In the Tweed R, shortfin glass eels were caught from June – October 2000 and from April – October 2001 (Table 3.2). The highest catch of newly arrived glass eels at this site was in July 2000 with 4.0 $\pm$ 0.8 glass eels per collector (mean $\pm$ s.e., Fig. 3.2). In the Lansdowne R, this species was caught from June – Sept 2000 and from April – October 2001 (Table 3.2). The highest catch of 2.1 $\pm$ 0.6 newly arrived glass eels per collector (mean $\pm$ s.e.) was recorded in May 2001. At Audley, shortfins were caught from June – October 2000 and from March – October 2001 (Table 3.2). June of both years recorded the highest catches of newly arrived glass eels with 39.3 $\pm$ 7.1 and 26.8 $\pm$ 4.5 eels per collector (mean $\pm$ s.e.) in 2000 and 2001 respectively (Fig. 3.2). In Mullet Ck, shortfin glass eels were caught from June – September 2000 and from March – September 2001. At this site, the highest catch of 8.2 $\pm$ 1.4 newly arrived glass eels per collector (mean $\pm$ s.e.) was recorded in June 2001 and June

2000 also recorded the highest catch of newly arrived eels for that year but with a smaller number of glass eels per collector (2.7 ± 1.1, mean ± s.e.). In Croobyar Ck, shortfin glass eels were caught in the first sampling occasion at this site (November 2000) and then from February – November 2001 (Table 3.2). Here, the highest catch of 4.2 ± 1.8 newly arrived glass eels per collector (mean ± s.e.) was recorded in July (Fig. 3.2). In Boggy Ck, shortfins were caught from June – November 2000 (zero catch in October) and then from March – October 2001 (Table 3.2). In June 2001 the highest catch of 1.0 ± 0.3 newly arrived glass eels per collector (mean ± s.e.) was recorded (Fig. 3.2).

Longfin glass eels were absent from the catch in the Tweed R., the northern most site, only in October of both years (Table 3.3). January 2001 produced the peak catch with 5.5 ± 1.3 newly arrived glass eels caught per collector (mean ± s.e., Fig. 3.3). In the Lansdowne R., this species was caught from June – August and November 2000, followed by February, April, June – October and December 2001 (Table 3.3). However, newly arrived glass eels were only caught in four months. The peak catch of newly arrived glass eels was in December 2001 with 2.1 ± 1.2 eels per collector (mean ± s.e., Fig. 3.3). At Audley, longfin glass eels were collected in all months except August and October of both years, as well as November and December 2001 (Table 3.3). Peak catches of newly arrived longfins were collected at Audley during March 2001 with 1.7 ± 0.4 glass eels per collector (mean ± s.e., Fig. 3.3) and this was closely followed by the catch in April 2001 (1.4 ± 0.5 glass eels per collector, mean ± s.e.). In Mullet Ck, longfin glass eels were caught in June 2000 and between October 2000 and October 2001 (Table 3.3). The peak catches of newly arrived glass eels during this period was in January with 29.1 ± 6.8 eels per collector (mean ± s.e., Fig. 3.3). In Croobyar Ck, longfin glass eels were caught from November 2000 to May 2001 as well as between July and December 2001 (Table 3.3). The greatest catch of newly arrived glass eels was during February 2001 with 148.7 ± 60.5 eels per collector (mean ± s.e., Fig. 3.3). Fewest longfin glass eels were collected at Boggy Ck, the southern most site, and the occurrence of longfins were recorded the least number of times at this site (Table 3.3). Longfin glass eels were collected from July – September 2000, February – May 2001 and August –

October 2001 (Table 3.3). Newly arrived glass eels were only recorded in March 2001 where 0.2 ± 0.1 glass eels per collector (mean ± s.e.) were caught (Fig. 3.3).

There was no consistent correlation between catches of newly arrived glass eels of either species and sea surface temperature, downstream water temperature or the difference between these two parameters (Tables 3.5, 3.6). For shortfins, significant correlations between downstream temperature and recruitment of glass eels were found for Mullet and Boggy creeks, meaning that a greater number of shortfins recruited to these sites when estuarine downstream temperature decreased. There was also a positive correlation between shortfin recruitment and the difference between sea surface temperature and downstream water temperature at Lansdowne R., Mullet and Boggy creeks. This means that a greater difference between the parameters leads to higher shortfin recruitment at these sites. No significant correlations were found between sea surface temperature and shortfin recruitment, however most sites recorded a negative correlation implying that shortfin recruitment occurs during cold water months (Fig. 3.4).

Table 3.5 – Relationship between newly arrived shortfins and sea surface temperature (SST), downstream temperature (DT) and the difference between these two parameters (SST-DT) at each site. Correlation coefficients (r) are shown with significant correlations shown in bold.

Site	***SST***		***DT***		***SST-DT***	
	r	**p**	**r**	**p**	**r**	**p**
Tweed R.	-0.227	0.416	-0.466	0.080	0.370	0.175
Lansdowne R.	0.303	0.272	-0.485	0.067	0.523	**0.045**
Audley	-0.123	0.662	-0.409	0.130	0.463	0.082
Mullet Ck.	-0.193	0.492	-0.645	**0.009**	0.726	**0.002**
Croobyar Ck.	-0.052	0.861	-0.316	0.272	0.391	0.167
Boggy Ck.	-0.240	0.390	-0.636	**0.011**	0.728	**0.002**

For longfins, significant positive correlations were found between sea surface temperature and new recruits at Audley and Croobyar Ck. meaning that an increase in SST causes an increase in longfin recruitment at these sites. Also, positive non-significant correlations between recruitment and SST were found at the remaining sites, implying that longfin recruitment occurs during the warm water months (Fig.

3.4). No correlations were found between the recruitment of newly arrived longfins and downstream water temperature or the difference between sea surface temperature and downstream water temperature. However, all sites recorded positive non-significant correlations between longfin recruitment and downstream estuarine water temperature, again implying that recruitment of this species to estuaries occurs during warm water months.

Table 3.6 – Relationship between newly arrived longfins and sea surface temperature (SST), downstream temperature (DT) and the difference between these two parameters (SST-DT) at each site. Correlation coefficients (r) are shown with significant correlations shown in bold.

Site	***SST***		***DT***		***SST-DT***	
	r	**p**	**r**	**p**	**r**	**p**
Tweed R.	0.497	0.059	0.468	0.079	-0.172	0.539
Lansdowne R.	0.458	0.086	0.316	0.251	-0.053	0.851
Audley	0.638	**0.011**	0.346	0.207	-0.083	0.768
Mullet Ck.	0.479	0.071	0.388	0.153	-0.245	0.379
Croobyar Ck.	0.600	**0.023**	0.405	0.151	-0.145	0.620
Boggy Ck.	0.101	0.721	0.253	0.364	-0.284	0.304

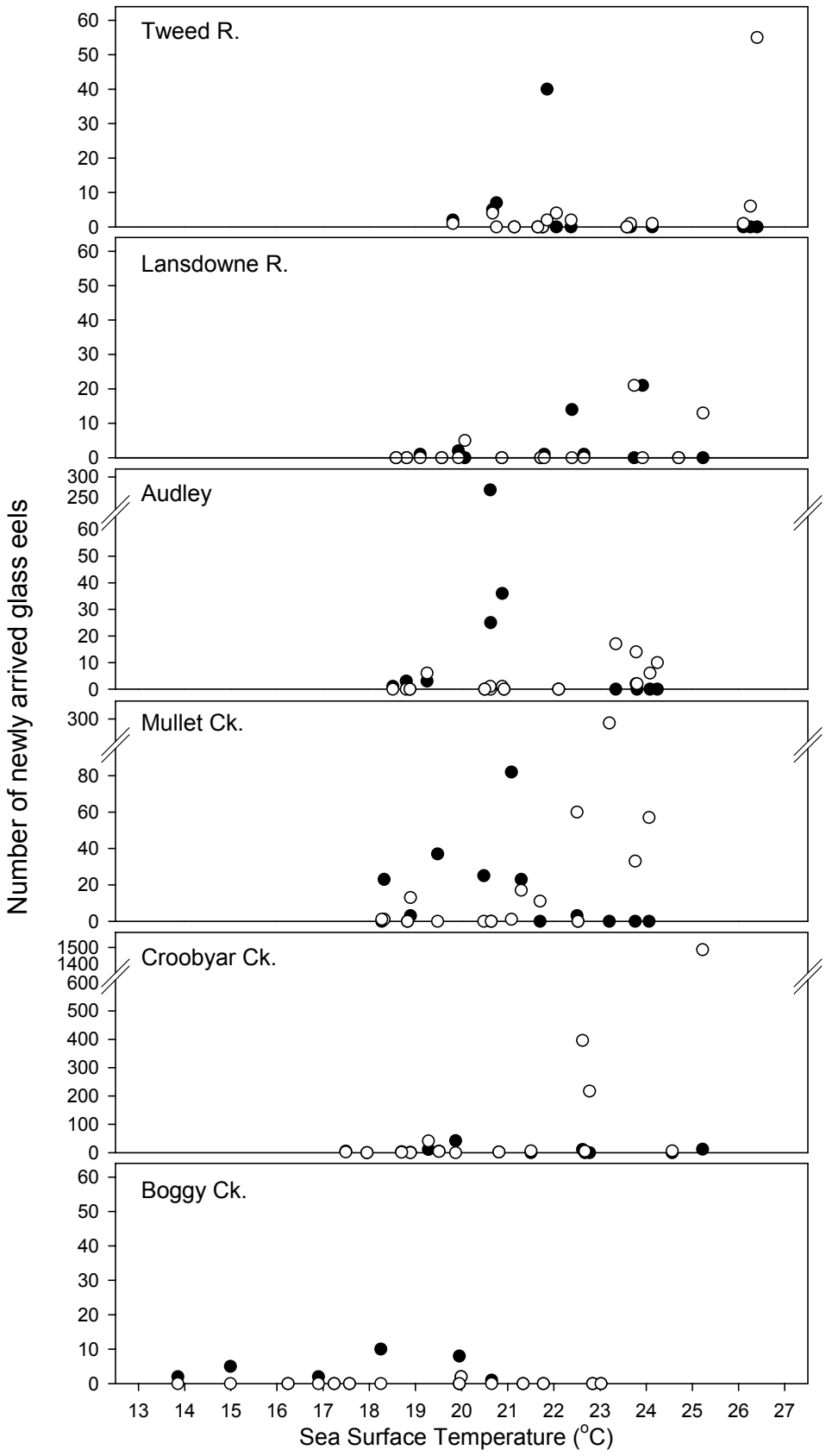

Fig. 3.4 – Relationship between recruitment of newly arrived shortfins (filled circles) and newly arrived longfins (open circles) with sea surface temperature.

3.4.3 *Long term seasonal and inter-annual recruitment patterns at Audley*

From 1998 to 2002 shortfin glass eels were caught at Audley between February and November (all years combined, Table 3.7) with a peak from April/May to August/September, depending on the year. Newly arrived shortfin glass eels were most abundant in either May (1998, 1999) or June (2000, 2001, 2002) although the CPUEs varied annually from 75.0± 0.0 (mean ± s.e.) in May 1999 to 3.7 ± 0.6 (mean ± s.e.) in June 2002 (Fig 3.5). The standard error for May 1999 is zero since, at the time of sampling, all eels were counted together and, therefore, the total was divided by two (the number of collectors used) to gain the mean number of glass eels per collector. The pattern in catches of shortfins is similar among years (Table 3.7, Fig. 3.5) with perhaps a small catch in late autumn, followed by a peak in catch, with subsequent months showing a sharp decrease in catches. Figure 3.5 also shows that catches of shortfins were similar among collectors, as indicated by the small standard errors.

Table 3.7 – Total number of newly arrived (n) and previously arrived (p) shortfin glass eels collected at Audley from 1998 – 2002. na = not sampled.

Year	***1998***		***1999***		***2000***		***2001***		***2002***	
Month	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**
Jan	na	na	0	0	0	0	0	0	0	0
Feb	na	na	0	0	0	0	0	0	2	0
March	na	na	0	0	1	0	0	1	1	0
April	na	na	10	0	0	0	2	2	19	0
May	58	4	150	7	0	0	25	3	2	3
June	35	9	27	0	393	61	268	24	37	0
July	29	2	2	0	25	9	36	36	na	na
Aug	7	8	8	6	9	2	3	3	na	na
Sept	0	4	8	0	1	8	3	1	na	na
Oct	0	0	0	0	1	2	0	2	na	na
Nov	0	0	2	0	0	0	0	0	na	na
Dec	0	0	0	0	0	0	0	0	na	na

The recruitment of longfins to Audley was less consistent than that for shortfins with high variability in catches among years (Fig. 3.6, Table 3.8). However, the longfin recruitment season at this site can be classified as being from January/February to April/May. Peak months for collecting newly arrived longfin glass eels were in either February (1999) or March (2000, 2001, 2002) although the CPUEs varied annually from 41.0 $\pm$ 1.0 (mean $\pm$ s.e.) in February 1999 to 1.7 $\pm$ 0.4 in March 2001 (Fig. 3.6). It is also interesting to note the apparent failure of longfins to recruit to Audley in the 2000/01 season which is evident by the low CPUEs and small numbers of glass eels that were caught (Fig. 3.6).

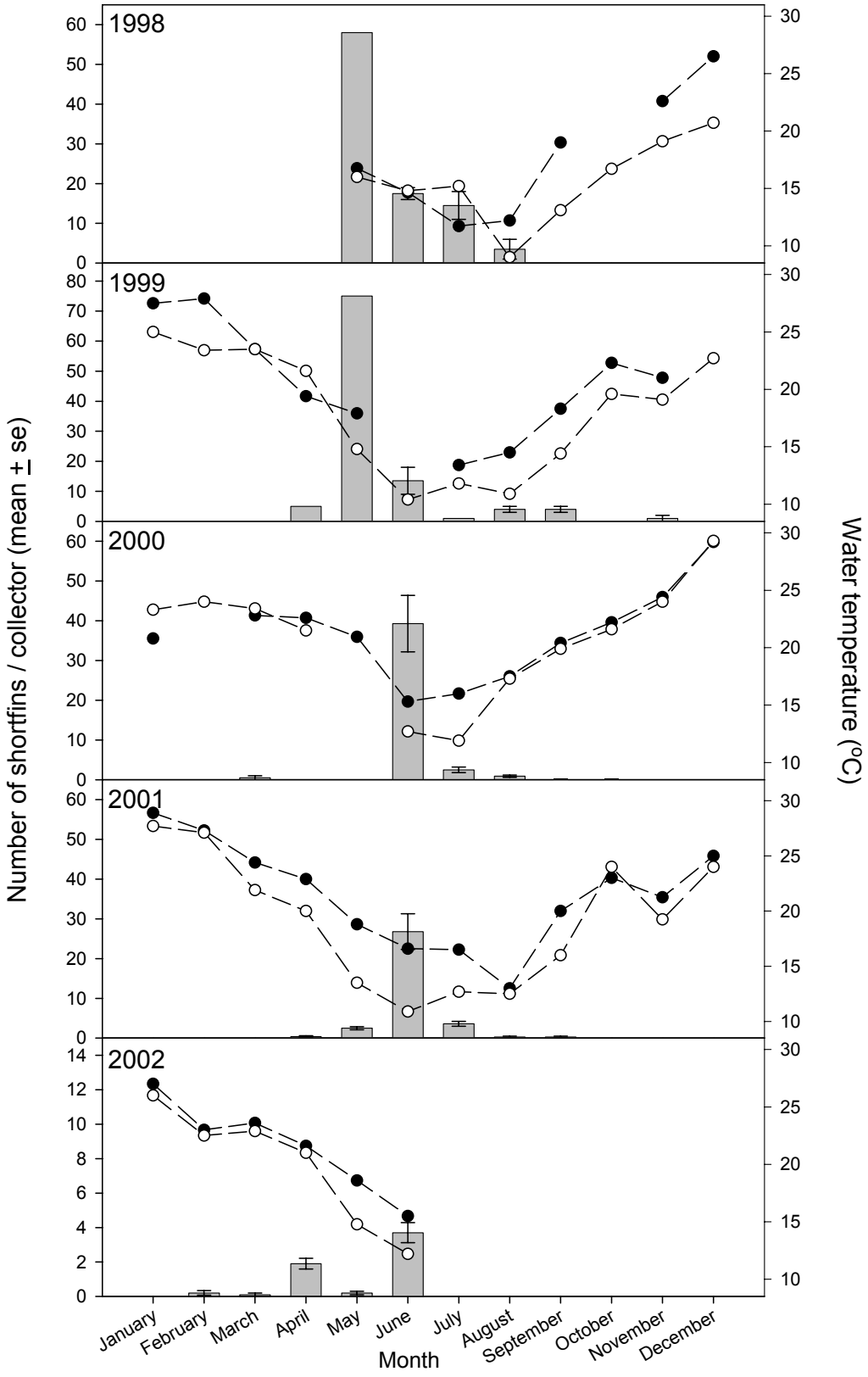

Fig. 3.5 – CPUE of newly arrived shortfin glass eels at Audley from 1998 – 2002. Filled circles indicate downstream temperature, open circles indicate upstream temperature.

Table 3.8 – Total number of newly arrived (n) and previously arrived (p) longfin glass eels collected at Audley from 1998 – 2002. na = not sampled.

Year	*1998*		*1999*		*2000*		*2001*		*2002*	
Month	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**	**n**	**p**
Jan	na	na	0	0	26	0	2	0	0	0
Feb	na	na	82	1	4	0	10	2	72	0
March	na	na	5	1	43	4	17	5	172	66
April	na	na	15	1	4	0	14	1	33	3
May	3	0	16	3	1	0	3	0	7	0
June	0	0	0	0	7	1	0	0	2	0
July	0	1	0	0	1	0	0	1	na	na
Aug	0	0	7	0	0	0	0	0	na	na
Sept	1	0	0	0	2	0	1	0	na	na
Oct	0	0	0	0	0	0	0	0	na	na
Nov	0	1	1	0	0	7	0	1	na	na
Dec	0	0	0	0	6	0	0	0	na	na

Allowing for one complete recruitment season for each species at Audley (November – October), the relative abundance of longfins was calculated (Table 3.9). Comparison among seasons shows that longfin relative abundance declines during each subsequent season.

Table 3.9 – Relative abundance of longfins at each site from November 2000 – October 2001.

	1998/99	*1999/00*	*2000/01*
Shortfins	244	521	409
Longfins	216	186	79
% Longfins	47%	26%	16%

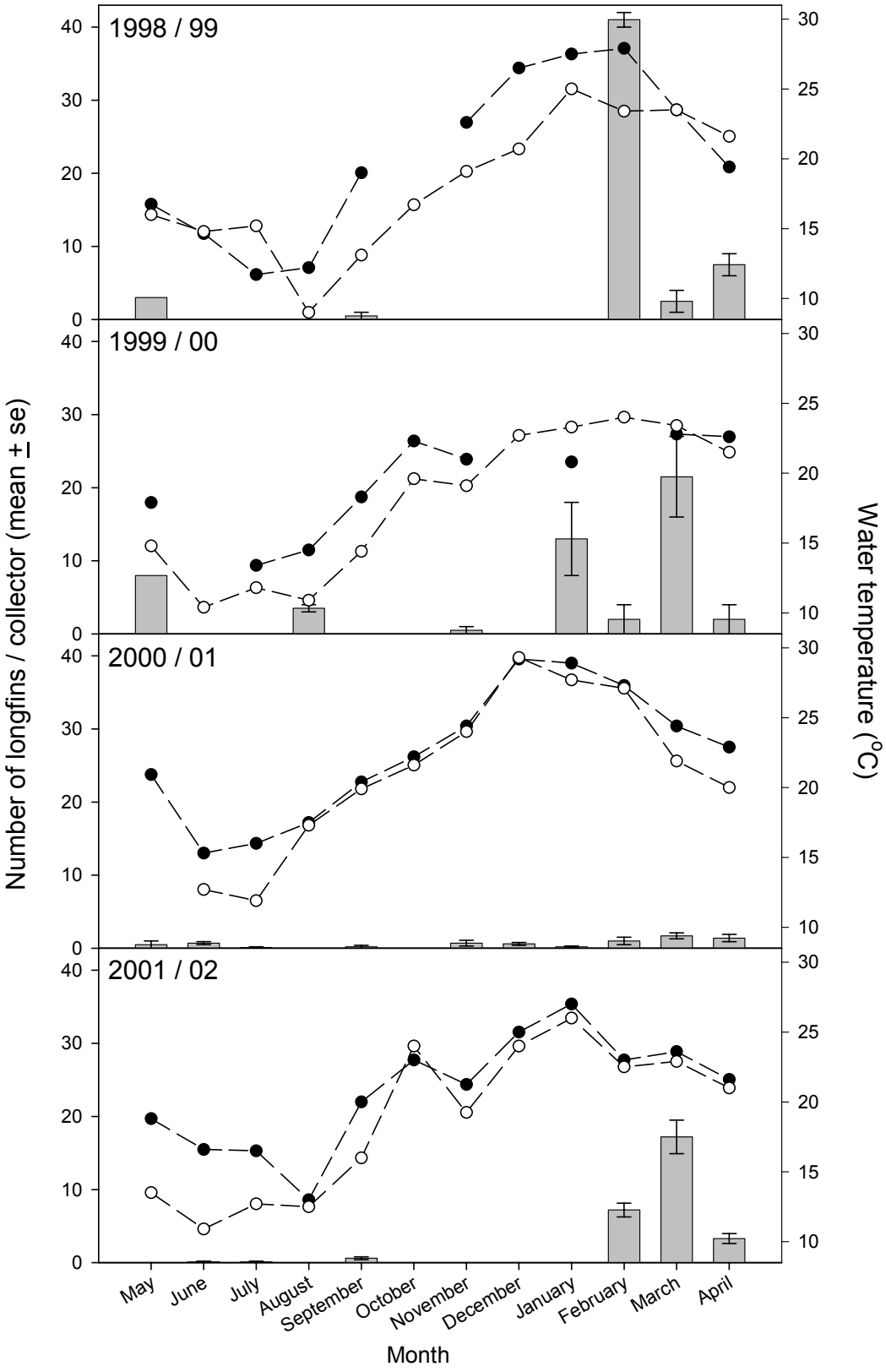

Fig. 3.6 – CPUE of newly arrived longfin glass eels at Audley from 1998/99 – 2001/02. Filled circles indicate downstream temperature, open circles indicate upstream temperature.

There was no consistent correlation between sea surface temperature, downstream water temperature, or the difference between these two parameters with the recruitment of newly arrived shortfins at Audley among years (Table 3.10). The year 2002 was the only one where a significant correlation was found between SST and shortfin recruitment. The predominantly negative correlations (non-significant) between shortfin recruitment and SST and downstream water temperature suggests that shortfins recruit to this estuary during the cold water months (Fig. 3.7).

Table 3.10 – Relationship between newly arrived shortfins and sea surface temperature (SST), downstream temperature (DT) and the difference between these two parameters (SST-DT) at Audley. Correlation coefficients (r) are shown and significant correlations are shown in bold.

Year	***SST***		***DT***		***SST-DT***	
	r	**p**	**r**	**p**	**r**	**p**
1998	-0.034	0.937	-0.499	0.265	0.620	0.137
1999	0.063	0.873	-0.240	0.504	0.298	0.403
2000	-0.450	0.142	-0.525	0.097	0.467	0.147
2001	-0.134	0.678	-0.398	0.200	0.445	0.147
2002	-0.837	**0.038**	-0.742	0.092	0.539	0.270

As with shortfins, the majority of correlations between newly arrived longfins and sea surface temperature, downstream water temperature, and the difference between these two parameters were not significant (Table 3.11) with the only significant correlation at Audley among years occurring between longfin recruitment and sea surface temperature in 2001. Also, although not significant, the predominantly positive correlations between the number of newly arrived longfins and SST and downstream water temperature indicates that longfins recruit to this site during the warm water months (Fig. 3.7).

Table 3.11 – Relationship between newly arrived longfins and sea surface temperature (SST), downstream temperature (DT) and the difference between these two parameters (SST-DT) at Audley. Correlation coefficients (r) are shown and significant correlations are shown in bold.

Year	*SST*		*DT*		*SST-DT*	
	r	**p**	**r**	**p**	**r**	**p**
1998	0.408	0.316	-0.035	0.941	0.248	0.592
1999	0.321	0.400	0.464	0.177	-0.522	0.121
2000	0.082	0.800	0.133	0.696	-0.123	0.719
2001	0.676	**0.016**	0.393	0.207	-0.140	0.664
2002	0.559	0.249	0.317	0.541	-0.013	0.980

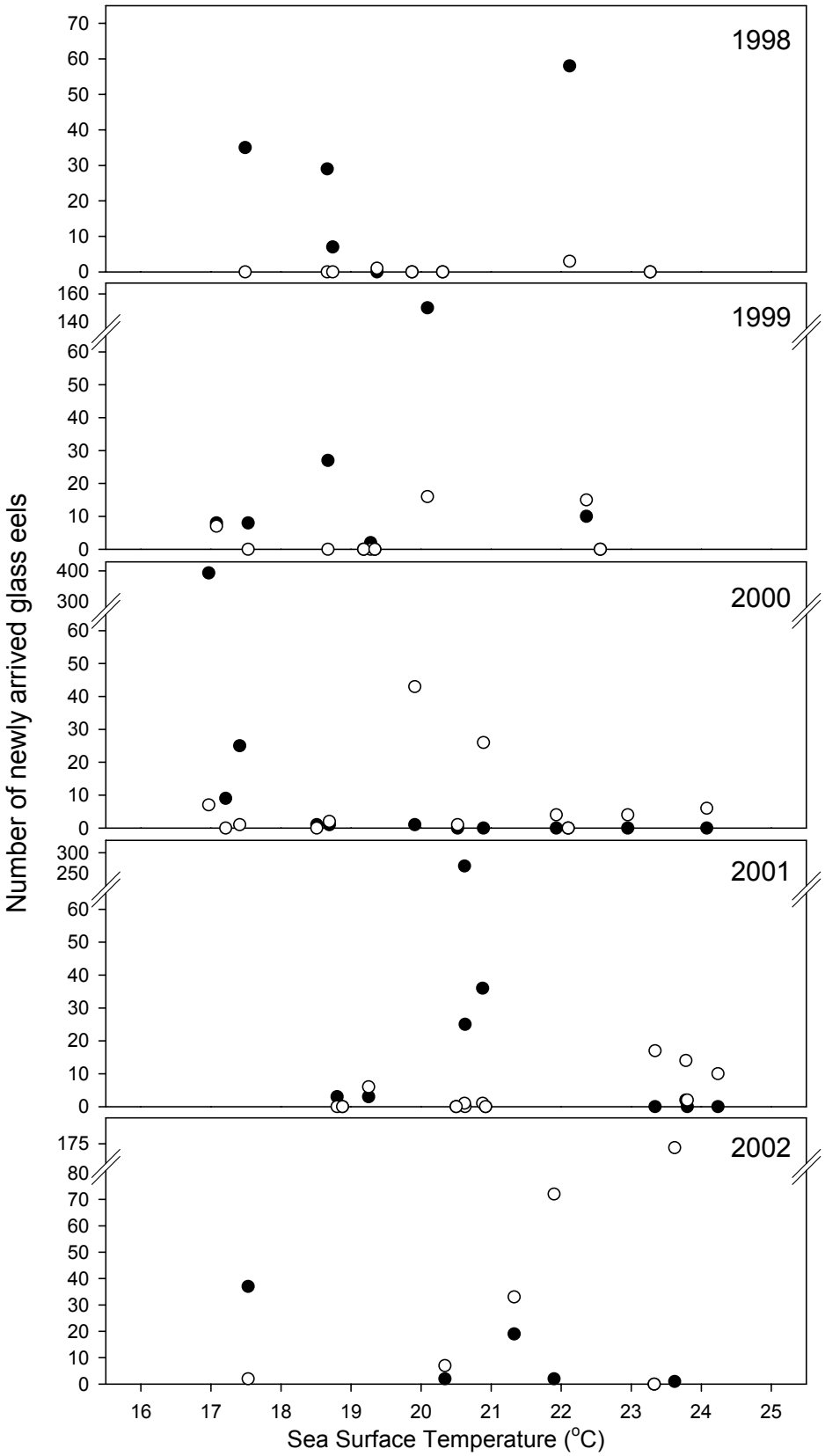

Fig. 3.7 – Relationship between recruitment of newly arrived shortfins (filled circles) and newly arrived longfins (open circles) with sea surface temperature.

3.4.4 *Changes in species composition with time*

The "crossover" from shortfins to longfins as the dominant species in the catch (ie percent composition of the catch was greater than 50%) was October for Mullet Ck, November for the Tweed and Lansdowne rivers, and December for Audley, all in the year 2000 (Fig. 3.8). Because sampling did not commence at Croobyar Ck until November, the exact timing of the crossover in the dominant species could not be determined for 2000 however it is assumed to be prior to November. The failure to catch newly arrived longfin glass eels until March 2001 at Boggy Ck suggests that this species may not exhibit a true recruitment season at this site. The crossover from longfins to shortfins as the dominant species in the catch was in March for Boggy Ck, April for the Lansdowne R., Mullet and Croobyar creeks, May for Audley and June for the Tweed R.

The longer temporal series at Audley shows that shortfins were the dominant species from May in 1998, 1999 and 2001, and from June in the years 2000 and 2002 (Fig. 3.9). Longfins became the dominant species in the catch from September 1998 in the 1989/99 season, although the catch of glass eels after this was zero until February 1999. Longfins were then the dominant species from January 2000 for the 1999/00 season and from December 2000 for the 2000/01 season. September 2001 also saw the arrival of a greater amount of newly arrived longfin glass eels compared to shortfins. Once again, however, the catch after this time was zero until February 2002 where longfins were the dominant species in the 2001/02 season.

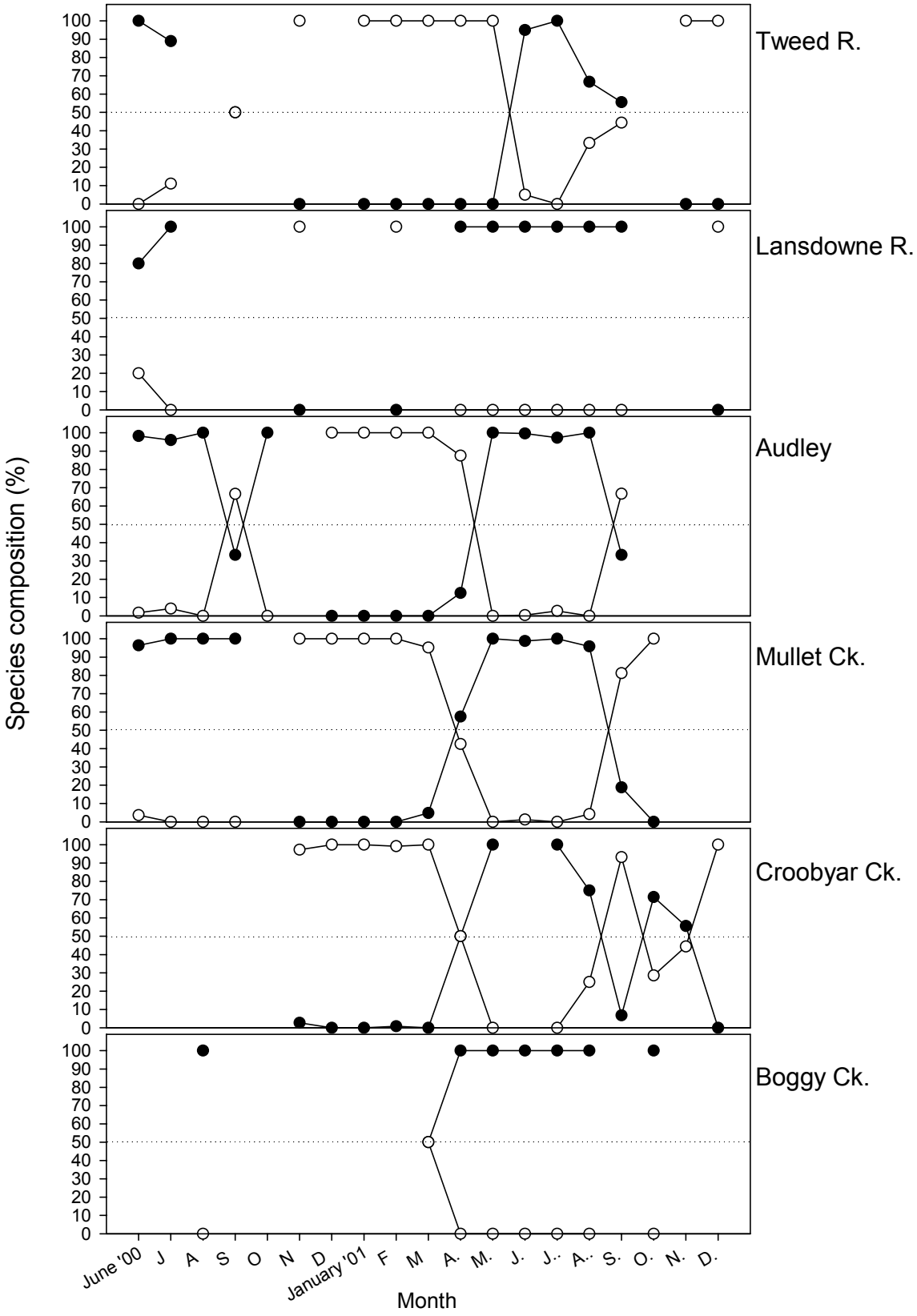

Fig. 3.8 – Percent change in species composition in catch at each site. Filled circles indicate shortfins, open circles indicate longfins. Where neither species is indicated, the catch was zero.

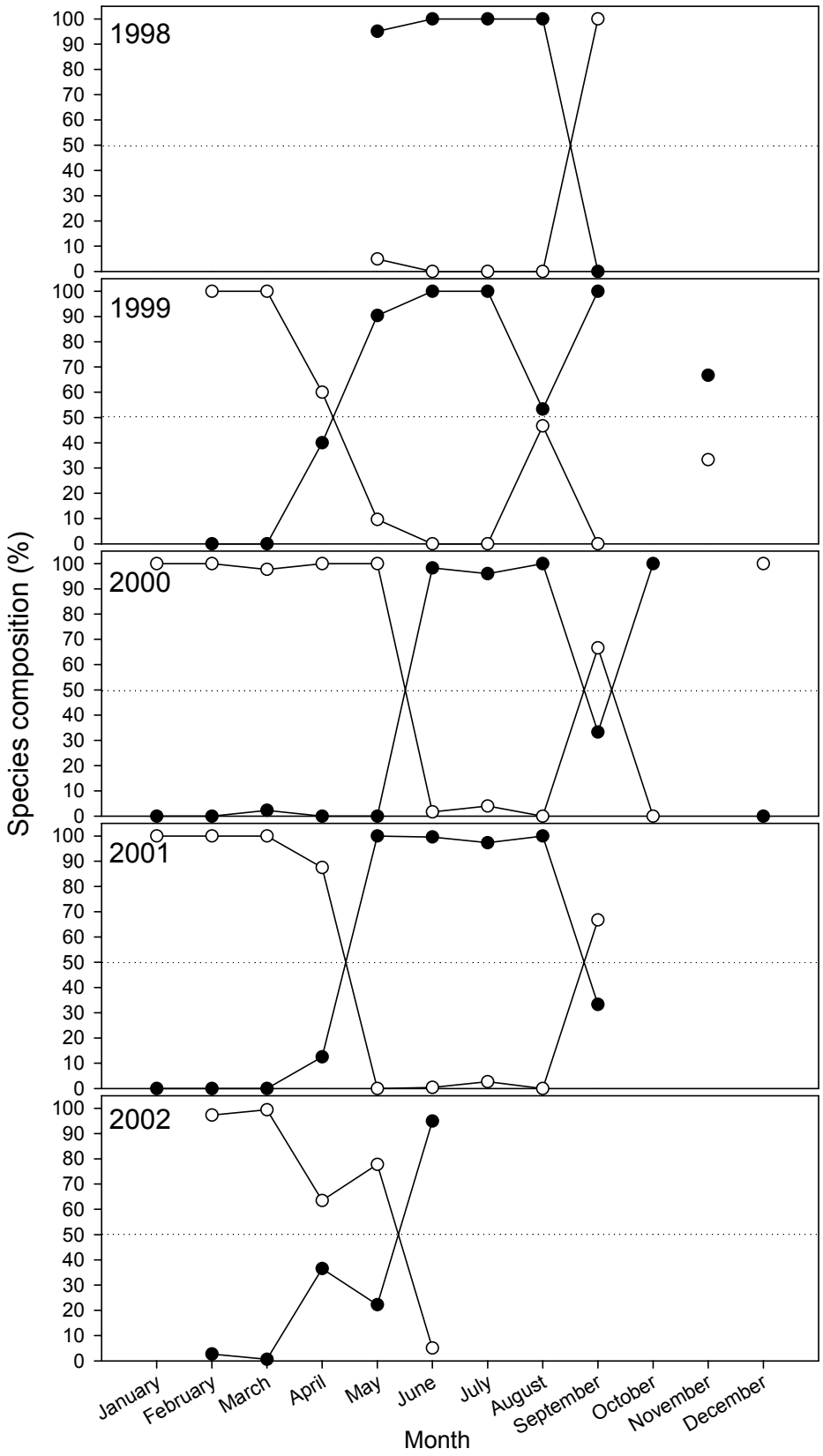

Fig. 3.9 – Percent change in species composition at Audley from 1998 - 2002. Filled circles indicate shortfins, open circles indicate longfins. Where neither species is indicated, the catch was zero.

3.4.5 *Condition (K)*

Condition of shortfin glass eels was negatively related to eel length ($r^2 = 0.007$, $p = 0.039$) so that eels of a lesser condition were longer. Thus, while there was no continual temporal decline in condition of shortfins within each site, there was a significant temporal decline in glass eel length with eels recruiting to these sites later in the season being shorter ($r = -0.193$, $F_{1,4} = 9.99$, $p < 0.00001$).

For longfins, there was no relationship between condition (K) and eel length ($r^2 = 0.0005$, $p = 0.190$) so declines in K were not due to eels recruiting later in the season being longer. Significant declines in K were seen at the Tweed R. ($r = -0.628$, $F_{1,3} = 18.74$, $p < 0.00001$), Audley ($r = -0.846$, $F_{1,3} = 109.27$, $p < 0.00001$), Mullet ($r = -0.802$, $F_{1,3} = 275.0$, $p < 0.00001$) and Croobyar ($r = -0.363$, $F_{1,3} = 76.17$, $p < 0.00001$) creeks. Newly arrived longfins were only caught at Lansdowne R. in February and at Boggy Ck. in March so regression analysis could not be performed. In January and February, longfins with the best condition were caught at Croobyar Ck. (K = 1.31 & 1.21 respectively), in March at Mullet Ck. (K = 0.87), and in April at Tweed R. (K = 0.84) and better condition often corresponded to pulses in recruitment at these sites (Table 3.3).

3.4.6 *Pigmentation*

There was a general temporal progression of Ege (1939) pigmentation stages within sites for shortfins, with glass eels becoming more pigmented later in the recruitment season at some sites (Appendix 1). From April to September, a temporal increase in pigmentation was seen at Lansdowne R., Audley, Mullet Ck. and Boggy Ck. The Tweed R. had late stage pigmented eels early in the season but catches were low, and higher catches had an increased representation of less pigmented glass eels. For Croobyar Ck., there were apparently two 'waves' of pigmentation stages, with the first wave beginning in April and an increase in pigmented eels to June, then a second wave beginning in July.

The ANOSIM two-way crossed analysis for both shortfins and longfins showed that there were significant differences between sites for pigmentation stages (averaged across all times; $ps < 0.001$) meaning, that when all months were combined, there

were significantly different trends in pigmentation frequencies between each site. There were also significant differences between months (averaged across all sites, ps < 0.001) meaning, that when sites were combined, there were significantly different trends in pigmentation frequencies between months. When each month was analysed separately (Tables 3.12 & 3.13 for shortfins and Tables 3.14 & 3.15 for longfins), a general progression of increasingly pigmented eels caught over time can be seen since higher pigmentation stages explain the dissimilarity between sites in the later part of the recruitment period.

Table 3.12 - ANOSIM pairwise tests for shortfins showing months where there were significant differences in pigmentation frequencies between sites, as indicated by the pairwise R values.

Shortfins	*Lansdowne R.*	*Audley*	*Mullet Ck.*	*Croobyar Ck.*	*Boggy Ck.*
Tweed R.	June (R = 0.813) September (R = 0.928)	June (R = 0.529)	July (R = 0.594) September (p = 0.667)	June (R = 0.843)	
Lansdowne R.		June (R = 0.898) July (R = 0.633) September (R = 0.942)	June (R = 0.882) July (R = 0.512) September (R = 0.818)	July (R = 0.763) September (R = 0.734)	July (R = 0.556) September (R = 0.61)
Audley				June (R = 0.922)	
Mullet Ck.				June (R = 0.905)	
Croobyar Ck.					

Table 3.13 - SIMPER analyses showing pigmentation stages of shortfins that contributed most to the dissimilarity in pigmentation frequencies between sites that were significantly different from each other. T = Tweed R., L = Lansdowne R., A = Audley, M = Mullet Ck., C = Croobyar Ck., B = Boggy Ck. Ege (1936) pigmentation stages are listed with the percent contribution that each stage contributed to the dissimilarity appearing in parentheses.

June	*Lansdowne R.*	*Audley*	*Mullet Ck.*	*Croobyar Ck.*	*Boggy Ck.*
Tweed R.	T – stage 1 (45.31%) L – stage 6 (38.40%) & stage 5 (11.86%)	T – stage 1 (32.48%) A – stage 3 (19.83%) & stage 4 (17.03%)		T – stage 1 (44.68%) C – stage 6 (35.74%)	
Lansdowne R.		L – stage 6 (39.99%) A – stage 1 (31.70%)	L – stage 6 (39.92%) M – stage 1(34.12%)		
Audley				A – stage 1 (30.79%) & stage 3 (8.94%) C – stage 6 (36.31%)	
Mullet Ck.				M – stage 1 (33.17%) C – stage 6 (36.39%)	
Croobyar Ck.					

Table 3.13 continued - SIMPER analyses showing pigmentation stages of shortfins that contributed most to the dissimilarity in pigmentation frequencies between sites that were significantly different from each other. T = Tweed R., L = Lansdowne R., A = Audley, M = Mullet Ck., C = Croobyar Ck., B = Boggy Ck. Ege (1936) pigmentation stages are listed with the percent contribution that each stage contributed to the dissimilarity appearing in parentheses.

July	***Lansdowne R.***	***Audley***	***Mullet Ck.***	***Croobyar Ck.***	***Boggy Ck.***
Tweed R.			T – stage 1 (37.15%) M – stage 5 (18.91%)		
Lansdowne R.		L – stage 6 (34.66%) A – stage 5 (17.24%)	L – stage 6 (32.22%) M – stage 5 (22.31%)	L – stage 6 (35.90%) C – stage 1 (18.06%) & stage 4 (14.50%)	L – stage 6 (36.62%) B – stage 5 (29.29%)
Audley					
Mullet Ck.					
Croobyar Ck.					

September	***Lansdowne R.***	***Audley***	***Mullet Ck.***	***Croobyar Ck.***	***Boggy Ck.***
Tweed R.	T – stage 1 (39.96%) L – stage 7 (28.56%)		T – stage 1 (33.97%) M – stage 7 (26.92%)		
Lansdowne R.		L – stage 7 (21.05%) & stage 6 (20.37%) A – stage 1 (38.84%)	L – stage 6 (24.71%) M – stage 4 (22.73%)	L – stage 7 (24.56%) & stage 6 (17.07%) C – stage 3 (17.21%)	L – stage 6 (42.76%) B – stage 7 (36.85%)
Audley					
Mullet Ck.					
Croobyar Ck.					

Table 3.14 - ANOSIM pairwise tests for longfins showing months where there were significant differences in pigmentation frequencies between sites, as indicated by the pairwise R values.

Longfins	*Lansdowne R.*	*Audley*	*Mullet Ck.*	*Croobyar Ck.*	*Boggy Ck.*
Tweed R.		January (R = 0.861) April (R = 0.752)	March (R = 0.887)	February (R = 0.637)	
Lansdowne R.		April (R = 0.723)		February (R = 0.667) April (R = 0.5)	February (R = 0.556)
Audley			January (R = 0.959)	April (R = 0.859)	April (R = 0.822)
Mullet Ck.				February (R = 0.68) March (R = 0.574) April (R = 0.536)	February (R = 0.622) April (R = 0.541)
Croobyar Ck.					February (R = 0.916)

Table 3.15 - SIMPER analyses showing pigmentation stages of longfins that contributed most to the dissimilarity in pigmentation frequencies between sites that were significantly different from each other. T = Tweed R., L = Lansdowne R., A = Audley, M = Mullet Ck., C = Croobyar Ck., B = Boggy Ck. Ege (1936) pigmentation stages are listed with the percent contribution that each stage contributed to the dissimilarity appearing in parentheses.

January	*Lansdowne R.*	*Audley*	*Mullet Ck.*	*Croobyar Ck.*	*Boggy Ck.*
Tweed R.		T – stage 1 (37.08%) A – stage 3 (30.24%)			
Lansdowne R.					
Audley			A – stage 3 (29.80%) M – stage 1 (29.23%)		
Mullet Ck.					
Croobyar Ck.					

Table 3.15 continued - SIMPER analyses showing pigmentation stages of longfins that contributed most to the dissimilarity in pigmentation frequencies between sites that were significantly different from each other. T = Tweed R., L = Lansdowne R., A = Audley, M = Mullet Ck., C = Croobyar Ck., B = Boggy Ck. Ege (1936) pigmentation stages are listed with the percent contribution that each stage contributed to the dissimilarity appearing in parentheses.

February	*Lansdowne R.*	*Audley*	*Mullet Ck.*	*Croobyar Ck.*	*Boggy Ck.*
Tweed R.				T – stage 6 (26.52%) C – stage 1 (32.98%)	
Lansdowne R.				L – stage 3 (19.91%) C – stage 1 (32.21%)	L – stage 1 (21.35%) B – stage 6 (33.33%)
Audley					
Mullet Ck.				M – stage 4 (24.89%) C – stage 1 (36.83%)	M – stage 4 (24.57%) B – stage 6 (34.89%)
Croobyar Ck.					C – stage 1 (31.08%) B – stage 6 (33.73%)

March	*Lansdowne R.*	*Audley*	*Mullet Ck.*	*Croobyar Ck.*	*Boggy Ck.*
Tweed R.			T – stage 3 (50.0%) M – stage 1 (18.68%)		
Lansdowne R.					
Audley					
Mullet Ck.				M – stage 5 (14.49%) C – stage 1 (29.21%)	
Croobyar Ck.					

Table 3.15 continued - SIMPER analyses showing pigmentation stages of longfins that contributed most to the dissimilarity in pigmentation frequencies between sites that were significantly different from each other. T = Tweed R., L = Lansdowne R., A = Audley, M = Mullet Ck., C = Croobyar Ck., B = Boggy Ck. Ege (1936) pigmentation stages are listed with the percent contribution that each stage contributed to the dissimilarity appearing in parentheses.

April	***Lansdowne R.***	***Audley***	***Mullet Ck.***	***Croobyar Ck.***	***Boggy Ck.***
Tweed R.		T – stage 6 (46.23%) A – stage 1 (34.91%)			
Lansdowne R.		L – stage 6 (34.93%) A – stage 1 (35.10%)		L – stage 6 (34.87%) C – stage 7 (36.67%)	
Audley				A – stage 1 (35.13%) C – stage 7 (26.79%)	A – stage 1 (34.75%) B – stage 7 (31.78%)
Mullet Ck.				M – stage 1 (14.24%) C – stage 7 (27.45%)	M – stage 1 (13.89%) C – stage 7 (32.75%)
Croobyar Ck.					

Analysis of pigmentation stages over the longer temporal data series at Audley showed no significant difference between years for shortfins ($p = 0.005$) and, when the outlier was removed, the sample statistic rose to $p = 0.007$. When months were considered, there was a significant difference between months ($p < 0.001$, Table 3.16) i.e. the percentage distribution of pigmentation stages did not follow the same pattern across months, irrespective of year. For longfins, there was also no significant difference between years ($p = 0.001$). When months were considered, there was a significant difference between months ($p < 0.001$, Table 3.16) i.e. the percentage distribution of pigmentation stages did not follow the same pattern across months, irrespective of year. That there was no difference between years for shortfins and longfins is attributable to the variation in pigmentation frequencies between months and that this variation occurred across all years sampled.

As with all sites, higher pigmentation stages accounted for the differences in shortfins at Audley towards the latter part of the recruitment period. However, longfins did not exhibit this pattern with stage 6, relatively pigmented eels, accounting for differences in the early part of the recruitment period and stage 3, relatively unpigmented eels, accounting for the differences between months in the latter part of the recruitment period. It must be noted that the pairwise R values for both shortfins and longfins are around 0.5 which indicates that the pigmentation frequencies between months are overlapping but still different.

Table 3.16 - ANOSIM pairwise tests for shortfins and longfins at Audley showing months where there were significant differences in pigmentation frequencies between months, as indicated by the pairwise R values, and results of SIMPER analyses showing pigmentation stages that contributed most to the dissimilarity in pigmentation frequencies between months. Shortfins table - A = April, M = May, J = June, Jy = July, Au = August, S = September; Longfins table – Ja = January, F = February, Mh = March, A = April, M = May. Ege (1936) pigmentation stages are listed with the percent contribution that each stage contributed to the dissimilarity appearing in parentheses.

Shortfins	***May***	***June***	***July***	***August***	***September***
April				R = 0.358 A – stage 1 (44.77%) Au – stage 6 (21.21%)	R = 0.488 A – stage 1 (41.65%) S – stage 6 (25.48%)
May					
June				R = 0.445 J – stage 1 (33.68%) Au – stage 6 (21.53%)	R = 0.579 J – stage 1 (31.34%) S – stage 6 (24.51%)
July					
August					

Longfins	***February***	***March***	***April***	***May***
January	R = 0.401 Ja – stage 1 (29.20%) F – stage 6 (19.49%)		R = 0.488 Ja – stage 1 (36.01%) A – stage 3 (31.16%)	
February				
March				
April				R = 0.414 A – stage 3 (24.38%) M – stage 1 (34.89%)

3.5 *Discussion*

3.5.1 *Spatial and temporal recruitment patterns*

The recruitment seasons for shortfin and longfin glass eels into selected NSW estuaries have been identified as being both spatially and temporally variable. The timing of catches and the catch per unit effort (CPUE) varied between species, sites and years. This variability is also highlighted in other Australian glass eel recruitment studies. There is no other documented research into the recruitment of glass eels to multiple sites in NSW. However, there have been studies that document the recruitment of these two eel species into estuaries of other eastern Australian states (Table 3.17) and the results presented in this study will be compared to them.

Table 3.17 – Summary of recruitment timing for shortfin and longfin glass eels in eastern Australian states. Bars indicate month when at least one eel was caught. Shortfins depicted by white bars, longfins depicted by shaded bars.

Study	*State*	*J*	*F*	*M*	*A*	*M*	*J*	*J*	*A*	*S*	*O*	*N*	*D*
Beumer & Sloane (1990)*	Qld	Not determined											
McKinnon et al. (2002)	Qld												
This study	**NSW**												
McKinnon et al. (2002)	NSW												
Pease et al. (2003)	NSW												
Beumer & Sloane (1990)	Vic												
McKinnon et al. (2002)#	Vic												
Sloane (1984a)	Tas												
McKinnon et al. (2002)	Tas	None recorded											

*- Not all months were listed for all sites, # - sampling period was from June – October only

In NSW, shortfins showed a more contracted recruitment compared to longfins, with the monthly catch of newly arrived glass eels increasing in the mid-coast sites and southern sites in this study. Beumer & Sloane (1990) also concluded that the duration of shortfin glass eel recruitment increased throughout the year as latitude increased, reinforcing its classification as a temperate species. However, when comparing the occurrence of shortfins between Australian studies, this latitudinal pattern is not immediately evident. This may, in part, be due to different sampling methods used to collect glass eels, the temporal scope of each study, and the varying oceanic and estuarine conditions that assist in the delivery glass eels to these areas. Thus, the longer temporal series at Audley provides vital information regarding the recruitment of both species.

Audley is situated approximately equidistant between the northern-most and southern-most sites in this study and shortfin recruitment at this site can be defined as being from April – September, depending on the year. This is in good agreement with other studies in NSW (McKinnon et al. 2002, Pease et al. 2003, Table 3.17). The data presented in Pease et al. (2003) provides the data for the 1998 and 1999 data presented here. The same site was used with a modification to the sampling method. Data for McKinnon et al. (2002) was collected in Port Hacking, the same estuary that the Audley weir separates, but was collected further downstream. Peak months of shortfin recruitment were found to be July in Queensland (McKinnon et al. 2002), May – July in NSW depending on site (McKinnon et al. 2002, this study), July/August in Victoria (Beumer & Sloane 1990, McKinnon et al. 2002) and in May or September/October in Tasmania depending on region (Sloane 1984a). Thus, from the comparison of spatial recruitment of shortfins between sites in this study, and the comparison of peak months of recruitment between this and other studies, there was no progression in peak recruitment from north to south, so the null hypothesis of a southwardly recruitment delay is rejected.

In NSW, longfins generally occurred in a wider range of months in the northern and mid-coast sites compared to the southern sites. However, it was more difficult to define their recruitment to seasons as low numbers of longfins were also caught throughout the shortfin season. The duration of longfin recruitment decreases with

increasing latitude with longfins available year round in Queensland and a restricted season recorded for both Victoria and Tasmania (Table 3.17). In this study, the recruitment season of longfins in NSW can be defined as being from January – May although, depending on site, longfins can recruit to the estuary outside of this range. An uncommon out of season recruitment event reported by Pease et al. (2003) may not necessarily be atypical. In 1999, longfins recruited to Audley through to August. This also occurred in 2000 and 2001 in this study although numbers were low. This out of season recruitment was also found at Mullet Ck. and Croobyar Ck. in 2001. Peak months of longfin recruitment were found to be in March in Queensland (McKinnon et al. 2002), June (McKinnon et al. 2002) and January – March (this study) in NSW, March in Victoria (Beumer & Sloane, 1990), and March in Tasmania (Sloane 1984a). Again, from comparing spatial recruitment of longfins between sites in this study, and the comparison of peak months of recruitment between this and other studies, there is no indication of a progression in peak recruitment from north to south, once more rejecting the null hypothesis.

The inter-annual variability in glass eel recruitment that is displayed at Audley in this study seems to be typical of *Anguilla* glass eel and elver recruitment worldwide. This variability has been shown for shortfins and longfins in Australia (McKinnon et al. 2002, Pease et al. 2003), for shortfins and New Zealand longfins in New Zealand (Jellyman & Ryan 1983, Jellyman et al. 2002), for the American eel in North America (Powles & Warlen 2001, Jessop 2003 a,b), for European eels in Europe (Ciccotti et al. 1995, de Casamajor et al. 2000), for Japanese eels in Japan (Kawakami et al. 1999) and for the tropical species *A. bicolor pacifica*, *A. celebesensis* and *A. marmorata* (Sugeha et al. 2001) in Indonesia.

Spatial patterns in the relative abundance of each species recruiting to the east coast of mainland Australia have not been determined by other studies so the variation in relative abundance of each species found in this study, with no region showing higher recruitment of a species, cannot be compared. However, studies in Tasmania showed that longfins comprised 23% of glass eels that recruited to that state's north-east region, comprised 26% of eels recruiting to the east region and comprised 3% of eels that recruited to the south-east (Sloane 1984a). In New Zealand, shortfin glass

eels dominated samples taken from freshwater areas (Jellyman & Ryan 1983, Jellyman et al. 1999, Jellyman et al. 2002, Jellyman & Lambert 2003).

The high recruitment of shortfin glass eels to Tasmania is reflected in the commercial fishery for yellow eels, where this species represents 97% of the total catch (Sloane 1984b). In New Zealand, Jellyman et al. (2002) reported that the dominant catches of shortfin glass eels was also evident in the wild fishery, with this species comprising approximately two-thirds of the wild harvest (by weight). However, in Jellyman & Lambert (2003) the catches of glass eels in the study estuary comprised 4% longfins, while sampling of the commercial catch comprised 98.7% yellow-eels of this species. In NSW, the wild harvest fishery is primarily for longfins, due to the higher value of the live export of this species to Asia, and is, on average, in the order of 150 tonnes (Gooley 2002). However, commercial fishing in freshwater areas of NSW catchments is prohibited, and so may exclude fishing in slow flowing river areas which are preferred by shortfins (Glova et al. 1998). The decrease in the relative abundance of longfins at Audley in this study is of concern and should be monitored. Pease et al. (2003) showed that the relative abundance of longfin glass eels at Audley were 32% and 68% in 1998/99 and 1999/00 respectively, however, abundance from the 1998 shortfin season was compared to the 1999 longfin season. Due to the lack of previous glass eel monitoring information, it is unknown whether this is due to an uncharacteristic increase in shortfin recruitment or a decrease in longfin recruitment. Therefore, it is important that fishery independent sampling be conducted in all habitats of estuarine and freshwater rivers to determine the link between the relative abundance of each species that recruit to the area and the relative abundance of each species in the sub-adult population, over a long temporal scale.

A correlation between temperature and CPUE has been shown to exist for a number of anguillid species. McKinnon and Gooley (1998) showed this correlation for shortfins, and it has also been shown for the European eel (Tosi et al. 1990) and American eel (Sorensen & Bianchini 1986). Alternatively, Jellyman (1977), Sloane (1984a), Beumer & Sloane (1990) and Pease et al. (2003), concluded that water temperature did not have a large influence on recruitment patterns of glass eels. In

the present study, no overall correlation was found between the number of newly arrived shortfins recruiting to the sampled sites and water temperature (ocean or estuary) and shortfins recruited to all sites and during periods when water temperatures reached as high as 25.4°C. Sloane (1984a) recorded that the recruitment of shortfins into Tasmanian streams occurred in the months where water temperatures were between 4°C and 22°C. The Beumer & Sloane (1990) data for Bruthen Ck. in Victoria showed that major invasions of shortfins occurred where temperatures ranged from 4°C to 16°C. McKinnon & Gooley (1998) suggest that the water temperature range 10°C – 14°C may provide the lower and upper limits, respectively, for maximum fishing returns of shortfins in Victorian estuaries. Pease et al. (2003) showed that the primary period of shortfin recruitment occurred when water temperatures were at or below 18.5°C. From these studies it is clear that there are no specific water temperatures that instigate and/or cease the recruitment of shortfin glass eels in eastern Australian waters. The positive (non-significant) correlations between the number of newly arrived shortfins and the temperature difference between the ocean and estuary found in this study, indicates that these glass eels may be able to sense this temperature difference and use this to guide them to the estuaries.

For longfins, recruitment has occurred when temperatures have reached a maximum of 29.3°C (this study) and there was no overall correlation between the number of newly arrived longfins and oceanic or estuarine water temperature. Likewise for NSW, Pease et al. (2003) showed that primary longfin recruitment to the estuary was when water temperatures rose above 18.5°C. Longfins have also been found to recruit to Tasmanian waters where temperatures ranged from 4.5°C to 22°C (Sloane 1984a). In Victoria, longfins recruited when water temperatures were between 5.5°C and 16°C (Beumer & Sloane 1990). Beumer & Sloane (1990) also showed that longfins recruited to Queensland's tropical waters when temperatures were between 18°C and 28°C with highest catches recorded when temperatures exceeded 22°C. Again, there appears to be no specific water temperatures that trigger and/or cease the recruitment of longfin glass eels in eastern Australian waters. It appears that longfins can recruit to estuaries where water temperatures are low and are able to recruit to estuaries where water temperatures are higher than can be sustained by

shortfins. The predominantly negative (non-significant) correlations between the number of newly arrived longfins and the temperature difference between the ocean and estuary found in this study may indicate that longfins prefer to enter estuaries with a similar warm water temperature from which they have been transported from the spawning grounds. Whilst no overall correlation between water temperature and recruitment of glass eels has been found in this study, it may provide a stimuli for which the eels can detect a suitable estuary, and water temperature may affect the long-term survival and growth of eels.

3.5.2 *Physical and morphological differences of glass eels*

The condition of larvae entering a population is thought to affect their survival and, hence, their contribution to the population (Pepin 1991, 1993). It follows, therefore, that better-condition larvae have a higher rate of survival and will contribute more to the population. In this study, shortfin condition was significantly related to glass eel length, and length decreased as the recruitment season increased. A decrease in lengths of estuarine captured glass eels as the recruitment season progresses has also been found by Chisnall et al. (2002) for shortfins and the New Zealand longfin eel, by Lefebvre et al. (2003) for the European eel and by Haro & Krueger (1988) and Jessop (1998) for the American eel and is due to shorter eels arriving later (Haro & Krueger 1988). Later arriving eels may be shorter due to a longer larval oceanic period (Jellyman 1977, Sloane 1984a) where the eels may not have obtained adequate nutritional intake to increase in length (Jessop 1998). This relationship cannot be determined without ageing of eels over their seasonal recruitment period and is a hypothesis that should be further studied. Larger eels may have superior swimming abilities that allow them to detrain from the oceanic currents and enter the estuaries earlier than their shorter counterparts (Jessop 1998).

In contrast, better-condition longfins generally occurred during recruitment pulses at sites, irrespective of latitude, in this study. This is inconsistent with the results found by Gooley & Ingram (2002) where better-condition longfins were found in the Albert R. (Qld), the northern most site, compared to Port Hacking (NSW). Also, Jellyman (1977) and Jessop (1998) showed that glass eels entering estuaries decreased in condition over time within each site as have been shown for longfins in this study. It

is undocumented however, if the decline in condition of eels directly affects their survival and further research is needed to test whether better-condition glass eels have a higher survival rate. If this hypothesis is true, management strategies would need to be implemented to combat the commercial harvest of better-condition eels so that these eels can contribute to the wild population whilst still maintaining the flow of viable eels for ongrowing in aquaculture facilities.

The general observation of increased representation of more heavily pigmented eels later in the recruitment season at some sites in this study is a pattern that has been widely documented for these and other Anguillid eel species (Sloane 1984a, Haro & Krueger 1988, Poole et al. 2004). However, the reasons for large variations in pigmentation of eels within and between sites are poorly understood and, perhaps for this reason, are often not attempted. Pigmentation of glass eels varied spatially and temporally at the six sampling sites with only a limited number of months having the same frequency distribution of pigmentation stages. This among-estuary difference is not surprising since pigmentation has been shown to vary within estuaries (Pease et al. 2003). That no differences in pigmentation frequencies at Audley was found between years in this study is supported by Pease et al. (2003), yet Lefebvre et al. (2003) showed vastly different monthly pigmentation composition between eels in their 2000/01 study and data from 1974/75.

Pigmentation stage is thought to reflect the duration of post-metamorphic life (metamorphosis from leptocephalus to glass eel) (Jellyman 1977) but this cannot be compared without ageing data (see Chapter 5). The hypothesis also exists that more advanced pigmentation stages of later arriving glass eels could be attributed to higher water temperatures promoting more rapid development (Strubberg 1913). The correlation between an increase in pigmentation with an increase in water temperature in controlled conditions has been previously shown (Jellyman 1977, Jellyman et al. 1999, Dou et al. 2003) and an increase in pigmentation in the wild with increasing oceanic and coastal water temperatures has been assumed (Haro & Krueger 1988, Desaunay et al. 1996, Jessop 1998). This cause and effect association cannot be made in this study since the same glass eels were not progressively monitored in waters where the temperature was increased. A further explanation of

pigmentation that more likely explains its role is proposed by Haro & Krueger (1988); that the development of pigment adapts the pelagic glass eel to a benthic existence and the increase in pigmentation may reflect increased contact with the substrate. Additionally, it would not be uncommon to catch more pigmented glass eels near the estuarine/freshwater interface (as in this study), since their accumulation in this region as they adapt to a freshwater existence (Sloane 1984a) is associated with the more heavily pigmented elver appearance (Tesch 2003). Tsukamoto (1990) found that temperate Japanese eels recruited at lower latitudes with more advanced pigment – a hypothesis that, again, does not hold true in this study since eel samples were recorded with a large range of pigment stages at all sites, irrespective of latitude.

3.5.3 *Changes in species composition with time*

As with recruitment variability between sites and years, the crossover in the dominant species in the catch also showed spatial and temporal variability. There is little recruitment of either species during October - December and this period demarcates the crossover from shortfins to longfins as the dominant species in the catch, depending on site. March to June was the crossover period from longfins to shortfins as the dominant species in the catch, depending on site. The longer Audley time series shows that the crossover from shortfins to longfins can occur at any time over a four month period (September – December) while the crossover from longfins to shortfins shows some degree of predictability with shortfins becoming the dominant species in May or June. A report by the QDPI (1995) showed that, in early April in south-east Queensland, longfins comprised over 50% of the catch and, thereafter until August, the catch was dominated by shortfins. This infers that the longfin to shortfin crossover period occurs at approximately the same time as in NSW. However, numbers of glass eels collected in the Queensland study during this period are unknown and, since only April – August was sampled, the shortfin and longfin recruitment seasons cannot be determined. April/May was the crossover from longfins to shortfins as the dominant species in the catch in Tasmania, depending on site (Sloane 1984a), but the crossover from shortfins to longfins cannot be deduced since there was only 12 months of sampling conducted. Again, this

infers that the longfin to shortfin crossover period occurs at approximately the same time.

Does this mean that glass eels are distributed throughout the coastal waters from Queensland to Tasmania in any one year at the same time? The answer would appear to be 'yes' judging by the simultaneous catches of each species at each of the six sites in this study and the timing of recruitment and crossover periods of each species from other Australian studies. Thus, the common denominator between all of these areas is that the same oceanographic currents deliver glass eels along this geographical range. A speculative transport model can be proposed based on the results from this study and on the models theorised for the two species of eels studied here and on the information gained from other Anguillid eels (Jellyman 1987, Arai et a 2001, Ishikawa et al. 2001, Shiao et al. 2002, Tesch 2003).

Aoyama et al. (1999) discovered overlapping distributions of nine shortfin and 12 longfin leptocephali in September 1995 in the western Pacific Ocean. This lends much support to the hypothesis that Australian glass eels spawn in these tropical areas with the spawning area of shortfins thought to be between Fiji and Tahiti (Castle 1963, Aoyama et al. 1999) or as near as New Caledonia (Schmidt 1928), however the exact spawning locations remain unidentified. The evidence of overlapping oceanic distributions suggests that these two eel species have similar dispersal routes via the South Equatorial Current (SEC, west flowing) and the East Australian Current (EAC, a western boundary current).

Current patterns within the EAC are complex with a series of bathymetric features including ridges, rises, land masses, shelves and islands which results in a high degree of seasonal and annual variation of the surface circulation (Jellyman 1987). The EAC frequently crosses onto the continental shelf and moves closer inshore as well as diverging and moving out to sea in several places along the NSW coast (Cresswell 1987). Dispersal of the leptocephali of the Australian shortfin and longfin eel is assumed to be by near surface currents, like that of the Japanese eel, European eel and American eel (Castonguay & McCleave 1987, Ishikawa et al. 2001), where Castonguay & McCleave (1987) caught *Anguilla* leptocephali in oceanic waters up to

depths of 275 m, depending on time of day. The speed of the SEC has been documented as being 0.5 – 0.6 m/s (Tchernia 1980) and as slow as 0.2 – 0.3 m/s (Shiao et al. 2002), and the distance from the sample sites in this study to the hypothesised spawning grounds is approximately 1500 – 2500 km. Therefore, it could take anywhere from 29 – 87 days for leptocephali to be passively transported directly from the spawning grounds to the coast of the northern site in this study, and 48 – 145 days to be transported to the coast of the southern site in this study via these currents, depending on the current speed. Longer oceanic drift times may be explained by the leptocephali being indirectly transported by complex oceanic currents, the formation of eddies and variable current speeds, thus explaining much of the inter-annual variability. This is also thought to be the case with the transportation of Japanese eel leptocephali from the spawning sites via the North Equatorial Current (Ishikawa et al. 2001). Also, the EAC is strongest between December and April (Hamon 1965) and speeds of this current may reach as high as 2 m/s (Oke & Middleton 2001). Eddies that form from this current have speeds from 0.3 m/s to 1.5 m/s and they may last up to one year (Cresswell & Legeckis 1986).

Shiao et al. (2001) concluded that, by taking these oceanic conditions into account, glass eels could be transported from southern Queensland, southwards to southern Victoria in 29 days. This spatial range encompasses the spatial range discussed in this study so it can be assumed that, with direct transport, glass eels of both east Australian glass eel species would be transported from the Tweed R. to Boggy Ck. in about 29 days. However, due to the complex nature of the oceanographic conditions, it would be highly speculative to predict when glass eels would be recruiting to estuaries solely on this information since location of glass eels in the EAC at any given time would be unknown. In fact, the uncharacteristic recruitment of longfins outside of the main recruitment season in this study may be explained by oceanographic conditions. Eddies commonly form along the NSW coast and some longfins may have become engaged in them. Thus, it is not unreasonable to assume that longfins were able to disengage from these eddies after six months of being entrained in them and continue their inshore migration.

Aoyama et al. (1999) assumed that some longfin larvae detrain earlier from the EAC due to its more northerly Australian distribution. In contrast, McKinnon et al. 2002 suggest that both species are transported northwards and southwards when the EAC divides between 14° and 18°S, with the more tropical longfin species able to show increased survival in these conditions compared to the more temperate shortfin. Then, with the south flowing EAC, both species are transported along Australia's east coast. The onset of metamorphosis is thought to occur when the leptocephali reach the continental shelf (Tesch 2003) and, for the European eel, is thought to be triggered by the arrival of leptocephali over the 1000m isobath (Schmidt 1928). Newly metamorphosed glass eels are thought to have active swimming capabilities (Tesch 2003) and, therefore, may be able to disengage from the EAC when stimulated by certain cues. Two of these cues may be organic odours that are released from estuaries and the change in salinity created by freshwater discharge. Eels have advanced olfactory capabilities and have been shown to be attracted to earthy odours that might commonly be discharged from rivers and estuaries (Creutzberg 1961, Sorensen 1986, Tosi et al. 1990, Sola 1995, Sola & Tongiorgi 1996, McCleave & Jellyman 2002). Steady prolonged swimming performance of shortfin and longfin glass eels has been recorded as 29 – 35 cm/s and 32 – 42 cm/sec respectively (Langdon & Collins 2000). Whilst the glass eels have these velocity capabilities, it is unclear whether they are actively swimming when entrained in the EAC or whether they are simply transported at the rate of the current itself. Burst swimming performance was measured at 64 – 79 cm/s for shortfins and 63 – 75 cm/s for longfins (Langdon & Collins 2000). These swimming speeds may be used by glass eels to detrain from the EAC when enticed by environmental stimuli.

The first significant newly arrived longfin recruitment event occurred at Croobyar Ck. on the 30th November 2000. Figure 3.10 illustrates the temperature of the sea surface currents at this time. Warm water from the EAC is colliding with the coast at the location where Croobyar Ck. is situated and colder water surrounding this southward moving EAC tract is colliding with the coast at the location where four of the other five sites are situated. Only small numbers of longfins were caught at other sites. Thus, in this instance, there is a good correlation between the intersection of the coast by the EAC and glass eel recruitment. The first significant shortfin

recruitment event occurred at Audley and Mullet Ck. on the 21st & 22nd June 2001 respectively. Figure 3.11a illustrates the temperature of the sea surface currents at this time. It is the colder water surrounding the warm water tract that is impinging with the coast at these sites at this time. Warmer water is colliding with the coast at the Lansdowne R. yet only one newly arrived shortfin eel was caught at this site at this time. This colder water proceeded to reach the Croobyar Ck. coastal region in July (Fig. 3.11b), the first significant shortfin catch at this site. Thus, it appears that shortfins do not travel in the warmest water of the EAC, rather, in the colder fringes and this may again be indicative of the primarily temperate nature of this species. It is difficult to answer the questions relating to glass eel recruitment and the oceanographic processes that drive this recruitment. In fact, development of relatively accurate transport models requires multi-scale, multi-dimensional studies, such as those reported in a series of papers published in a recent issue of Fisheries Oceanography (Crowder & Werner 1999). It is necessary to capture leptocephali (Jellyman et al. 1999) and newly metamorphosed glass eels at sea, as well as obtaining accurate oceanographic data, to solve the mystery of migratory routes and the influence of the EAC.

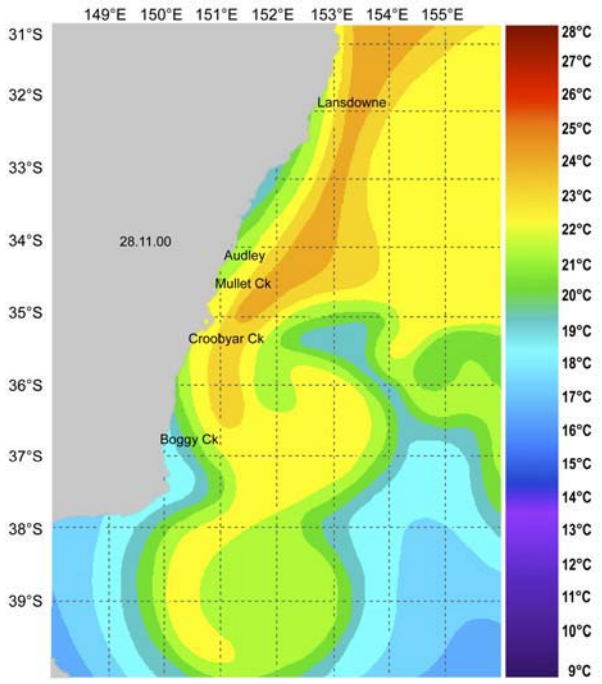

Fig. 3.10 – Sea surface temperature diagram for 28/11/00. Modified from diagram downloaded from www.aodc.gov.au.

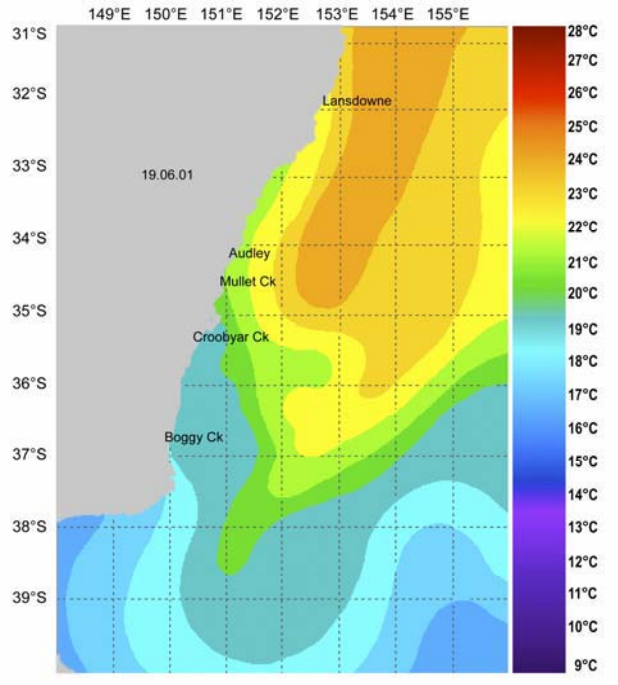

Fig. 3.11a – Sea surface temperature diagram for 19/6/01. Modified from diagram downloaded from www.aodc.gov.au**.**

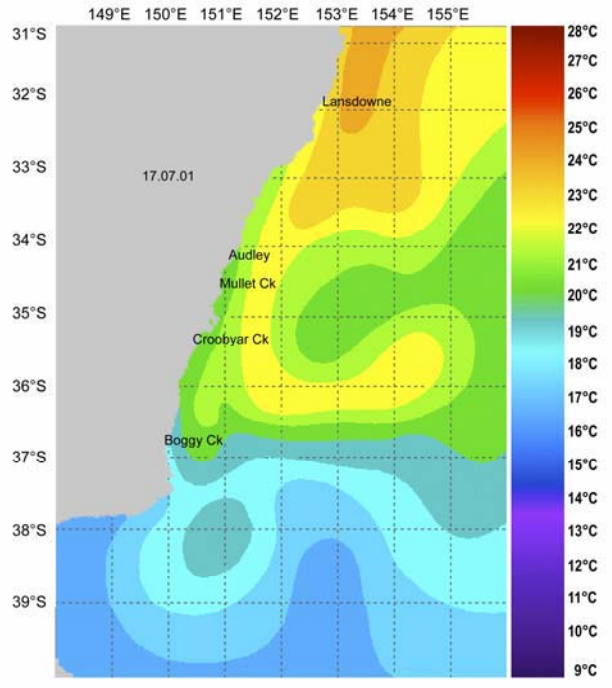

Fig. 3.11b – Sea surface temperature diagram for 17/7/01. Modified from diagram downloaded from www.aodc.gov.au.

The behaviour of glass eels changes when they reach waters that are affected by tidal currents. Glass eels use what is commonly referred to as selective tidal stream transport (McCleave & Kleckner 1982, McCleave & Wippelhauser 1987) to continue their upstream migration towards freshwater. Glass eels use the incoming tide in conjunction with active swimming to migrate towards freshwater. With the onset of the ebb tide, glass eels burrow into the substrate so as not to be pushed back downstream. At this point, glass eels do not seek preferred habitats and remain in the substrate until the subsequent incoming tide. Again, the use of the incoming tide and active swimming facilitates the eels' migration towards the salt-/freshwater interface. Glass eels remain here for a time to undergo physiological adaptations to survive in the freshwater environment. The availability of more complex and preferred habitats plays an important role at this stage (Chapter 4). Glass eels are able to explore their surroundings with their nightly movements into the water column and are able to locate more preferred habitats that, in turn, may increase their survival. Once their physiological adaptations are complete, most glass eels migrate into freshwater habitats.

3.6 *References*

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Chapter 4: Estuarine habitat preferences of *Anguilla australis* and *A. reinhardtii* glass eels as inferred from laboratory experiments

Silberschneider, V., Pease, B.C. & Booth, D.J. 2004. Estuarine habitat preferences of *Anguilla australis* and *A. reinhardtii* glass eels as inferred from laboratory experiments. *Environmental Biology of Fishes* **71**: 395-402.

4.1 *Abstract*

The habitat preferences of *Anguilla australis* (shortfin) and *A. reinhardtii* (longfin) glass eels were tested using circular tanks in an aquarium, containing four types of estuarine habitat (sand, mud, rocks/cobbles and seagrass). Shortfin eels either showed a tendency to occur in heterogeneous habitats, or in rocks/cobbles. Longfin glass eels showed a significant preference for rocks/cobbles in both experiments. Tests on shortfins and longfin glass eels in tanks with only rocks/cobbles available showed that eels were not clumped, indicating that individuals select habitat for re-settlement independently. Therefore, we assumed that the uneven distribution of glass eels observed in the habitat type experiments were the result of habitat preference. Given a choice of habitats in tank experiments, shortfin and longfin glass eels preferred habitats containing structure, and in particular, rocks/cobbles.

4.2 *Introduction*

Spatial variations in the distribution of fish and aquatic invertebrates partly reflect their behavioural responses to various aspects of habitat quality (such as type of substratum, availability of refugia and food resources) or to post-settlement mortality. Environments studied in this regard include coral reefs (Shulman 1985, Hixon & Beets 1993, Booth & Beretta 1994), estuaries (Kenyon et al. 1997, Laegdsgaard & Johnson 2001) and streams (Heggenes et al. 1991, Fonesca & Hart 2001). In open populations, spatial variations in distribution may also reflect differences in the supply of new recruits to particular habitats (Fonesca & Hart 2001).

Eels of the genus *Anguilla* are one of the dominant taxa in freshwater fish communities in coastal regions of New South Wales, south-eastern Australia (Gehrke & Harris 2000). They are long-lived and spend most of their lifetime in freshwater and tidal habitats within coastal catchments. The leptocephalus larvae of *Anguilla australis* (Richardson), commonly and hereafter referred to as 'shortfin', and *A. reinhardtii* (Steindachner), commonly and hereafter referred to as 'longfin', are transported to the east coast of Australia via the East Australian Current (Jespersen 1942, Castle 1963, Jellyman 1987, Beumer & Sloane 1990). The leptocephali then metamorphose into glass eels (small unpigmented-slightly pigmented post-larval eels) before recruiting to estuaries and migrating upstream to a wide range of estuarine and freshwater habitats (Beumer & Harrington 1980).

During the upstream migration, glass eels exhibit a crepuscular and daytime shelter seeking behaviour (Jellyman 1979, Silberschneider et al. 2001, Tesch 2003). Factors affecting upstream glass eel migration, and consequently extending their time in the estuary, include local hydrographic conditions (McCleave & Kleckner 1982) such as tidal flow and estuary length, freshwater inflow (Sloane 1984), barriers to migration (Naismith & Knights 1988, Haro et al. 2000), and the time taken for glass eels to undergo a physiological adaptation from a highly saline to a freshwater environment. This physiological adaptation time is thought to be at least two weeks and occurs at the estuary/freshwater interface (Deelder 1958, Jellyman 1979, Pease et al. 2003).

Glass eels will then remain in this area until they are physiologically able to move further upstream. A proportion of recruiting eels may remain in estuarine habitats until they reach sexual maturity (Arai et al. 2003, Kotake et al. 2003, Morrison et al. 2003, Tzeng et al. 2003). Thus, it is important to determine the preference for various estuarine habitats by glass eels as a first step in identifying which habitats should be conserved to maintain sustainable recruitment of these eel species to coastal catchments.

Habitat alteration and fragmentation have been suggested as contributing factors to the decline of *Anguilla rostrata* (American eel) populations (Haro et al. 2000), while the availability of daytime refuges was one of the main determinants of density, biomass and population structure of *A. anguilla* (European eel) at specific sites (Knights & Bark 2001). However, the value of specific types of habitat for growth and survival of eels has not been quantified (Haro et al. 2000). Much of the information on habitat preferences of *A. australis* and *A. dieffenbachii* is from studies in New Zealand, where sampling was conducted in freshwater lakes (Chisnall 1996, Glova et al. 1998, Jellyman & Chisnall 1999, Broad et al. 2001) and preference tests were performed under controlled conditions using freshwater (Glova 1999, 2001). However, these studies have focussed on pigmented elvers and yellow-stage eels in freshwater; only limited information was obtained on glass eels (Jellyman & Chisnall 1999). There have been no studies that document estuarine habitat use and/or preferences by glass eels (defined in this study as post-larval eels that have not yet attained a pigmentation stage of VI_B1 (Strubberg 1913)). The aim of the present study was to determine whether shortfin and longfin glass eels show a preference for any particular type of estuarine habitat.

4.3 *Methods*

Glass eels used in the laboratory experiments were collected below the base of the Audley causeway on the Hacking River, southern Sydney, New South Wales, south-eastern Australia using the artificial habitat collectors described in Chapter 2. The causeway is located 12 km upstream from the ocean and the water immediately below it ranges in salinity from 0 to 35 ppt. Shortfin and longfin glass eels were collected during their peak estuarine recruitment seasons (May-August and February-April, respectively) and transported in plastic buckets to the aquarium facility at the Cronulla Fisheries Centre (approx. 20 min. drive). Glass eels were then placed in an 86 L holding tank with flow-through ambient water from the Port Hacking estuary, where salinity ranged from 5 to 35 ppt. Separate batches of glass eels were collected for each of the habitat preference experiments. The size of each batch varied but comprised enough eels to provide a sample size of between 24 and 30 eels for each experimental tank (a density of 0.28 - 0.35 glass eels L^{-1}). These densities were considered conservative since, in tank culture trials, Ingram et al. (2001) showed that stocking densities of glass eels at approximately 15 glass eels L^{-1} did not influence the growth or survival rates of shortfins.

Experiments were performed using four 86 L fibreglass tanks (Fig. 4.1). All tanks were supplied with filtered seawater from the Port Hacking estuary, had central outflow pipes ('standpipes') to maintain constant water levels, and air-stone diffusers to aerate the water. To prevent the glass eels from escaping, the top of each standpipe was covered with fine mesh netting, which was also placed around the outside of the tanks and extended inwards over the top of the tanks. The experimental tanks were distributed on one side of an enclosed room with no windows. Each tank was divided into four equal sections with perspex dividers (385 x 75 x 4 mm) that were sealed to the perspex base (Fig. 4.1). Each substratum/habitat was placed in one of the four sections within 1 cm from the top of the divider so that all combinations of habitat placement were investigated (Table 4.1). The four types of substratum/habitat used consisted of two homogeneous (sand and mud) and two heterogeneous (rocks/cobbles and seagrass) types, with heterogeneous habitats defined as those habitats with more complex structure. The

seagrass habitat consisted of a mixture of *Zostera sp*. and *Halophila sp*. in a sand substratum. All habitat types are representative of the predominant substrata/habitats in the Port Hacking estuary, with each substratum/habitat removed from the same area to maintain consistency between tanks and experiments. Each of these substrata/habitats was collected from the Port Hacking estuary near the Cronulla Fisheries Centre and were placed in the tanks 1 - 2 days prior to the commencement of the experiments to allow sediments to settle out of the water column.

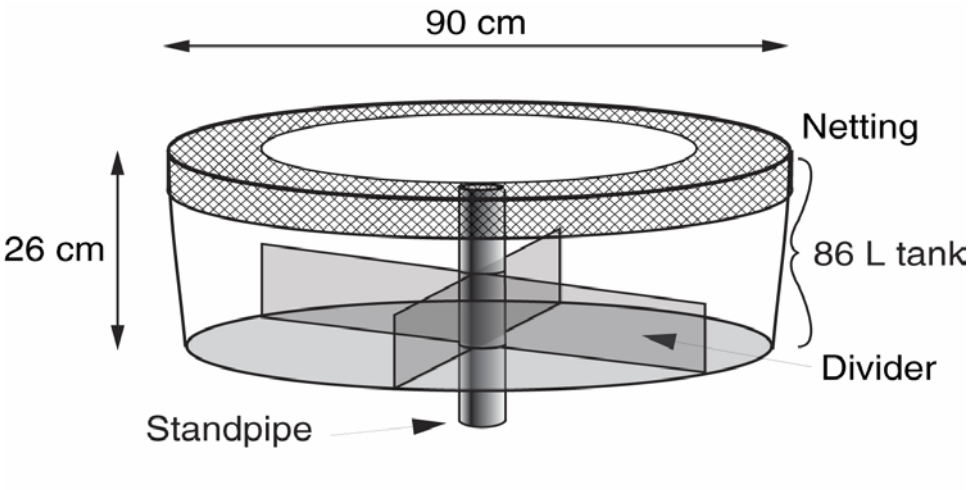

Fig. 4.1 - Diagram of a modified tank used in laboratory habitat preference experiments.

Table 4.1 – Layout of habitats in each experiment for (a) shortfin and (b) longfin glass eels where S = sand, SG = seagrass, RC = rocks/cobbles and M = mud. Note that tanks are circular so that habitats in sections 1 and 4 are adjacent.

(a)	***Experiment 1***				***Experiment 2***			
	Tank				*Tank*			
Section	1	2	3	4	1	2	3	4
1	SG	M	RC	S	RC	SG	S	M
2	S	RC	SG	M	SG	S	M	RC
3	RC	S	M	SG	M	RC	SG	S
4	M	SG	S	RC	S	M	RC	SG

(b)	***Experiment 1***				***Experiment 2***			
	Tank				*Tank*			
Section	1	2	3	4	1	2	3	4
1	SG	M	RC	S	RC	SG	S	M
2	S	RC	SG	M	SG	S	M	RC
3	RC	S	M	SG	M	RC	SG	S
4	M	SG	S	RC	S	M	RC	SG

For shortfin eels, Experiment 1 was conducted from 8 to 9 July and Experiment 2 was conducted from 12 to 13 July 2001. Glass eels collected for these experiments were part of a glass eel recruitment study (Chapter 3) and were allowed to acclimate in the aquarium for 17 days. The acclimation period depended on satisfying the sampling requirements for the recruitment study and allowing for the collection of substrata/habitats for the experiment. After acclimation, 30 glass eels were dip-netted and released in each of the tanks by rotating the dip-net around the standpipe so that the glass eels were not released over one particular habitat. For longfins, Experiment 1 was conducted from 4 to 5 April and Experiment 2 was conducted from 8 to 9 April 2002. Because longfins experienced a high rate of mortality shortly after capture, they were treated with a formalin bath (1:10 000) to eradicate any ectoparasitic infestations, and were used in experiments only after mortalities had reached zero. These treated longfin eels were allowed to acclimate for 9 days. After acclimation, 24 glass eels were dip-netted and released into each of the tanks as described above. Feeding was not attempted prior to the experiments and glass eels of both species were released into the tanks at around midday.

Lights in the room were set to a 10:14 hour light:dark cycle for shortfins and a 12:12 hour light:dark cycle for longfins, which were the approximate natural cycles for the two respective times of year. After 24 hours had elapsed, tanks were checked for signs of glass eel movement, the netting was removed, and a partition that exactly fitted the shape of the tank and isolated each substratum/habitat was quickly inserted. The water was siphoned off or scooped out and each habitat was thoroughly checked for the presence of glass eels. Glass eels collected from each section/habitat were counted, euthanased with benzocaine or clove oil, and stored in labelled plastic jars containing 95% ethanol for confirmation of species.

Shortfin and longfin glass eels were tested for habitat preferences monospecifically, as there are only one or two months of the year when their recruitment overlaps. Glass eels would undergo more stress if identified prior to experimentation, as they would have to be anaesthetised and viewed microscopically to identify them to species.

Analysis of variance (ANOVA) using a 4 x 4 x 4 Latin square analysis (i.e. 4 tanks x 4 sections x 4 habitat types), based on the number of eels in each section, was performed on the results of each experiment for each species (SAS version 6.12). When statistical differences were found among sections, a Duncan's Multiple Range Test was performed to determine which treatment means were significantly different (SAS version 6.12). In all tests, $p < 0.05$ was considered significant.

A separate series of experiments was done to determine whether any habitat preference detected in the previous series of experiments was due to a habitat choice or to aggregative behaviour of the glass eels (i.e. to test whether the individual eels were acting independently of each other). Two tanks were set up with the same habitat type in each section. Rocks/cobbles was chosen for this experiment because most glass eels in the experiments above were found in this habitat (see Results). Experiments were done using shortfins from 20 to 21 and 24 to 25 June 2002, and using longfins from 9 to 10 and 10 to 11 April 2002 (i.e. two experiments per species). In each experiment, 28 glass eels were released into each tank. Chi-square analyses (Statsoft 2001) were used to test for deviations in the observed numbers of eels in each section from the expected frequencies. Expected frequencies were calculated based on the assumption that the glass eels were distributed evenly through all sections. Any aggregative behaviour would result in an uneven distribution, indicating that habitat preferences of individuals were dependent on the preferences of other individuals.

4.4 *Results*

After glass eels had been released into the tanks they were observed swimming around the tank close to the substratum for approximately 2 minutes before they disappeared into the substratum/habitat. When the automatic lights switched off, in staggered two minute intervals, glass eels were observed coming out of the substratum/habitat and swimming around the tank, some near the surface and others closer to the substratum. Glass eels were not impeded by the dividers. On first inspection of each tank the following morning, there was no sign of glass eels

swimming around the tank. Thus, it was concluded that glass eels exhibited normal nocturnal behaviour.

Identification of glass eels on completion of the experiments confirmed that only one species had been used for each experiment. During the entire experimental series, nine shortfins were not recovered but all longfins were recovered. Mean length of the shortfins was 52.5 mm $\pm$ 0.4 (s.e), with V_B as the most common pigmentation stage (only head and tail pigmentation; Strubberg 1913). Mean length of the longfins was 48.3 mm $\pm$ 0.07, with $VI_{A.IV}$ 1 as the most common pigmentation stage (distinct development of ventrolateral pigment; Strubberg 1913).

In Experiment 1, two of the four tanks had more shortfin glass eels in rocks/cobbles than in seagrass while, in the remaining two tanks, there were more shortfins in seagrass than in rocks/cobbles (Table 4.2a). Only one and two eels were found in the mud habitat and one eel was found in the sand habitat. Despite the variation of glass eels in each habitat, there was no significant difference between the number of shortfins in any of the habitats (Table 4.3, $p > 0.05$). In Experiment 2, there were significantly more shortfins in rocks/cobbles than in any other habitat (Tables 4.2a, 4.3, $p < 0.05$) with a total of 88 eels in rocks/cobbles and 23 in seagrass. There was only one glass eel found in the mud habitat, and no eels in the sand habitat.

Table 4.2 Numbers of (a) shortfin and (b) longfin glass eels collected from each habitat type after each experiment.

(a)	***Experiment 1***				***Experiment 2***				
	Tank				*Tank*				
Habitat type	1	2	3	4	1	2	3	4	Totals
Seagrass	23	1	17	4	13	3	3	4	68
Rocks/cobbles	5	29	11	25	13	26	24	25	158
Sand	0	0	0	1	0	0	0	0	1
Mud	1	0	2	0	0	1	0	0	4
Totals	29	30	30	30	26	30	27	29	231

(b)	***Experiment 1***				***Experiment 2***				
	Tank				*Tank*				
Habitat type	1	2	3	4	1	2	3	4	Totals
Seagrass	3	4	1	6	2	0	1	0	17
Rocks/cobbles	20	19	23	18	22	24	23	24	173
Sand	1	1	0	0	0	0	0	0	2
Mud	0	0	0	0	0	0	0	0	0
Totals	24	24	24	24	24	24	24	24	192

There were significantly more longfin glass eels present in the rocks/cobbles habitat than in all other habitats in both Expts 1 & 2 (Tables 4.2b, 4.3, $p < 0.05$). In Expt 1, there were a total of 80 glass eels in rocks/cobbles. Also, the means for seagrass were significantly larger than the means for mud in Expt 1 (Table 4.3, $p < 0.05$). In Expt 2, a total of 93 longfins were found in rocks/cobbles with the remaining three eels found in seagrass (Table 4.2b), with no significant difference between the means of seagrass, mud and sand.

Table 4.3. Comparisons of mean number of eels per habitat type and tests of significance from ANOVA (Latin square) and Duncan's tests for habitat preferences of shortfin and longfin glass eels in Experiments 1 & 2 when tested in tanks. Significant differences between habitats are indicated in bold. A, B, C indicates Duncan's test groupings of significantly different means of glass eels between habitat types.

	Shortfins		***Longfins***	
Habitat type	*Experiment 1*	*Experiment 2*	*Experiment 1*	*Experiment 2*
Mud	0.75^{B}	0.25^{B}	0^{C}	0^{B}
Seagrass	$11.25^{A,B}$	5.75^{B}	3.5^{B}	0.75^{B}
Rocks/cobbles	17.5^{A}	22.0^{A}	20.0^{A}	23.25^{A}
Sand	0^{B}	0^{B}	$0.5^{B,C}$	0^{B}
Habitat $F_{3,3}$	4.64	21.14	99.91	794.25
Tank $F_{3,3}$	0.00	0.04	0.00	0.00
Section $F_{3,3}$	1.86	0.95	0.60	0.75
Habitat p value	0.0525	**0.0014**	**< 0.0001**	**< 0.0001**
Tank p value	> 0.9999	0.9878	> 0.9999	> 0.9999
Section p value	0.2369	0.4753	0.6357	0.5609

The ANOVA results also showed no significant difference in the number of glass eels among sections within tanks in the presence of the four different habitat types (Table 4.3, $p > 0.05$ in all cases). Similarly, there was no significant difference between the observed and expected frequencies of shortfin or longfin glass eels among sections within tanks when each tank was filled with rocks/cobbles only, indicating that there was no aggregative behaviour. In these single habitat experiments for shortfins, χ^2 and p values ranged from 3.43 – 7.71 and 0.0523 – 0.3301 respectively. For longfins, χ^2 and p values ranged from 0.86 – 4.29 and 0.2322 – 0.8358 respectively.

4.5 *Discussion*

The results from the tank experiments suggest that, given a choice of habitats, both shortfin and longfin glass eels prefer habitats with heterogeneous structure, in particular rocks/cobbles. The uniform distribution of shortfin and longfin glass eels in all experimental tanks when only the rocks/cobbles habitat was present facilitates the conclusion that glass eels 'preferred' rocks/cobbles when given a choice of four habitats. Glass eels did not exhibit aggregative behaviour, thus each eel presumably made an individual choice. Shortfin glass eels also displayed a greater preference for seagrass than for the other types of substratum/habitat in two of the four tanks in Expt 1. The results from Glova (1999, 2001) support these findings. Small shortfin eels (< 100 mm) and *A. dieffenbachii* (the New Zealand longfin eel) preferred watercress, cobbles and, to a lesser extent, woody debris compared to more homogeneous habitats when tested in replicate channels. Glova (1999) also found that, when the species were mixed, the proportion of small shortfins in watercress was greater than New Zealand longfins and, conversely, the proportion of longfins in cobbles was greater than shortfins. Thus, shortfins appear to inhabit macrophytes as well as rocks/cobbles.

Field sampling using habitat collectors (unpublished data) during the same time period as the tank experiments did not show distinct habitat preferences. Shortfins were found in all habitats tested, and longfins were found in all habitats except rocks. Cairns (1941) observed that elvers are often found buried in sand and mud substrata, as well as under logs and boulders in the lower reaches of freshwater rivers in New Zealand. Beumer & Harrington (1980) noted that glass eels seek shelter within the estuary in mud or vegetation. Thus, there is evidence to suggest that there may be differences between results obtained in controlled conditions compared to the natural environment.

It has been well documented that glass eel migration through the estuary occurs at night during new moon periods on flood tides (Jellyman 1979, Sorensen & Bianchini 1986, Tesch 2003). It was proven that when the ebb tide begins, glass eels seek cover in the substratum and remain there until the following night's flood tide so as

not to be forced back downstream (Creutzberg 1961). Thus, in the wild, glass eels may seek a particular habitat in which to hide, but may be forced to hide in less desirable habitats if the flood tide delivers them to an area where the preferred habitat does not occur. The study by Fonesca & Hart (2001) on the colonisation and habitat preference of black fly larvae found that processes governing the supply of colonists to substrata sometimes prevent them from reaching their preferred habitats. If fluid-mediated transport does not reliably deliver organisms to their preferred habitats, then the ability to disperse again or move about locally following settlement is likely to be a critical factor affecting fitness (Fonesca & Hart 2001).

It is suggested that the onset of the ebb tide induces glass eels that are travelling through the estuary towards freshwater to seek shelter in the substratum with only a very limited time to search the surrounding area for available habitat. However, once glass eels reach the estuarine/freshwater interface, their behaviour is modified and they have time to search and select highly preferred habitat types. My observations during a separate study at the Audley causeway (Silberschneider et al. 2001) showed that glass eels were accumulating around the freshwater outflows during the night and were sheltering in the surrounding substratum/habitat during the day, presumably whilst undergoing their physiological adaptation to freshwater. It was during this time that glass eels were caught in large numbers in artificial habitat collectors. Thus, I concluded that glass eels located the collectors during their nightly movements out of the substratum and found them to be a favourable alternative habitat. In turn, these nightly movements would also enable glass eels to find preferred habitat types which almost certainly provide more suitable refuge from predation.

I believe that the observed habitat preference behaviour is primarily based on the desire to use the most effective shelter for minimising the probability of predation. Other research has shown that post-settlement mortality is reduced in structurally diverse habitats because they provide a refuge from predation (Tupper & Boutilier 1997, Moksnes et al. 1998, O'Beirn et al. 1998, Steele 1999, Lindholm et al. 2001). Thus, glass eels may be more visible and accessible to predators when in unstructured or homogeneous habitats (e.g. sand and mud) compared to

heterogeneous habitats (e.g. rocks and seagrass) which contain small interstitial spaces to shelter in. Glova (2001) tested the cover preferences of juvenile eels in the presence of subadult longfin eels and found that small eels co-occurred with subadult longfins in watercress, presumably because small eels found adequate shelter in this heterogeneous habitat.

The ability of glass eels to burrow into the substratum, as well as their ability to live in small interstitial spaces (Glova 2001), potentially allows them to use all available habitats in estuaries. However, this study is the first to identify the preference of glass eels for different estuarine habitats. Rocks/cobbles and, to a lesser extent, seagrass are the preferred habitats of shortfins, and rocks/cobbles are the preferred habitat of longfin glass eels. It is believed that these preferred habitats offer increased shelter from predation compared to homogeneous sand and mud habitats. Because glass eels will spend at least two weeks in the estuary (Sloane 1984, Pease et al. 2003) and a proportion may remain in the estuary until they reach sexual maturity (Arai et al. 2003, Kotake et al. 2003, Morrison et al. 2003, Tzeng et al. 2003, Walsh et al. 2003), maintenance of preferred glass eel habitats will help to ensure the sustainability of eel populations in the coastal catchments of south-eastern Australia.

4.6 *References*

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Chapter 5: Age structure of recruiting *Anguilla australis* and *A. reinhardtii* glass eels to New South Wales

5.1. *Abstract*

Determining the age of juvenile fish is important in understanding their previous life history, including growth rate and date of recruitment. However, determining the ages of glass eels (in days) based on otolith increments is laborious and time consuming. While daily increment deposition in the otoliths of some anguillids has been validated, it has been assumed for shortfin (*Anguilla australis*) and longfin (*A. reinhardtii*) glass eels that recruit to the eastern states of Australia. Consequently, I validated daily increment deposition in otoliths of these species. Glass eel transport from their spawning grounds in the south-western Pacific Ocean is probably via the South Equatorial Current (SEC) and south flowing East Australian Current (EAC). With the south moving EAC, it was hypothesised that glass eels arriving at six sampling sites along the New South Wales (NSW) coast (nine degrees of latitude) would show significant difference in their ages among sites at different latitudes. For shortfins, eels recruiting to Tweed R. (most northerly location) were significantly older than eels at the remaining sites, counter to expectation. For longfins, there was no significant difference in the ages of eels between sites. Also, interannual patterns in ages of new recruits showed that shortfins recruiting to Audley in June were significantly older in 2000 than in June 2001, meaning that current speeds may have been slower in 2000 or that shortfins recruiting in that year spawned further west than eels in 2001. There was no difference in the ages of longfins that recruited to this site in February between 1999, 2000, and 2001. Subsequently, hatch times for each species were estimated from the total ages. Shortfins and longfins were estimated to have hatched from October – January and July – September, respectively, which is in good agreement with other studies of estimated hatch times of these species, as well as with the timing of leptocephali catches near the supposed spawning grounds. Longfins that recruited out of season were calculated to have hatched December – February. Patterns in the variability of age at recruitment and hatching times of the two species are discussed in relation to their geographic range,

extent of spawning grounds, spawning season, and delivery of leptocephali by ocean currents.

5.2. *Introduction*

Knowledge of fish age is one of the fundamental criteria used by ecologists and fisheries scientists to determine key processes within the life history of a fish species. In the early life history stages, which are typically pelagic and thus difficult to sample, information on age structure can be used to improve our understanding of recruitment processes and the effect a change in the environment can have on fish growth and survival (Jones 1992). In adults, knowledge of age can be used by fisheries scientists to determine the effect of fishing on stocks and the efficiency of management strategies with regard to maximising yield while ensuring future sustainability (Jones, 1992). Age data for fish can also be used to back-calculate hatch dates and growth rates, to link growth rates to environmental variables and to provide mortality estimates (Campana & Jones 1992).

Scales, opercular bones, fin rays, vertebrae and otoliths can be used to age fish but, for most fish, otoliths remain the structure of choice (Campana & Nielson 1985). Otoliths are acellular and grow in a more reliable manner than other hard parts of fish, and are not subject to resorption and remodelling (Campana & Nielson 1985, Secor et al. 1995). Otoliths show daily and annual increments (depending on the age of the fish) through cyclic deposition of calcium carbonate and protein (Campana & Nielson 1985), and are a permanent record of the life history of a fish, thus providing a unique tool in early life history and recruitment studies (Jones 1992, Secor et al. 1995).

The morphology of anguillid glass eel otoliths has been previously described (Lecomte-Finiger 1992, Arai et al. 1999a). The otolith core, or nucleus, has been described as a deep hole, after etching, with the deep circular groove surrounding this hole termed the hatch check (Fig. 5.1, Lecomte-Finiger 1992, Arai et al. 1999a).

This check has been inferred to occur at hatch of the leptocephali (transparent, laterally flattened, leaf-shaped larvae) since sagittae of newly-hatched larvae were found to be the same diameter as the check observed in elvers (Umezawa et al. 1989). Outside this check is a series of distinctive concentric growth increments (Lecomte-Finiger 1992). The region that encompasses these structures is defined as the leptocephalus growth zone (LGZ) in this thesis (Fig. 5.2). The otolith structure then changes and a diffuse ring, often with a metamorphosis check, is followed by an increase, then gradual decrease, of increment widths that are more difficult to differentiate towards the otolith edge. Thus, the region that encompasses these structures is defined as the post-metamorphic growth zone (PMGZ) in this thesis (Fig. 5.2). The change in increment widths has been shown to occur during the metamorphic period in starry flounder (Campana 1984) and in the conger eel (Lee & Byun 1996). Metamorphosis is presumably a stressful event in the life of the eel so any check in this zone is thought to occur during this time.

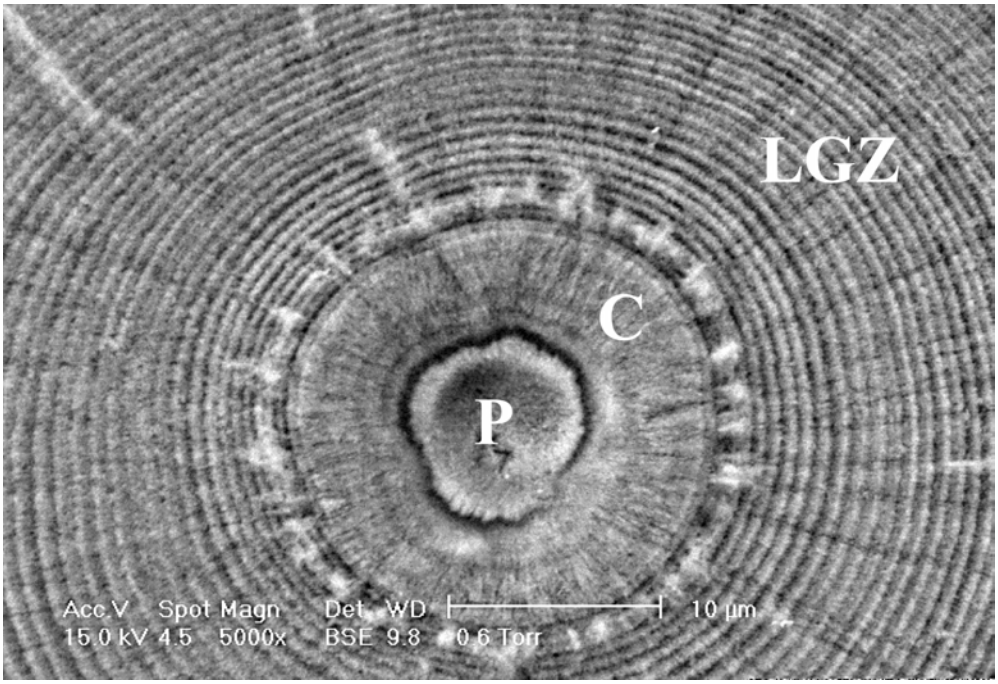

Fig. 5.1 – Environmental Scanning Electron Microscope (ESEM) image of the primordium (P), core (C) and part of the leptocephalus growth zone (LGZ).

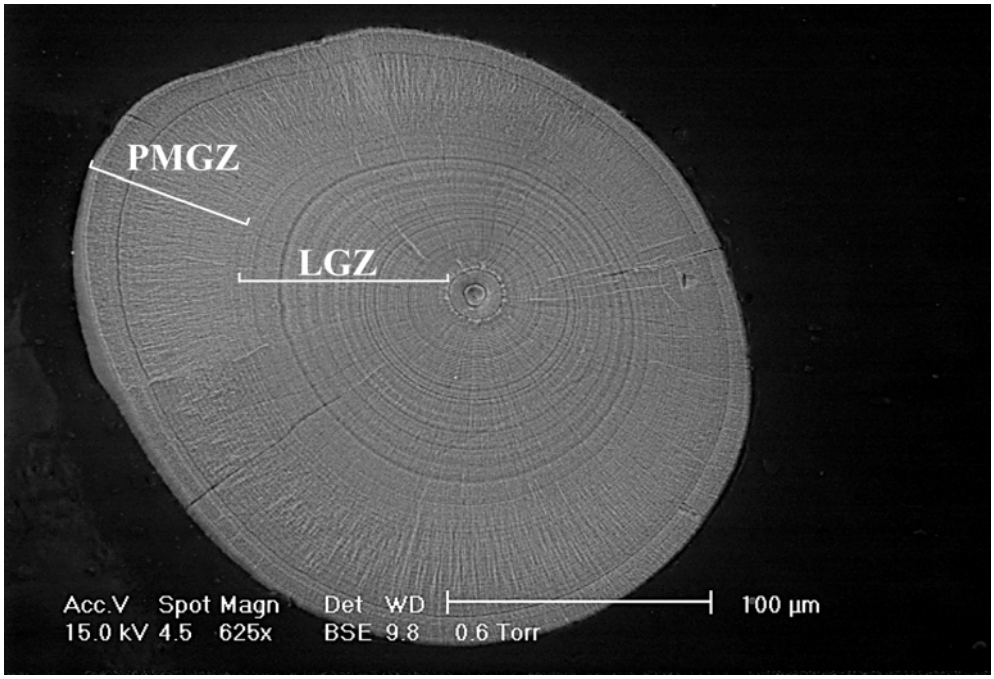

Fig. 5.2 – Whole otolith showing leptocephalus growth zone (LGZ) and post-metamorphic growth zone (PMGZ).

Many authors suggest that a deeply etched ring towards the edge of a glass eel or elver otolith is a transition ring, freshwater check, or elver check (Lecomte-Finiger 1992, Kawakami et al. 1998), yet it is not always observed (Desaunay et al. 1996, Arai et al. 1999b, Cieri & McCleave 2001, McKinnon et al. 2002). Check formation may reflect periods of stress to the fish or ontogenetic changes in feeding and activity (Campana & Nielson 1985, Geffen & Nash 1995). The ultimate cause of check formation may differ between intertidal and subtidal species, and between areas with different tidal regimes, because of differences in the importance of various environmental cues such as salinity (Geffen & Nash 1995). Due to the unpredictability of this formation in otoliths of eels, any check marks near the otolith edge were not considered in the data analysis in this chapter.

To estimate the age of wild caught fish, the age at first increment formation and the accuracy of increment deposition should be known. First increment deposition is best determined by laboratory rearing but can also be assumed to be at hatch or at yolk-sac absorption (Jones 1992). For Anguillid eels, time at first increment deposition has proved difficult since researchers have not successfully closed the life-cycle loop. Attempts to artificially induce maturation of the Japanese eel began in the 1960's but the ability to get preleptocephali to survive beyond the depletion of their yolk-sac and oil droplet stores was not successful until 2000 (Tanaka et al. 2001). Optimal rearing conditions are yet to be developed for eels and has lead to uncertainty in age validation experiments (Shinoda et al. 2004).

Daily increment deposition in the otoliths of anguillid glass eels remains controversial due to the inability to satisfy a key component of any age study: the estimation of accuracy between the true and estimated age of the eel. McCleave et al. (1998) suggest that increment deposition may not be daily due to the unknown process by which leptocephali gain nutrition since nutrition seems to have negative effect on ring deposition (Umezawa & Tsukamoto 1991). As discussed above, the true age of eels cannot be determined since eels have not been reared in captivity through all life-history events. However, Jones (1992) states that daily increment deposition in otoliths of fish appears to be a universal phenomenon under all but the most severe conditions. Daily increment deposition has been validated in the

leptocephalus phase of growth for the Japanese eel (*Anguilla japonica*, Umezawa et al. 1989) although experiment duration was only six days, and has been validated in the glass eel or elver phase of growth for the American eel (*A. rostrata*, Martin 1995, Cieri & McCleave 2001), a tropical eel (*A. celebesensis*, Arai et al. 2000) and Japanese eel (Tsukamoto 1989). Daily increment deposition in the glass eel phase of growth has been assumed for Australian shortfins (*A. australis*) and longfins (*A. reinhardtii*) (Arai et al. 1999b, Shiao et al. 2001, 2002, McKinnon et al. 2002) but has not been validated. Therefore, since this is a vital gap in our knowledge and a prerequisite to ageing, one aim of my ageing study is to validate daily increment deposition in the glass eel phase of growth for shortfin and longfin glass eels.

In this study, age of eels was used to infer key processes in recruitment, and otolith properties were used to identify mechanisms to simplify the ageing of these eels. Hypotheses regarding the timing of glass eel recruitment with respect to the southward moving East Australian Current (EAC) were discussed in Chapter 3. It was expected that glass eels would travel in a large cluster within the EAC and, when favourable cues were detected, such as a particular odour or temperature, eels would disengage from the cluster towards the estuary. However, no latitudinal delay in the timing of glass eel recruitment was found. This finding indicates that the total age (daily increments in LGZ and PMGZ) of all glass eels recruiting to the New South Wales (NSW) coast during the same monthly lunar period should be similar. Therefore, the null hypothesis for spatial comparisons is that the age of individuals recruiting during the same lunar period does not differ significantly among sites during this study, nor do they differ from each other for the number of increments in the LGZ (indicating larval duration) or the number of increments in the PMGZ.

Since the peak recruitment of each eel species to Australia is during approximately the same season (Chapter 3), and that this recruitment is assumed to be by the same currents, it was expected that the age of eels would not differ interannually. Therefore, interannual variability was examined using the null hypothesis that there was no significant difference among years in the number of LGZ increments, the number of PMGZ increments and total ages of eels caught during the new moon period at the same site. Also, monthly field sampling identified a recruitment pulse

of longfins outside their peak recruitment period (Chapter 3; Pease et al. 2003b). Consequently, the number of increments within the LGZ and PMGZ, and total ages of longfins were compared for eels caught in February 1999 (the typical peak recruitment period, Chapter 3) with those caught in August 1999. The null hypothesis is that there was no significant difference in the ages of eels caught during these two periods.

Fisheries biologists often attempt to find a link between fish length or weight with age or between otolith properties, such as otolith weight or radii distance of the sectioned otolith, and age to get an immediate indication of the population stock structure and reduce study costs due to the involved ageing process. Therefore, total age is compared to length and pigmentation stage of the eels to determine if one of these external characteristics is indicative of age. Also, it was hypothesised that there would be a significant correlation between otolith radius, LGZ and PMGZ distances and their respective daily increment counts, since the former can be used as proxies for growth in some species.

5.3. *Methods*

5.3.1. *Daily increment validation in the laboratory*

Artificial habitat collectors were placed at Audley weir on 13 April 2002 and were sampled the next day. Twenty glass eels were collected and transported live to the NSW Fisheries aquaria where they were kept in a 20 L bucket with aeration. On 16 April, glass eels were immersed in a solution of the fluorescent dye, alizarin complexone (AC), at a concentration of 80 mg/L. The AC powder was diluted in ambient seawater then left over night under aeration to ensure total dissolving of the powder and stabilisation of the pH. Glass eels were occasionally fed during the holding period. After a maximum of 86 days the glass eels were euthenased and their otoliths were removed and aged as described in the following section.

5.3.2. *Otolith preparation*

As outlined previously (Chapter 3), those eels with a pigmentation stage of up to, and including, $VI_{AII}4$ (Strubberg 1913), which is equivalent to Ege (1939) pigmentation stage 4, are defined in this thesis as newly-arrived glass eels. To test for spatial differences in ages, five otoliths were taken from newly arrived glass eels at sites where there were sufficient catches of these eels. For shortfins, otoliths were taken from glass eels in June 2001 samples at the Tweed R., Audley, Mullet and Boggy creeks. For longfins, otoliths were taken from glass eels in February 2001 samples at the Tweed and Lansdowne rivers, Audley, Mullet and Croobyar creeks. To test for temporal differences in ages, five otoliths were taken from shortfins collected at Audley in June 2000 from longfins collected at Audley in February 1999 and 2000, as well as from August 1999 samples.

Sagittal otoliths were removed from glass eels using a stereo dissecting light microscope and teasing the otoliths away from the head. Each otolith was placed on a separate, labelled slide using thermoplastic glue (Crystal Bond®), with the sulcus facing down. Otoliths were ground down and polished in a two-stage process. Stage 1 involved using 3 µm lapping film to grind the otolith until the core was almost reached. Stage 2 involved polishing the otolith to remove scratches with a Kent 3 Automatic Lapping and Polishing Unit, using a polishing pad and colloidal silica for 2 minutes. When scratches were no longer visible, otoliths were etched by immersion in 5% EDTA solution for 30 – 40 seconds. Each sample was thoroughly rinsed in water between each preparation stage.

Each sample was placed in a Philips XL30 ESEM (Environmental Scanning Electron Microscope) for capturing a series of images for each otolith. Within the ESEM, a backscatter detector and 15 kV accelerating voltage were used. Images of between 400x and 800x were used to take measurements of maximum otolith diameter (passing through the core), maximum otolith radius, leptocephalus growth zone (LGZ) and post-metamorphic growth zone (PMGZ). Images of between 3500x and 7000x magnification were used to count what were assumed to be daily increments. Check marks were not visible in all otoliths and some otoliths had multiple check marks near the otolith edge. Consequently, the distance from metamorphosis to any

check mark is not presented here. Measurements and daily increment counts were done along the longest radius using Scion Image software and, in some cases, the contrast was enhanced to aid detection of increments. Daily increments were counted twice on each otolith. A set of initial trial readings was used to develop an understanding of variability among individual otoliths and to develop a protocol for increment determination ie. identifying a countable increment as opposed to natural variation in the colour of the otolith as enhanced by etching, and identifying areas on otoliths where distinguishable marks could be used to section-off counted areas when multiple images were used. Second readings were done after all first readings were completed and the results are presented. Otoliths marked with AC were also viewed through a compound light microscope at a magnification of 100x with a fluorescent light and filter to view the AC mark.

A one-way analysis of variance (ANOVA) was used in separate tests of spatial and temporal differences in ages using Statistica version 6.0 (Statsoft 2001). If a significant difference was found, the Tukey test was then used to show where the difference was. All tests were done with a significance level of $p = 0.05$. Regression analysis was used to compare otolith measurements with their respective increment counts and to compare total age to eel length and weight. The non-parametric Gamma statistic was used to compare total age with Ege (1939) pigmentation stage because of the ordinal nature of the pigmentation data. Total age was only compared with pigmentation stage for eels that had attained a Strubberg (1913) pigmentation stage of up to, and including, $VI_{AII}4$ (equivalent to Ege (1939) pigmentation stage 4) since these eels are defined as new recruits (Chapter 3).

Where applicable, the Kolmogorov-Smirnov (K-S) test was used to test for normality and Bartlett's test was used to test for homogeneity. While most analyses used are robust with respect to departures from normality and homogeneity (Zar 1999), the assumptions were met.

5.4. *Results*

5.4.1. *Daily increment validation*

Fig. 5.3 shows a combination of ESEM image and light microscope image (using fluorescent light) for the same otolith. The AC mark matches a check seen on the ESEM image, and this check was probably formed by the stress of immersing the glass eel in the AC solution.

Due to the difficulty and precision necessary to prepare otoliths, only eight otoliths were deemed satisfactory. Satisfactory otoliths were neither under- or over ground, under- or over etched but where all increments along the longest axis were visible. Of these eels, three were identified as shortfins and five were longfins. Increment deposition appears to be daily for longfin and shortfin eels in the glass eel phase of growth (Fig. 5.4).

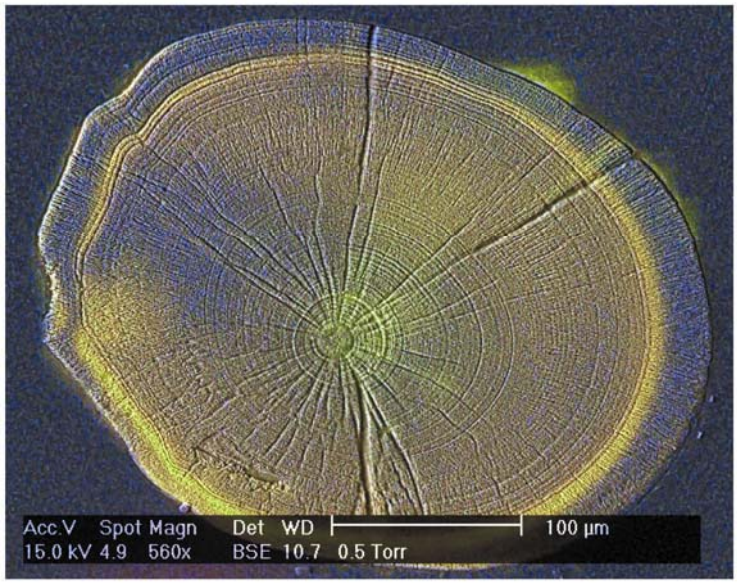

Fig. 5.3 – Glass eel otolith stained with alizarin complexone (AC), as seen by fluorescent ring, and overlayed with the ESEM image of the same otolith. Note that the dark check mark on the ESEM image corresponds to the fluorescent mark.

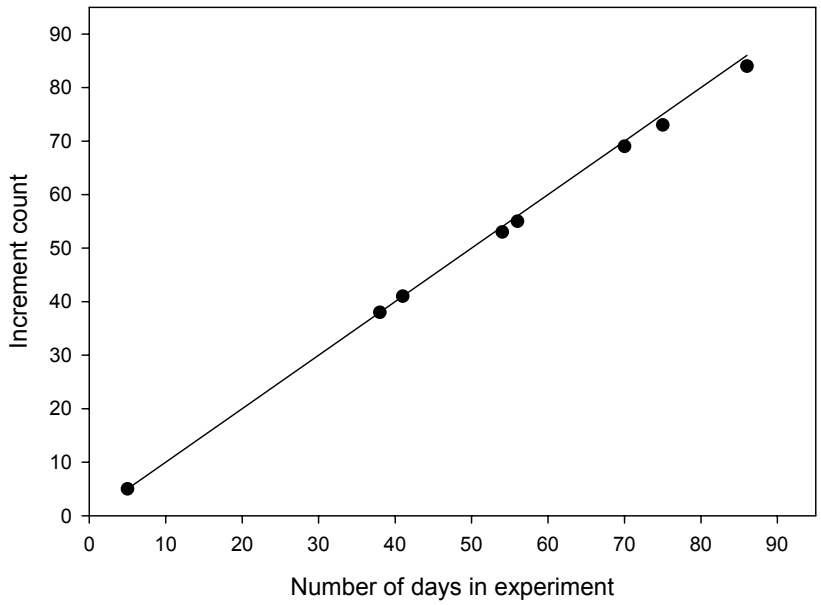

Fig. 5.4 – Relationship between experimental period and the number of increments counted distal to the AC mark. Line indicates equal increment counts and experimental days.

5.4.2. *Otolith morphology and measurements*

Fig. 5.5 illustrates the otolith and a series of images taken along the radius for increment count and width analyses. For both shortfins and longfins there was no significant difference in otolith diameter, radius, leptocephalus growth zone (LGZ) and post-metamorphic growth zone (PMGZ) between sites (all p's > 0.05, Table 5.1).

A relationship was found when comparing otolith radii, LGZ and PMGZ distances with the number of increments found in each region (Fig. 5.6, $r^2 = 0.20$, $p = 0.03$; $r^2 = 0.26$, $p = 0.01$; and $r^2 = 0.43$, $p = 0.0009$ respectively), with a trend of an increase in the number of increments counted with an increase in length of each region observed for shortfins. This indicates that these parameters could be used as an indication of eel age, larval duration, and age of the glass eel phase respectively. This pattern did not hold true for longfins especially when radii were related to total ages (Fig. 5.7, $r^2 < 0.00001$, $p = 0.63$) and the number of increments in the LGZ were compared to the length of these regions ($r^2 = 0.04$, $p = 0.16$). A positive trend was seen for the number of increments in the PMGZ related to its width ($r^2 = 0.12$, $p = 0.05$).

Table 5.1 – Otolith diameter, radius, leptocephalus growth zone (LGZ) and post-metamorphic growth zones (PMGZ) at 4 and 5 sites for shortfins and longfins, respectively, with results of ANOVA included. Latitude and longitude of each site is in parentheses. Power < 0.2 for each ANOVA.

Shortfins						***Longfins***					
Site	**Otolith**	**Diameter (µm)**	**Radius (µm)**	**LGZ (µm)**	**PMGZ (µm)**	**Site**	**Otolith**	**Diameter (µm)**	**Radius (µm)**	**LGZ (µm)**	**PMGZ (µm)**
Tweed R.	1	267.58	138.23	95.95	42.28	Tweed R.	1	308.87	166.27	99.06	67.21
(153°22’3”E	2	291.66	167.01	108.68	58.33	(153°22’3”E	2	272.63	163.26	99.00	64.26
28°20’5”S)	3	242.25	140.21	93.09	47.12	28°20’5”S)	3	292.40	168.81	120.34	48.47
	4	282.60	147.24	98.00	49.24		4	241.13	136.63	85.89	50.74
	5	280.17	162.07	94.51	67.56		5	279.56	155.55	98.45	57.10
Audley	1	255.25	143.16	91.75	51.41	Lansdowne R.	1	277.32	159.23	104.84	54.39
(151°03’2”E	2	256.01	131.55	87.32	44.23	(152°31’5”E	2	254.86	144.81	98.43	46.38
34°04’3”S)	3	234.49	118.34	78.04	40.30	31°47’4”S)	3	252.35	144.11	88.50	55.61
	4	240.38	138.45	86.73	51.72		4	274.29	154.73	84.93	69.80
	5	259.70	143.02	90.19	52.83		5	254.9	135.96	98.19	37.77
Mullet Ck.	1	234.89	133.57	82.47	51.10	Audley	1	272.04	145.79	88.57	57.22
(150°48’3”E	2	255.31	118.84	66.36	52.48	(151°03’2”E	2	268.08	148.86	87.96	60.90
34°28’5”S)	3	267.96	144.65	105.08	39.57	34°04’3”S)	3	291.86	153.62	90.07	63.55
	4	235.15	138.44	70.61	67.83		4	278.45	137.12	90.16	46.96
	5	264.93	155.92	96.38	59.54		5	271.13	152.72	85.42	67.3
Boggy Ck.	1	285.72	149.08	87.76	61.32	Mullet Ck.	1	284.43	161.42	97.49	63.93
(149°52’2”E	2	223.44	123.32	88.63	34.69	(150°48’3”E	2	278.52	156.32	102.56	53.76
36°53’1”S)	3	318.72	178.22	103.01	75.21	34°28’5”S)	3	270.09	148.76	102.47	46.29
	4	281.81	153.12	96.48	56.64		4	253.91	133.91	76.52	57.39
	5	242.79	121.61	69.80	51.81		5	267.92	144.50	100.99	43.51
						Croobyar Ck.	1	270.61	149.50	100.98	48.52
						(150°24’5”E	2	261.72	143.48	91.55	51.93
						35°18’3”S)	3	275.87	145.24	91.28	53.96
							4	281.61	160.78	103.65	57.13
							5	267.50	155.08	86.34	68.73
$F_{3,16}$		1.416	1.007	1.464	0.488	$F_{4,20}$		0.901	1.023	1.174	0.497
p value		0.27	0.42	0.26	0.70	p value		0.48	0.42	0.35	0.74

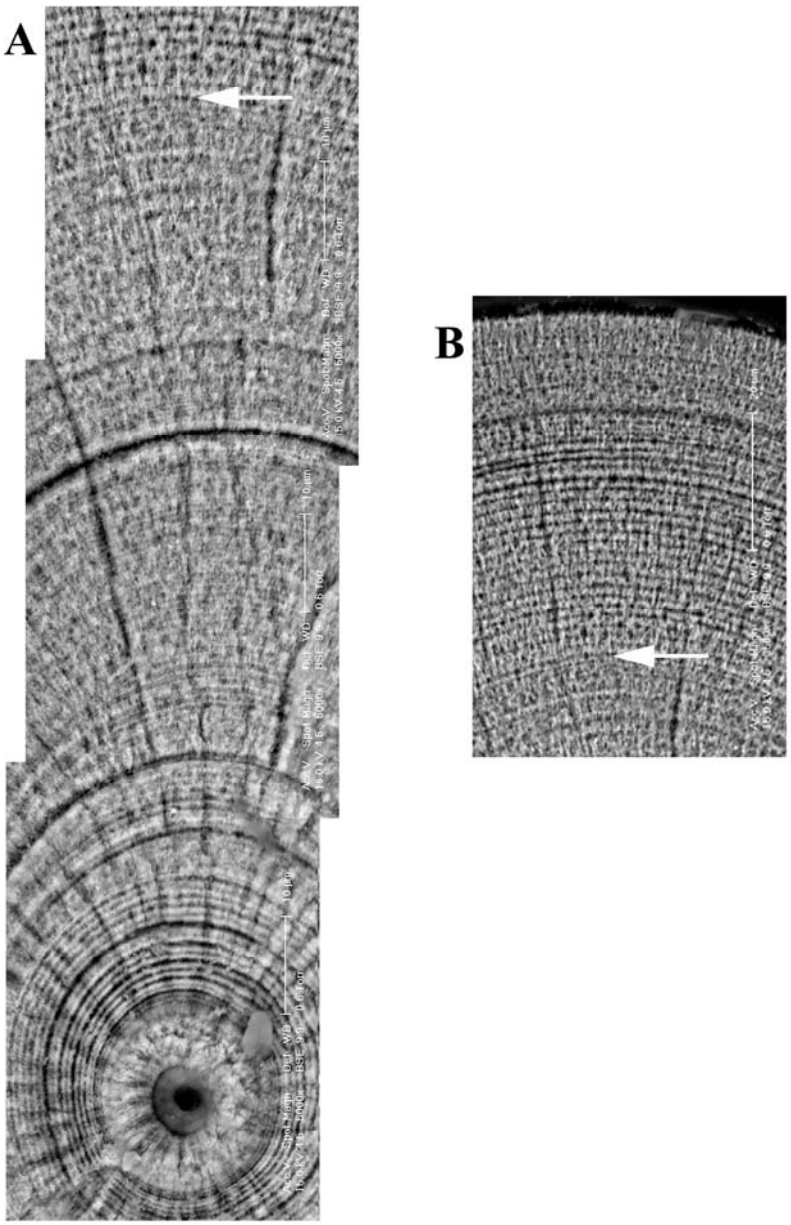

Fig. 5.5 – A) Shows a composite of ESEM images of an *A. reinhardtii* otolith's LGZ (144 increments counted) taken at 5000x magnification. B) Shows an ESEM image of the same glass eel's PMGZ (48 increments counted) taken at 3500x magnification. Arrow indicates end of LGZ in both images.

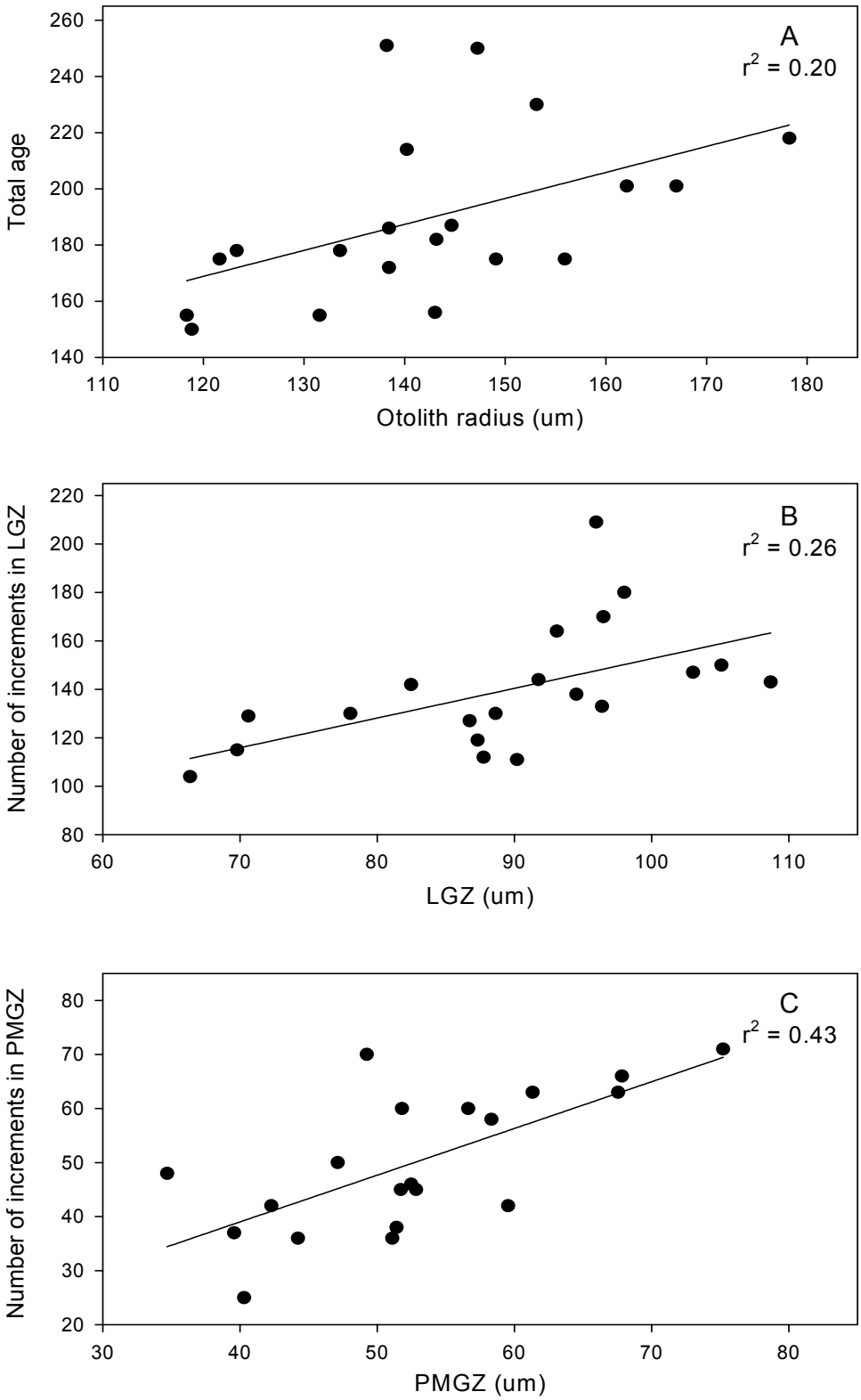

Fig. 5.6 – Comparison of shortfin A) otolith radii, B) LGZ and C) PMGZ with the number of increments counted in each region. n = 20 for each comparison.

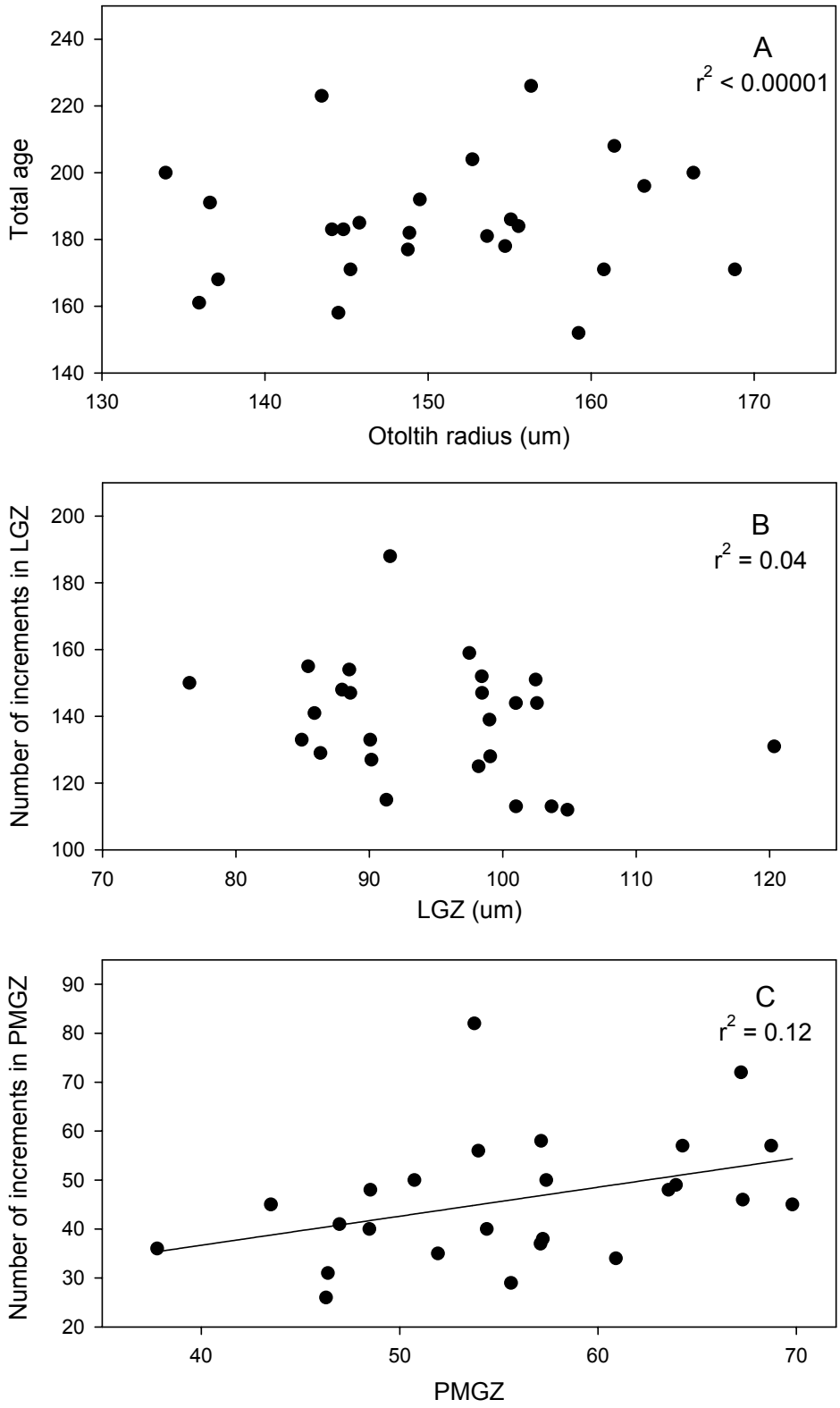

Fig. 5.7 – Comparison of longfin A) otolith radii, B) LGZ and C) PMGZ with the number of increments counted in each region. n = 25 for each comparison**.**

5.4.3. *Spatial patterns in age*

For shortfins, there was a significant difference in total ages (measured in days) of newly recruited glass eels between sites (Table 5.2). The total ages of eels caught at the Tweed R. were unexpectedly significantly older than those caught at Audley ($p = 0.005$) and Mullet Ck. ($p = 0.03$). The ages of eels caught at Boggy Ck. were not significantly different to those from the Tweed R. or from Audley and Mullet Ck. The differences in total age were attributed to differences in both the leptocephalus and glass eel phase of growth, depending on sites. Glass eels that recruited to the Tweed R. had a significantly longer leptocephalus phase than eels recruiting to Audley ($p = 0.04$). However, eels recruiting to Mullet and Boggy creeks were not significantly different from either site. In the glass eel phase of growth, eels that recruited to the Tweed R. and Boggy Ck. were significantly older than eels that recruited to Audley (p's = 0.04 and 0.01 between Audley and each site respectively). Again, for Mullet Ck., ages of eels in the glass eel phase of growth were not significantly different from either group.

Unlike shortfins, newly recruited longfin glass eels showed no significant differences in total ages ($p = 0.39$), leptocephalus age ($p = 0.95$) or in the number of increments counted in the glass eel phase of growth ($p = 0.24$), among sites (Table 5.3).

Table 5.2 – Shortfin total age, LGZ and PMGZ increment counts at each site with results of ANOVA included. p values in bold show a significant difference. For each comparison, means with a different superscript letter were found to be significantly different from each other.

Site	*Otolith*	*Age*	*Mean*	*s.e*	*LGZ*	*Mean*	*s.e*	*PMGZ*	*Mean*	*s.e*
Tweed	1	250			180			70		
R.	2	201			143			58		
	3	201	223.4^{A}	11.3	138	166.8^{C}	13.0	63	56.6^{E}	4.9
	4	251			209			42		
	5	214			164			50		
Audley	1	155			119			36		
	2	182			144			38		
	3	155	164.0^{B}	5.5	130	126.2^{D}	5.5	25	37.8^{F}	3.7
	4	172			127			45		
	5	156			111			45		
Mullet	1	150			104			46		
Ck.	2	175			133			42		
	3	187	175.2^{B}	6.7	150	$129.8^{C,D}$	8.2	37	$45.4^{E,F}$	5.5
	4	178			142			36		
	5	186			120			66		
Boggy	1	178			130			48		
Ck.	2	230			170			60		
	3	175	$195.2^{A,B}$	11.9	115	$134.8^{C,D}$	10.8	60	60.4^{E}	3.7
	4	218			147			71		
	5	175			112			63		
$F_{3,16}$		7.851			3.571			5.301		
p value		**0.002**			**0.038**			**0.010**		

Table 5.3 – Longfin total age, LGZ and PMGZ increment counts at each site with results of ANOVA included. Power < 0.2 for each ANOVA.

Site	*Otolith*	*Age*	*Mean*	*s.e*	*LGZ*	*Mean*	*s.e*	*PMGZ*	*Mean*	*s.e*
Tweed R.	1	200			128			72		
	2	196			139			57		
	3	171	188.4	5.1	131	137.2	3.4	40	51.2	6.3
	4	191			141			50		
	5	184			147			37		
Lansdowne	1	152			112			40		
R.	2	183			152			31		
	3	183	171.4	6.3	154	135.2	8.0	29	36.2	2.9
	4	178			133			45		
	5	161			125			36		
Audley	1	185			147			38		
	2	182			148			34		
	3	181	183.4	5.3	133	142.0	5.2	48	41.4	2.6
	4	168			127			41		
	5	201			155			46		
Mullet Ck.	1	208			159			49		
	2	226			144			82		
	3	177	193.8	11.9	151	143.4	8.0	26	50.4	9.0
	4	200			150			50		
	5	158			113			45		
Croobyar	1	192			144			48		
Ck.	2	223			188			35		
	3	171	188.6	9.5	115	137.8	13.8	56	50.8	4.3
	4	171			113			58		
	5	186			129			57		
$F_{4,20}$		1.081			0.167			1.507		
p value		0.392			0.953			0.238		

The increment width pattern within each otolith was similar for both shortfin and longfin glass eels (Figs. 5.8, 5.9). Increment width increased to a maximum of 1.7 μm at around 10 – 30 days from the beginning of the leptocephalus stage, then declined gradually to a minimum increment width, after which, increment widths remained fairly constant. Decreased increment widths during this period indicate the depletion of yolk-sac and oil droplet stores. For shortfins, the age and length of time at which this minimum occurred and remained constant varied among sites, with a minimum increment width of approximately 0.2 μm reached by glass eels recruiting to Tweed R. and this increment width remained fairly constant between age 90 and 135 days. At the other sites, the minimum increment widths and duration of this minimum, respectively, were 0.25 μm between age 60 and 110 days at Audley, 0.25 μm between age 40 and 110 days at Mullet Ck., and 0.2 μm between age 35 and 115 days at Boggy Ck. For longfins, the age and length of time at which the minimum increment width occurred and remained constant also varied between sites, with a minimum increment width of approximately 0.2 μm reached by glass eels recruiting to Tweed R. and this increment width remained fairly constant between age 60 and 120 days. At the other sites, the minimum increment widths and duration of this minimum, respectively, were 0.2 μm between age 30 and 110 days at Lansdowne, 0.2 μm between age 70 and 130 days at Audley, 0.2 μm between age 60 and 115 days at Mullet Ck., and 0.2 μm between age 50 and 110 days at Croobyar Ck.

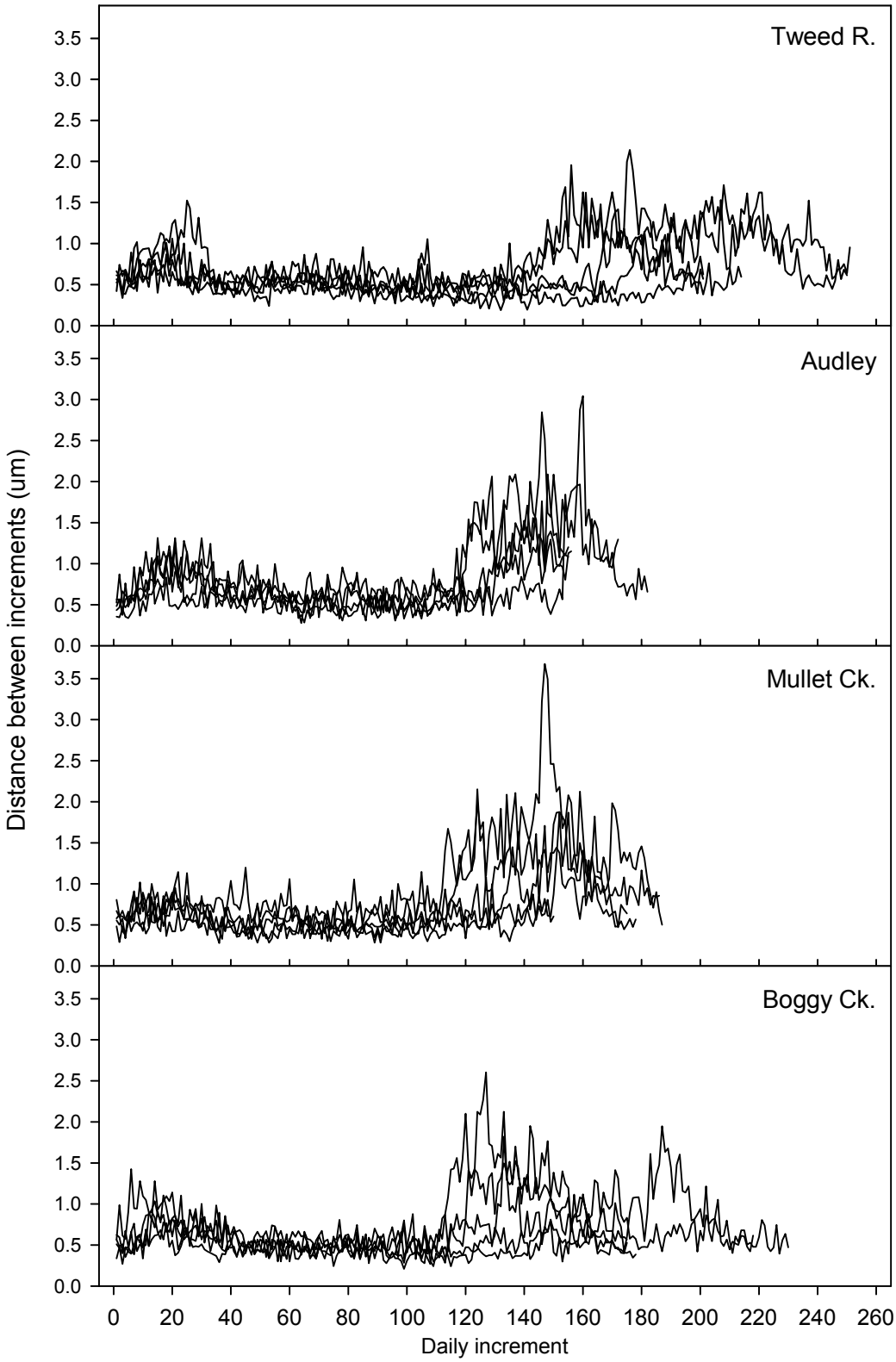

Fig. 5.8 – Distance between each increment in the otoliths of shortfins at each site. Increment series for each eel is indicated by a separate line. n = 5 for each site.

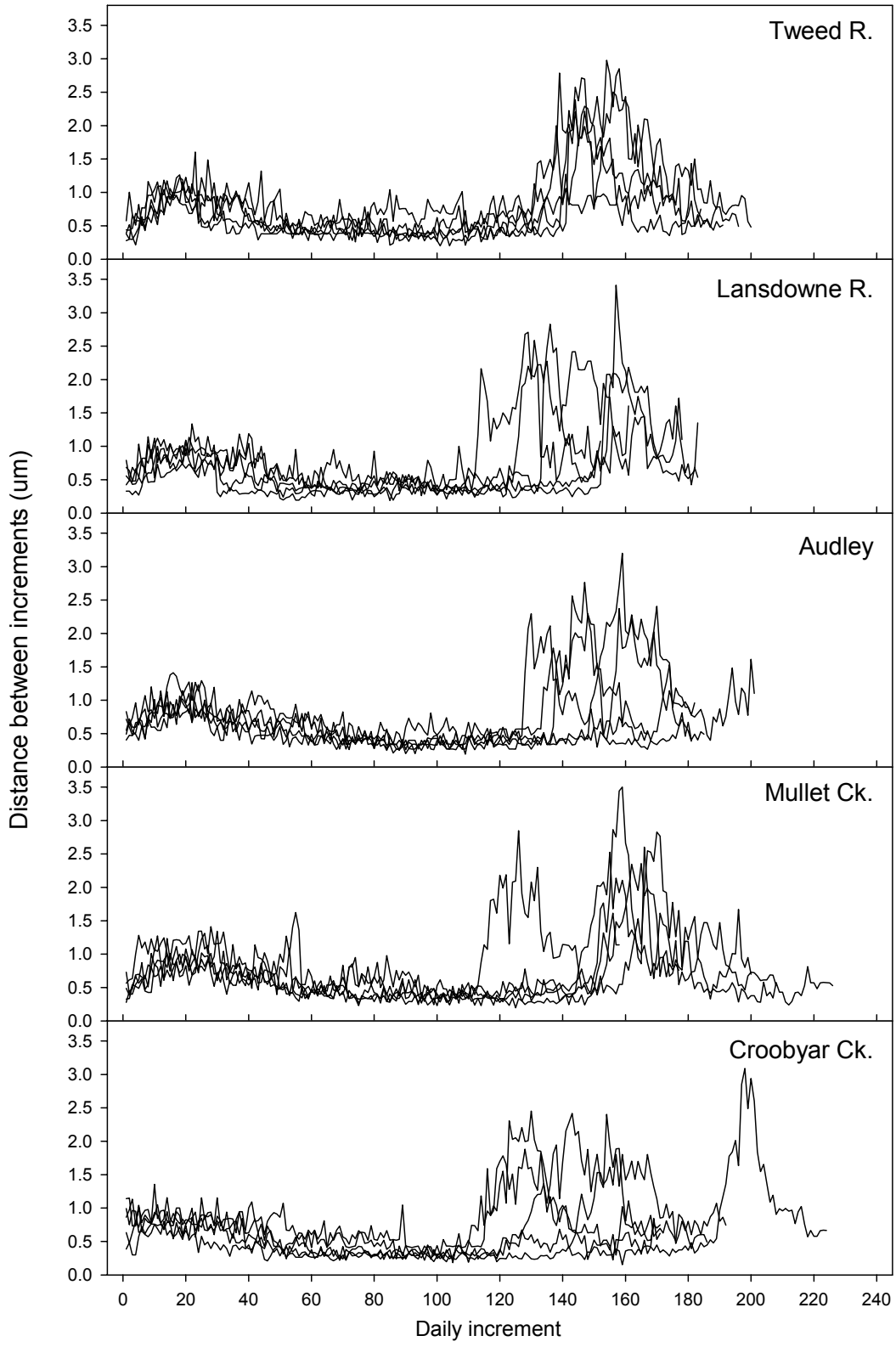

Fig. 5.9 – Distance between each increment in the otoliths of longfins at each site. Increment series for each eel is indicated by a separate line. n = 5 for each site.

The constancy of otolith increment widths was followed by a dramatic increase in increment width. For shortfins, a peak in increment width of up to 2.2 μm was observed for those eels recruiting to Tweed R., and this increase in width occurred when the eel was approximately 150 – 220 days old, depending on the otolith. A peak increment width of about 3.0 μm was observed at Audley which occurred between 130 and 160 days, at Mullet Ck a maximum increment width of about 3.75 μm was achieved and the increase in increment width occurred between 120 and 160 days, while a peak width of 2.6 μm was measured in otoliths of eels that recruited to Boggy Ck. with the increase in increment width occurring between 130 and 190 days. For longfins, the peak increment width from Tweed R. samples was approximately 2.9 μm with the dramatic increase in increment widths occurring between 140 and 160 days. At Lansdowne, 3.4 μm and 110 – 160 days were the peak increment width and age range of when peak increment width occurred respectively. At Audley the peak increment width was about 3.2 μm with increment increase occurring between 130 and 160 days and at Mullet Ck. a peak width of 3.5 μm was recorded and increment increase occurred between 125 and 170 days. At Croobyar Ck., 3.1 μm was the maximum increment width recorded and peak in increment width occurred between 120 and 200 days, depending on the total age of the eel. A decline in increment widths was observed for both species after these peaks.

5.4.4. *Temporal patterns in age*

A significant difference was found in total ages of shortfins recruiting to Audley between years (Table 5.4). Glass eels that recruited to Audley in 2000 were significantly older than eels that recruited in 2001. This difference was due to significantly more increments counted in the post-metamorphic phase of growth in 2000 than in 2001. There was no difference in the number of increments counted in the leptocephalus phase of growth between years.

Table 5.4 – Comparison of shortfin total age, LGZ age and PMGZ age at Audley between years, with results of ANOVA included. p values in bold show a significant difference. Means with a different superscript letter, within the same variable, were found to be significantly different from each other. Power < 0.3 for LGZ ANOVA.

Year	*Otolith*	*Total age*	*Mean*	*s.e.*	*LGZ age*	*Mean*	*s.e.*	*PMGZ age*	*Mean*	*s.e.*
2000	1	179			114			65		
	2	211			145			66		
	3	186	195.2^{A}	5.7	138	141.4	7.6	48	53.8^{C}	4.9
	4	199			157			42		
	5	201			153			48		
2001	1	155			119			36		
	2	182			144			38		
	3	155	164.0^{B}	5.5	130	126.2	5.5	25	37.8^{D}	3.7
	4	172			127			45		
	5	156			111			45		
$F_{1,8}$		15.466			2.614			6.812		
p value		**0.004**			0.145			**0.031**		

For longfins, there was no significant difference in the total age of eels that recruited to Audley in different years (Table 5.5), nor was there any difference in the number of increments counted in the leptocephalus or post-metamorphic phases of growth between years (all p's > 0.05)

Table 5.5 – Comparison of longfin total age, LGZ age and PMGZ age at Audley between years, with results of ANOVA included. Power < 0.2 for each ANOVA.

Year	*Otolith*	*Total age*	*Mean*	*s.e.*	*LGZ age*	*Mean*	*s.e.*	*PMGZ age*	*Mean*	*s.e.*
1999	1	178			134			44		
	2	203			165			38		
	3	185	189.2	4.1	139	139.8	6.5	46	49.4	4.3
	4	191			131			60		
	5	189			130			59		
2000	1	183			129			54		
	2	176			106			70		
	3	219	185.0	9.2	177	133.0	11.9	42	52.0	5.0
	4	183			132			51		
	5	164			121			43		
2001	1	185			147			38		
	2	182			148			34		
	3	181	183.4	5.3	133	142.0	5.2	48	41.4	2.6
	4	168			127			41		
	5	201			155			46		
$F_{2,12}$		0.209			0.314			1.802		
p value		0.815			0.736			0.207		

Also, there was no significant difference in the total ages of longfins that recruited to Audley in both February and August 1999 (Table 5.6). Similarly, no difference was found between the number of increments counted in the leptocephalus or post-metamorphic phases of growth between months (all p's > 0.05).

Table 5.6 – Comparison of longfin total age, LGZ age and PMGZ age at Audley between months in 1999, with results of ANOVA included. Power < 0.3 for each ANOVA.

Month	*Otolith*	*Total age*	*Mean*	*s.e.*	*LGZ age*	*Mean*	*s.e.*	*PMGZ age*	*Mean*	*s.e.*
February	1	178			134			44		
	2	203			165			38		
	3	185	189.2	4.1	139	139.8	6.5	46	49.4	4.3
	4	191			131			60		
	5	189			130			59		
August	1	238			183			55		
	2	223			168			55		
	3	180	208.2	10.2	140	155.2	9.3	40	53.0	3.9
	4	196			132			64		
	5	204			153			51		
$F_{1,8}$		0.174			1.858			0.383		
p value		0.687			0.210			0.553		

5.4.5. *Relationship between total age and glass eel length, weight and pigmentation stage*

There were no relationships between the total age of shortfins and their corresponding length, weight or pigmentation stage ($r^2 = 0.04$, p = 0.16; $r^2 = 0.04$, p = 0.17; and Gamma = -0.03, p = 0.87 respectively, Fig. 5.10). Also, when pigmentation stage was compared to the number of increments in the PMGZ, there was no relationship between the two parameters (Gamma = 0.27, p = 0.18). The lack of relationships between the above parameters with increment counts was also true for longfins, where $r^2 = 0.002$ (p = 0.30) when total age was related to eel length, $r^2 = 0.03$ (p = 0.16) when total age was related to eel weight and Gamma = 0.23 (p = 0.14) when total age was related to pigmentation stage (Fig. 5.11). However, there was a significant relationship between pigmentation stage and number of increments counted in PMGZ for longfins (Gamma = 0.30, p = 0.05).

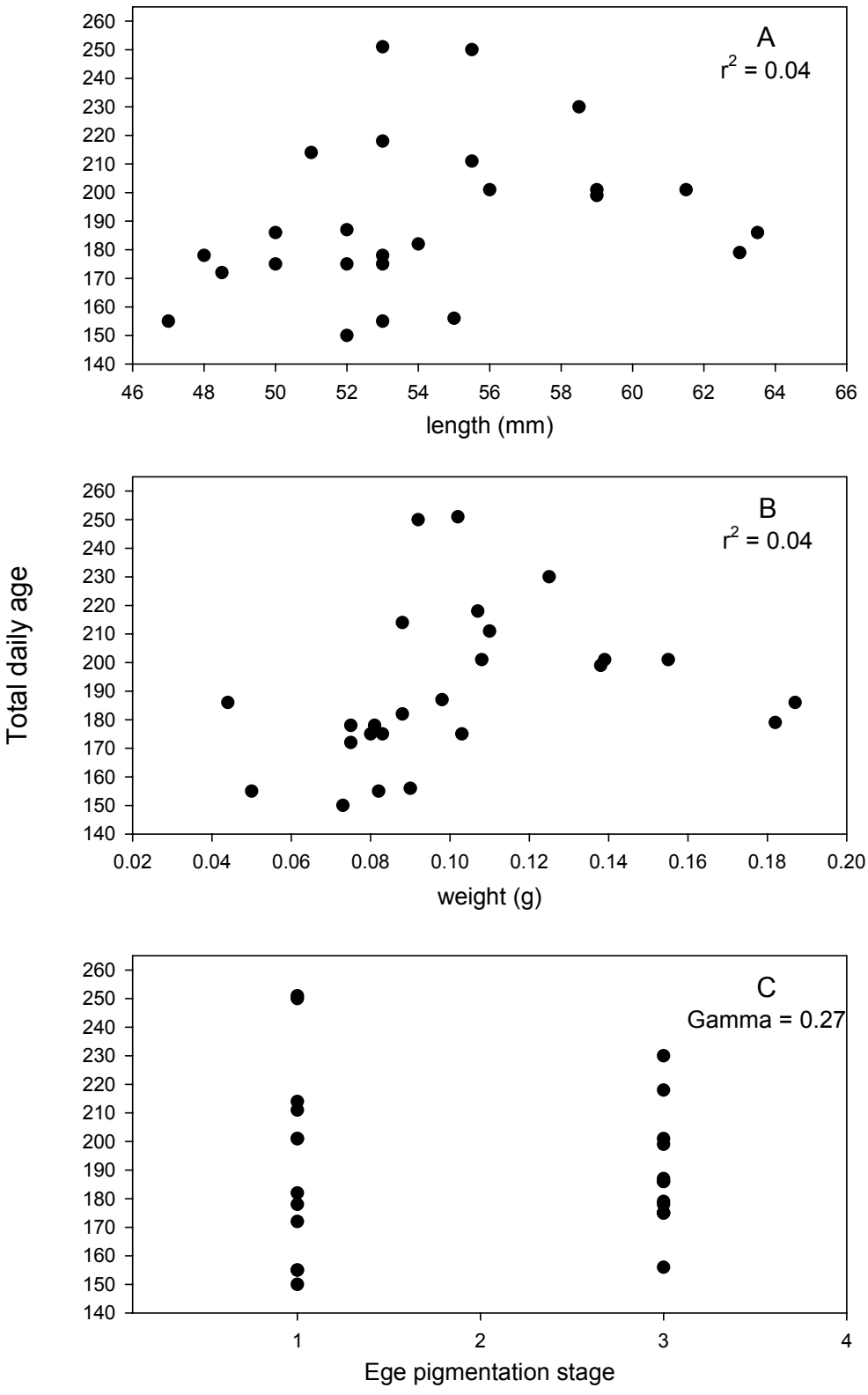

Fig. 5.10 – Total age of shortfins related to glass eel A) length, B) weight and C) pigmentation stage. n = 25 for each comparison.

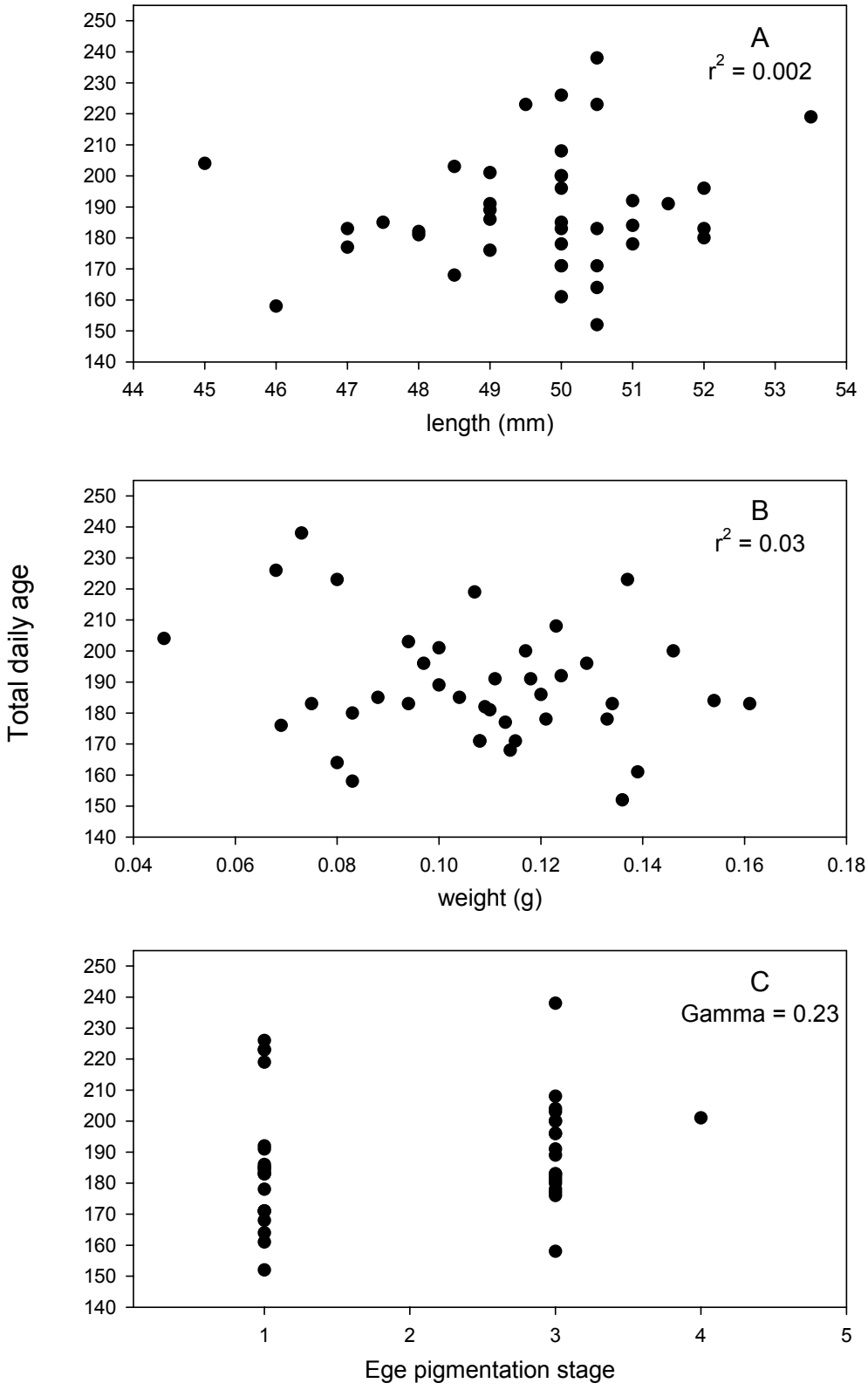

Fig. 5.11 – Total age of longfins compared to glass eel A) length, B) weight and C) pigmentation stage. n = 40 for each comparison.

5.4.6. *Estimated hatch dates*

Spatial comparisons of estimated hatch dates showed that shortfins that recruited to NSW in June hatched between the previous October to January, with hatch dates overlapping between sites (Table 5.7). Longfins that recruited to NSW during February were estimated to have hatched between July and September of the previous year, with good consistency between sites.

Table 5.7 – Spatial comparison of estimated hatch times, for each species caught in 2001, based on total ages. Latitude and longitude of each site is in parentheses. S = shortfins, L = longfins. Bars indicate hatch date range. Shortfins depicted by white bars, longfins depicted by shaded bars.

Site	*Est. hatch dates*	*M J J A S O N D'00 J'01 F M A*
Tweed R. (153°22'3"E 28°20'5"S)	S - 17/10/00-6/12/00 L - 9/8/00-7/9/00	
Lansdowne R. (152°31'5"E 31°47'4"S)	S - Not determined L - 25/8/00-25/9/00	
Audley (151°03'2"E 34°04'3"S)	S - 22/12/00-18/1/01 L - 12/8/00-14/9/00	
Mullet Ck. (150°48'3"E 34°28'5"S)	S - 18/12/00-24/1/01 L - 18/7/00-24/9/00	
Croobyar Ck. (150°24'5"E 35°18'3"S)	S - Not determined L - 20/7/00-10/9/00	
Boggy Ck. (149°52'2"E 36°53'1"S)	S - 10/11/00-4/1/01 L - Not determined	

The temporal comparison of hatch dates between years at Audley showed that shortfins recruiting to this site in June hatched between December and January each year (Table 5.8). For longfins that recruited to Audley in February, estimated hatch dates were between July and September, with overlapping hatch dates observed between years. However, longfin glass eels that recruited to Audley in August 1999 were estimated to have hatched between December and February.

Table 5.8 – Temporal comparison of estimated hatch dates, for each species caught at Audley, based on total ages. S = shortfins, L = longfins. Bars indicate estimated hatch date range. Shortfins depicted by white bars, longfins depicted by shaded bars.

Year	*Est. hatch dates*	*M*	*J*	*J*	*A*	*S*	*O*	*N*	*D*	*Next year*	*J*	*F*	*M*	*A*
February 1999	S - Not determined L - 31/7/98-25/8/98				▨									
August 1999	S - Not determined L - 30/12/98-26/2/99									▨	▨			
2000	S - 5/12/99-6/1/00 L - 5/7/99-29/8/99				▨					□	□			
2001	S - 22/12/00-18/1/01 L - 12/8/00-14/9/00					▨				□				

5.5. *Discussion*

This study is the first to document the ages of shortfin and longfin glass eels arriving at multiple sites within NSW and this is discussed in relation to recruitment of these eels from the spawning grounds by oceanic currents. It is also the first to document the interannual variability in ages of both species as they arrive at a single site. Interestingly, the ageing of longfins that recruited outside of their peak recruitment season in this study, lends much to dispelling the theory that these eels were caught in oceanic eddies, and is discussed here, for the first time, in relation to mechanisms that may be specific to this eel species.

The East Australian Current (EAC) is strongest between January and April (Hamon 1965), which is the time of peak longfin recruitment, and it may be that the EAC provides more regular delivery of leptocephali to the east coast of Australia during this time. In winter, which is the peak recruitment time of shortfins and when the EAC is not as strong, shortfins may be able to more easily detrain from the current, after receiving environmental cues, or may be more easily affected by other oceanographic conditions such as eddies. This may account for significant differences found for shortfin ages between sites. In this study, the mean age of shortfins and longfins at their time of metamorphosis from leptocephalus to glass eel, which is thought to occur on the continental shelf, was between 126 – 167 days and 135 – 143 days, respectively, depending on site. Similar results were found by McKinnon et al. (2002) where mean age of shortfin and longfins at metamorphosis was 105 – 176 days and 118 – 126 days respectively. Shiao et al. (2002) reported mean age at metamorphosis as 160 – 189 days for shortfins and 136 – 148 for longfins. However, Shiao et al. (2002) did not list the total age (age at capture) of shortfin and longfin glass eels aged in their study, despite saying that these ages would be compared, which was an unfortunate oversight. They do list time of estuarine arrival but this may differ from total age if a freshwater check (as they define it) was observed near the otolith margin.

In Chapter 3, it was calculated that the delivery of leptocephali to oceanic waters off the northernmost site would take up to 87 days and take up to 145 days to reach the

southernmost site. Ages of leptocephali reaching NSW estuaries were calculated at a maximum of 167 days. If delivery of leptocephali to NSW is passive, then the greater time taken to reach these areas compared to the times calculated based on current speeds, can be attributed to environmental conditions. The calculated times assume that currents are uni-directional and that the larvae are oriented toward some destination. This is not the case and may explain the greater number of days taken by leptocephali to reach the continental shelf (where they are assumed to metamorphose) than the calculated times suggest.

Total age of shortfins recruiting to Audley in June and July has been reported as 243 $\pm$ 19.7 (mean days $\pm$ s.d, Shiao et al. 2001) and within the range 197.3 $\pm$ 22.4 – 208.3 $\pm$ 27.2 (mean $\pm$ s.d) in June and August respectively (McKinnon et al. 2002). In this study, shortfins that recruited to Audley in June had a total age from 164.0 $\pm$ 5.5 to 195.2 $\pm$ 5.7 (mean days $\pm$ s.e) in 2001 and 2000 respectively. The variability between total age may be due to interannual variation in the length of time it takes glass eels to reach the estuary and the estuary/freshwater interface, with samples in Shiao et al. (2001) taken from 1999, samples from McKinnon et al. (2002) taken from 1998 and samples in this study taken from 2000 and 2001. This hypothesis is supported by a significant difference between years in this study (Table 5.4).

For longfins, total age has been reported as 166.6 $\pm$ 17.7 (mean days $\pm$ s.d) for eels recruiting in February 1999 to a site in Port Hacking that is 5 km downstream from Audley (McKinnon et al. 2002). In this study, total age was found to be 189.2 $\pm$ 4.1 (mean days $\pm$ s.e) for longfins collected at Audley in the same sampling period. However, Audley was sampled the day before the downstream site so the glass eels sampled were from separate estuary recruitment events. This study found no significant difference in the total age of longfins that recruited to Audley in different years. Similarly, no annual variability was found in the age at metamorphosis or estuarine arrival time for longfins recruiting to the Albert R. in Queensland for years where the same month was sampled (Shiao et al. 2002). This indicates that the same transport route may have been taken from the spawning ground and, if so, that oceanic currents may not have deviated wildly during these times.

The lack of significant differences found in ANOVAs for longfins may be attributed to low power from small sample sizes. The power level of 0.8 is generally accepted (Zar 1999) and to achieve this power for age of longfins between sites, 22 otoliths per site would need to be compared. However, in McKinnon et al. (2002) a total of 51 otoliths from 3 sites (no individual n's given but assume less than 22 per site) was a large enough sample size to detect a significant difference between total age and sites, and number of increments in the PMGZ and sites. Shiao et al. (2002) used sample sizes of 26 – 33 for this species and, even though significant differences among sites were found, the sample size was not large enough to completely separate sites in the ANOVA. Therefore, despite the low power of the ANOVAs, I believe that the results give a good indication of the age structure of this species due to the good agreement between results presented here and other studies.

Variation in total ages of glass eels between studies, both within the same sample period and interannually, may also be due to differences in otolith preparation and counting techniques that can lead to incorrect increment counts. However, due to the good agreement between the studies compared above, and the significant difference in ages of shortfins between years in this study, I conclude that there is close correspondence in preparation methods and increment counting procedures used in this study with those studies mentioned above.

Back-calculated hatch dates indicate that shortfins caught in June hatched between the previous October and January, while longfins collected in February had estimated hatch dates between July and September of the preceding year. Other studies of shortfins recruiting to Australian estuaries have estimated hatch dates from November to January (Arai et al. 1999b), August to February (Shiao et al. 2001, 2002) and October to March (McKinnon et al. 2002). Longfins recruiting to Australia in February in this study were also found to have similar hatching dates (July – October) to longfins aged previously (McKinnon et al. 2002). However, it can also be shown that longfins have an extended hatching period. Longfins collected in August in this study, when a recruitment pulse of these glass eels was detected, had an estimated hatch date of December – February. If the hatching dates are from the same population, then the hatch time for longfins in this study ranges

from July – February. Shiao et al. (2002) also found this species of eel to hatch from March through to the following January.

The lack of newly hatched leptocephalus samples, and indeed the enduring mystery of when and where these south Pacific eels spawn, can only lead us to make assumptions about the validity of the estimated hatch dates. The estimated hatch dates predicted for shortfin and longfin eels in this study are in good agreement with oceanic catches of leptocephali, where Aoyama et al. (1999) collected, and genetically identified, shortfin and longfin leptocephali (21 – 34 mm total length) in September. Although the spawning period for some shortfins collected in June are proposed to have hatched in October, the research cruise to collect leptocephali was only done from July to September. Also, it may be that shortfins that recruited earlier to the sampled estuaries in this study may have hatched earlier than October. Indeed, simultaneous catches of both species of leptocephali near the spawning grounds could be expected given the overlapping recruitment period of these two species to NSW (Chapter 3). Ageing of shortfin and longfin glass eels over their entire recruitment period would probably lead to overlapping estimated hatch dates.

There is some conjecture over whether daily increment deposition across the metamorphosis region is continuous. Cieri & McCleave (2000) suggest that calcium may be resorbed from the otoliths of *Anguilla* during metamorphosis partly due to the discrepancy between the radii of leptocephalus and glass eel otoliths. However, no statistical test is given to show that the perceived discrepancy is significant. The resorption theory is also used as an explanation for the discrepancy between back-calculated spawning dates of the Atlantic *Anguilla* with existing field observations highlighted in McCleave et al. (1998). It is clear that there is good agreement between the results of glass eel ages from this study with those found in the literature that are based on the study species. The estimated hatch dates are also comparable to the existing field observations for leptocephali of these species. Furthermore, Arai et al. (1999a) state that the maximum duration of metamorphosis is likely to be, at most, 18 days. If resorption does occur, and the metamorphosis period is 18 days, then the back-calculated hatch dates would still be comparable to field observations.

Timing of silver eel migration from freshwater to the spawning grounds, and the pathways used to arrive there, are relatively unknown since silver eels have rarely been caught during their oceanic migration. However, migrations of silver eels from freshwater to seawater have been stated to be in summer to autumn for shortfins (Todd 1980, Sloane 1984b, Watene et al. 2003), the New Zealand longfin eel (Todd 1980, Watene et al. 2003), the American eel (McGrath et al. 2003a, b) and the European eel (Aoyama et al. 2000, Durif et al. 2003, Tesch 2003). During the study of Australian longfin reproductive biology by Walsh et al. (2003), all seven silver males and 24 silver females captured in fresh and estuarine waters with open access to the ocean were obtained from January through May and from January through June, respectively (Pease, unpublished data). A single female silver eel was also captured from the ocean in March, indicating that the primary out-migration of silver longfins also occurs in summer/autumn. Recently, silver phase Japanese eels have been caught in the ocean during winter, presumably on their migration to the spawning grounds (Sasai et al. 2001).

If silver eel migration is predominantly in autumn, then the arrival of these eels to the spawning grounds would take longer for eels that originated furthest from these areas, provided that all these eels survived. Therefore, if mature eels from areas close to the spawning ground migrated seaward at the beginning of autumn, and some eels furthest from the spawning grounds began their migration at the end of autumn, there would be, at least, a 3 month spawning period that would be reflected in the ages of eels that recruit to estuaries during the migration period. Also, the predicted spawning region covers a large area (Aoyama et al. 1999), so some eels may spawn closer to Australia than others. This study found that ages of shortfins recruiting to different estuaries during the same month were significantly different. However, the implications of this spatial factor are confounded with temporal variability in the velocity of the delivery currents (SEC & EAC). The variation in ages of eels that recruit to NSW may reflect that younger eels hatched closer to Australia and older eels hatched more easterly. If this was the primary factor causing variability in age at recruitment, one would expect the variability of age at recruitment to be higher in longfins than shortfins because longfins have a greater

latitudinal range. However, longfins have no significant spatial or temporal variation in age at recruitment.

It was hypothesised in Chapter 3 that the uncharacteristic recruitment of longfins outside of their peak recruitment period may be explained by these later recruiting eels being engaged in eddies that commonly form off the NSW coast. If true, there would be a corresponding increase in their total ages, and this would manifest itself in a longer leptocephalus stage. However, this study found no significant difference between the total ages of longfins or in the ages of leptocephalus phase of growth in eels that recruited in February and August. The mean total age and number of increments observed in the leptocephalus phase of growth was higher in August than February, indicating that current velocities are indeed slower or more variable in winter compared to summer. Thus, it appears that the difference in recruitment timing of longfins seen in Chapter 3 and Pease et al. (2003b) is due to the extended spawning and hatch times of this more tropical species rather than their entrapment in an eddy.

The otoliths of shortfin and longfin glass eels analysed in this study were consistent with descriptions of otoliths of other anguillid eel species in terms of size and appearance (Tabeta et al. 1987, Tsukamoto et al. 1989, Umezawa & Tsukamoto 1990, Budimawan 1997, Arai et al. 1999b). Australian shortfin otolith maximum radii have been measured within the ranges 122 – 178 μm (this study), 150 – 190 μm (Sloane 1984a) and 165 – 179 μm (Arai et al. 1999b). Longfin otolith radii have been measured within the range 134 – 169 μm (this study) and at 160 μm (Sloane 1984a). Daily increment formation in otoliths during the glass eel phase of growth has been validated here for both species. The slightly lower number of expected increments during the validation experiment can be attributed to the difficulty in discerning increments at the otolith margin.

The daily increment width patterns shown for otoliths of Australian shortfin and longfin glass eels in this study are similar to the patterns described previously for these species (Arai et al. 1999b, Shiao et al. 2001, Shiao et al. 2002) and for other anguillid eel species (Tabeta et al. 1987, Umezawa & Tsukamoto 1990, Lecomte-

Finiger 1992, Cheng & Tzeng 1996, Arai et al. 1997, 2000, Wang & Tzeng 2000). Increment widths in otoliths of both species in this study showed a small peak in increment width at 10 – 30 days of age. This is similar to results from other studies such as Arai et al. (1999b) which showed this peak to form in shortfin otoliths 20 – 40 days from the hatch check, Shiao et al. (2001) which showed a peak in shortfin increment width of 0.8 μm 40 – 50 days after hatch, and Shiao et al. (2002) stated that, for shortfins and longfins, the increment width increased to 0.8 – 1.0 μm around 30 days of age. Thereafter, increment widths in otoliths in this study decreased, and this is thought to indicate the depletion of yolk-sac and oil droplet stores. Increment widths then remained fairly constant, or slightly decreased, to about 0.2 – 0.25 μm between 35 and 135 days for shortfins and about 0.2 μm between 30 and 130 days for longfins in this study. Again, good agreement was found between these results and those of other studies, where Arai et al. (1999b) showed an increment width decrease until age 130 – 200 days of age, Shiao et al. (2001) showed that increment widths decreased to 0.2 μm and Shiao et al. (2002) showed an increment width decline to 0.3 μm after approximately 170 days for shortfins and 150 days for longfins. Beyond this period, increment widths show a dramatic increase and then drop sharply. In their longfin age validation study, Pease et al. (2003a) noted that the marine nucleus was opaque (when viewed under reflected light) and this is probably formed by the area prior to the dramatic increase in increment widths.

The strontium content in anadromous and diadromous fish structures differs between their seawater and freshwater residencies (Bagenal et al. 1973, Secor et al. 1995) and past environmental histories can be reconstructed by analysing strontium and calcium ratios (Sr:Ca) (Radtke et al. 1990, Secor et al. 1995). Since the patterns between increment widths have been shown to be similar between studies, I assume that the relationship between increment widths and Sr:Ca patterns described in other studies (Arai et al. 1999b, Wang & Tzeng 2000, Shiao et al. 2001, Shiao et al. 2002) may also apply in this study. That is, when dramatic changes Sr:Ca have been shown to correspond with dramatic changes in increment widths, and have therefore been related to life history events, changes in increment widths in otoliths in this study can also be related to those events.

Sr:Ca at the core has been found to be low (recorded at approx. 0.0085 for shortfins, Arai et al. 1999b, 0.009 – 0.01 for both species, Shiao et al. 2001, 2002), gradually increasing to a peak towards the end of the LGZ (0.0172 for shortfins, Arai et al. 1999b, 0.018 – 0.02, Shiao et al. 2001, 2002) and then dramatically decreasing (approx. 0.0058, Arai et al. 1999b, 0.006 – 0.007, Shiao et al. 2001, 2002) at the edge of the otolith. The sharp decrease in Sr:Ca is accompanied by a sharp increase in increment width and this is thought to indicate the metamorphosis from leptocephalus to glass eel (Tzeng & Tsai 1994, Tzeng 1996, Arai et al. 1997, Arai et al. 1999b, Shiao et al. 2002). This occurs in the PMGZ of otoliths and, as stated in the Introduction, there is often a check mark visible in this zone. Under reflected light, the PMGZ is seen as a translucent zone outside of the marine nucleus (Pease et al. 2003a) and a check mark viewed in this zone is used as the "zero" age reference in annual ageing studies of later life history stages (Pease et al. 2003a).

Calculating the age of glass eels is a laborious and time-consuming process. Therefore, it may be of value to attempt to determine a relationship between age and characteristics of the eel that are more easily measured, such as distances of each defined otolith section, or between length, weight or pigmentation stage of the glass eel. As seen from this study, there is a relationship between shortfin otolith radii, LGZ and PMGZ and the number of increments in these regions. This does not seem to be the case for longfins. Bishop et al. (2000) found that the number of increments in the otoliths of 233 leptocephali (4 species, no *Anguilla spp*.) were positively related to otolith radii, and Tsukamoto et al. (1989) and Tsukamoto (1990) found a linear relationship between otolith radii and age for the Japanese eel. However, like Tsukamoto et al. (1989), I advise caution in using this method to determine age, since only a small number of samples were used. Small sample size may contribute to no difference being found in the longfin analyses.

It has also been shown in this study that glass eel total age cannot be determined by length, weight or pigmentation stage of the eel. Tsukamoto (1990) found no correlation between age and length, or age and pigmentation stage, for the Japanese eel, but Shiao et al. (2001) did find a positive correlation between total age and length of shortfins. Lefebvre et al. (2003) assumed that the increase in pigmentation

stages as the recruitment season of the European eel progressed was due to older glass eels recruiting later. No ageing of eels was attempted in that study and it would seem, based on the available research (McCleave et al. 1998), that such an assumption should be avoided. Jellyman (1977) assumed that pigmentation stage reflects the post-metamorphic sea life of glass eels, although no ageing of eels was done, and, even though only newly arrived glass eels were aged in this study, a correlation between post-metamorphic sea life and pigmentation was shown for longfins. A correlation between pigmentation and number of increments in the PMGZ for shortfins, and a stronger correlation for longfins, may be possible if ageing is done over the entire pigmentation range. Pease et al. (2003b) showed that length of longfins was negatively correlated with pigmentation and it therefore seems most likely that pigment reflects duration of glass eel estuarine residency (see Chapter 3). Yet, without an identifiable check mark on the otolith, this cannot be determined within the scope of this study.

There is a generally held belief that eel species that recruit to multiple countries, including the two species of eels studied here, are derived from panmictic stocks (Tzeng & Tsai 1992, Lintas et al. 1998, Dijkstra & Jellyman 1999, McKinnon et al. 2002) but recent genetic research indicates that this may not be the case (Daemen et al. 2001, Wirth & Bernatchez 2001). Do silver eels that migrate from all rivers and estuaries of NSW, and other eastern Australian states, manage to reach the spawning grounds or do silver eels from other Pacific nations, that lie closer to the spawning grounds, contribute primarily to the eel recruitment in eastern Australia? Genetic studies may hold the answers to this question, however conclusive evidence is not yet available for Australian shortfin and longfin eels. The length of time necessary for silver eels to reach the spawning grounds is also unknown. This question may be answered by continued radio-tracking of a migrating eel or by tagging the eel on its outward journey followed by its capture at the spawning grounds. However, it is doubtful that either of these questions will be answered conclusively due to the expense, length of time and intensive study that is required.

Chapter 3 and Pease et al. (2003b) show that the seasonal recruitment period of shortfins is not highly variable. This chapter shows that the age at recruitment of this

species is spatially and temporally variable. The most likely explanation is that there are more variable delivery currents in winter which is when this temperate species primarily recruits to NSW. This variability may be confounded by the size of the spawning grounds but this area is likely to be smaller than that for tropical longfins since longfins have a greater latitudinal range. The more restricted (less variable) spawning season of shortfins may also be a reflection of their more limited latitudinal range.

The more tropical longfin eel has an extended and variable recruitment season compared to shortfins (Chapter 3, Pease et al. 2003b). The extended spawning season of longfins may be related to their more extensive latitudinal range. Greater latitudinal range lends to a greater range in the time taken for eels to reach the spawning grounds but despite the variable and extended recruitment period, the age at recruitment for longfins does not significantly vary spatially or temporally. This indicates that the spawning area is probably no larger for this species than it is for shortfins and that the primary reason for the extended and variable recruitment is the greater range in the time of the spawning migration and associated spawning season. Lower variability in age at recruitment is most likely related to the fact that longfin recruitment is primarily during the summer when delivery currents are stronger and less variable.

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Chapter 6: General Discussion

This thesis is the first to identify spatial and temporal recruitment patterns of shortfin and longfin glass eels to multiple sites in New South Wales (NSW). It is also the first to validate daily increment deposition in the otoliths of both these species. The sampling technique designed and employed in this study to collect glass eels has subsequently been used in France to aid their glass eel monitoring program (Dekker 2002). The identification of important estuarine habitats for shortfin and longfin glass eels in estuaries was established through experimentation in this thesis and showed that the eels exhibited preferences that continue through to their freshwater existence. Ageing of glass eels of both species, and estimations of hatch dates, in this study agreed well with other studies on shortfin and longfin glass eel age and corresponds to timing of leptocephali catches in the Pacific. The ageing of eels also yields a better understanding of factors that affect eel recruitment to NSW, with oceanic conditions, namely current velocity, determined to be the primary cause of variability.

The compact size, versatility, and ease of use of the artificial collectors used to sample glass eels here enabled six estuaries, spread over the range of the NSW coastline, to be sampled during the new moon period of each month. Such sampling proved important in NSW especially since both shortfin and longfin glass eels recruit to these estuaries and since longfins occasionally recruited to the sampled sites outside of their typical recruitment period. Furthermore, the timing and abundance of glass eel recruitment can lay the foundations for sustainable glass eel fisheries. For example, if longfin eels are the predominant species in northern NSW rivers, due to its more tropical distribution and as commercial landings suggest, then a shortfin glass eel fishery in this region may not be seen as detrimental to the ecology of freshwater and estuarine environments since adult shortfins have a more temperate distribution. Alternatively, a balance between a longfin glass eel and yellow eel fishery in this same region would need to be found since over exploitation of the juvenile phase could dramatically affect the community structure of the estuary/freshwater environment and affect the success of the latter fishery. To determine this balance, studies on the natural mortality of eels as they traverse these

two life stages needs to be done. Also, many aquaculturists deal with only one species of eel. Therefore, it is of paramount importance that harvesters know peak recruitment timing of each species to maximise the likelihood of catching the preferred species.

The five year Audley temporal recruitment series is the first of its kind in Australia and showed consistent shortfin glass eel arrival between years. However, longfin recruitment extended over the year and the apparent failure of longfins to recruit to Audley in 2000/01 is of concern. Recruitment failure is generally thought to be due to the collective effects of overexploitation of glass eels and escaping spawners, loss and pollution of freshwater habitats, the *Anguillicola crassus* parasite, inappropriate restocking practices, and changes in oceanic conditions (Castonguay et al. 1994, Tzeng 1996, Atlantic States Marine Fisheries Commission 2000, Feunteun 2002). The result of this failure of glass eels to recruit to estuaries may not become clear until approximately 5 years later, the age at which longfins enter the commercial fishery (Pease et al. 2003), when there may be a decline in the commercial catch of yellow eels. This enforces the virtues of long term glass eel recruitment monitoring programs and, since recruitment failures can only be immediately identified in this way, management plans can be devised and implemented at a time when this glass eel year class is expected to appear in the commercial fishery. Perhaps further research could explore the connection between glass eel recruitment and the age structure of the yellow eel population within the same estuary.

Recruitment failure and declines in the commercial yield of eels has been widely documented in recent years (Castonguay et al. 1994, Dekker 2000, 2003, 2004). Analyses of 100 years of European eel commercial landings and 50 years of glass eel recruitment indices of this species for Lake IJsselmeer by Dekker (2004) did not solve the mystery of European eel decline but was at least able to isolate key events and tease out possible hypotheses for the decline. The eel stock began to decline in this lake in about 1960 but recruitment failure did not occur until the 1980's (Dekker 2000, 2004). The initial decline is thought to be due to an increase in natural mortality, although not from the *A. crassus* parasite which was introduced in the mid-1980's. The gradual decline in the eel stock was hardly noticed but the recruitment

failure was noticed immediately, presumably because of the implementation of a glass eel monitoring program. Indeed, a 90% decline in European glass eel recruitment throughout Europe since the 1980's (Dekker 2003) was identified through individual studies and monitoring programs introduced by many European countries (Dekker 2000). While oceanic factors having contributed to the European eel decline prior to the 1980's was excluded (Dekker 2004), it still remains a viable hypothesis for explaining the failure of glass eels to recruit to estuaries (Castonguay et al. 1994, Knights 2003).

Recently, Knights (2003) made a speculative assessment of long term oceanic factors on recruitment of eels to the Northern Hemisphere. The apparent negative correlation with the glass eel recruitment index and the North Atlantic Oscillation Index (NAOI), as well as other oceanic climatic conditions, appear to have had the greatest affect on recruitment as opposed to impacts of pollution, habitat loss or degradation, and overfishing (Knights 2003). While these latter impacts may have an effect on local stocks, they do not explain the continent wide declines discussed earlier. Alternatively, Jellyman et al. (2002) found no evidence that the El Niño Southern Oscillation phenomenon (ENSO) was directly associated with annual variation in glass eel recruitment in New Zealand although it may affect recruitment direction. In North America, the inability to test for any such continent wide declines in the recruitment of the American eel is due to the use of only one area for assessing an index of recruitment (Castonguay et al. 1994) and the shortage of relevant long term oceanic data (Knights 2003).

For effective management of the eel stock and the commercial eel fishery, long term monitoring of glass eel recruitment, stock and fishing mortality is needed, coupled with oceanic climate studies (Dekker 2000, Knights 2003). Furthermore, focussing on glass eel recruitment and silver eel escapement at several spatial scales (the "hydrosystem" including the whole catchment) over long temporal scales (a minimum of 5 - 15 years) has been suggested (Feunteun 2002). Accordingly, management of the eel can only be successful through the collaboration of local, national and international authorities. Australia and New Zealand are in an enviable position. Despite the importance and long history of eel harvesting among the native

populations of both countries, the more recent commercial eel fisheries have remained relatively small (around 1800 tonnes in 2002, FAO 2004) compared to Europe and Africa (over 7000 tonnes in 2002, FAO 2004). However, there is evidence to suggest that the New Zealand longfin eel is overfished (Hoyle & Jellyman 2002) but it is unknown if this decline is occurring at the same rate as elsewhere. Also, both countries have close international ties and good working relationships among eel researchers. Any collaborative eel management strategies should therefore be less complex even though state and regional differences need to be overcome.

Commonwealth legislation (Wildlife Protection Act 1982) requires that Australian eel fisheries be managed on an ecologically sustainable basis since eels harvested by commercial fisheries migrate through Commonwealth managed waters and are exported. The Australian and New Zealand Eel Reference Group (ANZERG) has been formed to provide a national and regional perspective on eel resource management as well as to promote sustainable eel industry development. It is through this medium that effective eel management in this region can be developed. In contrast, management of the European eel is difficult due to the multiple international governments and legislations that need to be overcome. Internationally, the International Council for the Exploration of the Seas (ICES) and the European Inland Fisheries Advisory Commission (EIFAC) have advised that the current European eel fishery is unsustainable and outside of safe biological limits (ICES 2001 in Starkie 2003). Because of the decline in the European eel, each European nation will need to make changes to its eel management and commercial fishery since there is no international eel fishery. This is a difficult task considering the different political agendas and allegiances in the region and that social demand to restore a depleted stock does not occur simultaneously in Europe (Feunteun 2002). The Atlantic States Marine Fisheries Commission (ASMFC) developed a coast-wide U.S. management plan because of the concern over the population status of the American eel (Haro et al. 2000). A major objective of this plan is the implementation of a long term monitoring program for glass eel recruitment, spawning stock biomass and for mortality rates (Atlantic States Marine Fisheries Commission 2000, Haro et al. 2000).

The above proposed management strategies assume that each species of eel comprises a single, randomly mating population, or panmictic stock (Tzeng & Tsai 1992, Lintas et al. 1998, Dijkstra & Jellyman 1999, McKinnon et al. 2002). However, recent preliminary research (Daemen et al. 2001, Wirth & Bernatchez 2001) indicates non-random mating and restricted gene flow among eels from different locations with Wirth & Bernatchez (2001) identifying three broad groups for the European eel (Mediterranean, North Sea and Baltic, and Iceland). This type of genetic stock structure information is currently lacking for Australian shortfin and longfin eels but is vital for eel management, especially for shortfins since stocks of this species are shared between Australia and New Zealand. Furthermore, research to determine the exact spawning location of each species of eel should be continued, especially if genetic information suggests stock separation.

Russell & Potter (2003) argue for the precautionary approach to be applied to eel management as set out by the United Nations (UN) and Food and Agriculture Organisation (FAO). As such, they propose that this approach applies not only to the commercial fishery, but also to the management of freshwater, estuarine and coastal habitats, aquaculture and restocking practices, as well as management to reduce exploitation or enhance stocks to counteract the effects of changing oceanographic conditions. Additionally, Feunteun (2002) proposed that for sustainable restoration and management of the European eel, the need to have a local restoration program that characterised the stocks and defined the restoration targets, defined restoration actions and monitored population parameters, and a coordinated continent wide approach that compared trends and adapted restoration techniques and targets.

The status of the shortfin and longfin population in Australia is poorly understood due to the lack of any long term glass eel recruitment monitoring information. Therefore, there is no bench mark level at which to assess any current recruitment patterns. I propose, in line with the precautionary approach, that a glass eel monitoring program be implemented, at specific sites, that span the geographic distribution of each species in both Australia and New Zealand. Again, this is assuming panmixia of the stock until any contrary evidence is produced. In NSW, the five year monitoring of glass eel recruitment to Audley should be continued as

information from this site currently provides the longest series of temporal monitoring data for both species in Australia. This river system is also closed to commercial fishing so can provide a good comparison with other sites for fishing mortality calculations. Furthermore, I believe that monitoring at the other sites sampled in this study should be continued since the sites are within the geographic recruitment range for both species in NSW and there is potential for the aquaculture industry to operate throughout the region depending on the species to be harvested. As outlined by Dekker (2002) it is only with a network of monitoring stations that we can discriminate local effects from global changes in recruitment in order to inform local and international management. Ageing information, as shown in this thesis, can assist in identifying global changes in recruitment by determining whether a change in recruitment is related to changes in current velocity or direction that transport eels from the spawning sites to estuaries. Accordingly, it would be useful to retain a sub-sample of glass eels caught from any monitoring program for ageing purposes.

The importance of complex habitats for both species of glass eel was outlined in Chapter 4 and the suspicion that habitat loss and degradation, as well as pollution, has led to the decline in the survival of eels was outlined. In fact, 33% of eel habitats available in Europe are estimated to be inaccessible for natural or artificial reasons (Moriarty & Dekker 1997 in Feunteun 2002). Thus, I recommend that strict development and pollution guidelines be implemented in estuaries and rivers that are identified as key eel residency areas. Perhaps the biggest issues in New Zealand are the unresolved problems associated with downstream passage of sexually mature eels through the turbines of Hydroelectric dams and, as with Australia, the lack of long term recruitment databases (the virtues of which are discussed above). The main issues identified with silver eel passage are the high mortalities of eels as they pass through the turbines and unsuccessful timing of spillway opening with downstream passage of mature females (Boubée et al. 2003, Watene et al. 2003). Therefore, management of each river system needs to allow for both successful upstream and downstream passage. Upstream passage from estuarine to freshwater habitats can be ensured by the destruction of weirs and other barriers that are no longer in use, and the construction of eel passages on those barriers that cannot be removed. Further

research needs to be done for successful downstream passage through turbines and spillways. Manual relocation of sexually mature eels is a possibility but the expense of this program may preclude its long term success.

As outlined throughout this thesis, the life history of the eel is unique, complex and poorly understood. Consequently, the management of the eel should be based on its life history as a whole and not compartmentalised so that each stage is managed as a discrete unit. Therefore, I recommend a more streamlined approach to eel management. In NSW alone, glass eel harvest is monitored by the aquaculture division and yellow eel commercial harvest in estuaries and coastal freshwater impoundments is managed by estuary managers. It is through ANZERG that I believe our obligation to maintain a sustainable eel population and fishery can be met and that we can achieve this by learning from the mistakes made in other regions and by adopting a "whole of eel" approach to management. The cornerstone of this management strategy should be the implementation of a large scale, long term glass eel monitoring program based on the information compiled in this thesis.

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Appendix 1

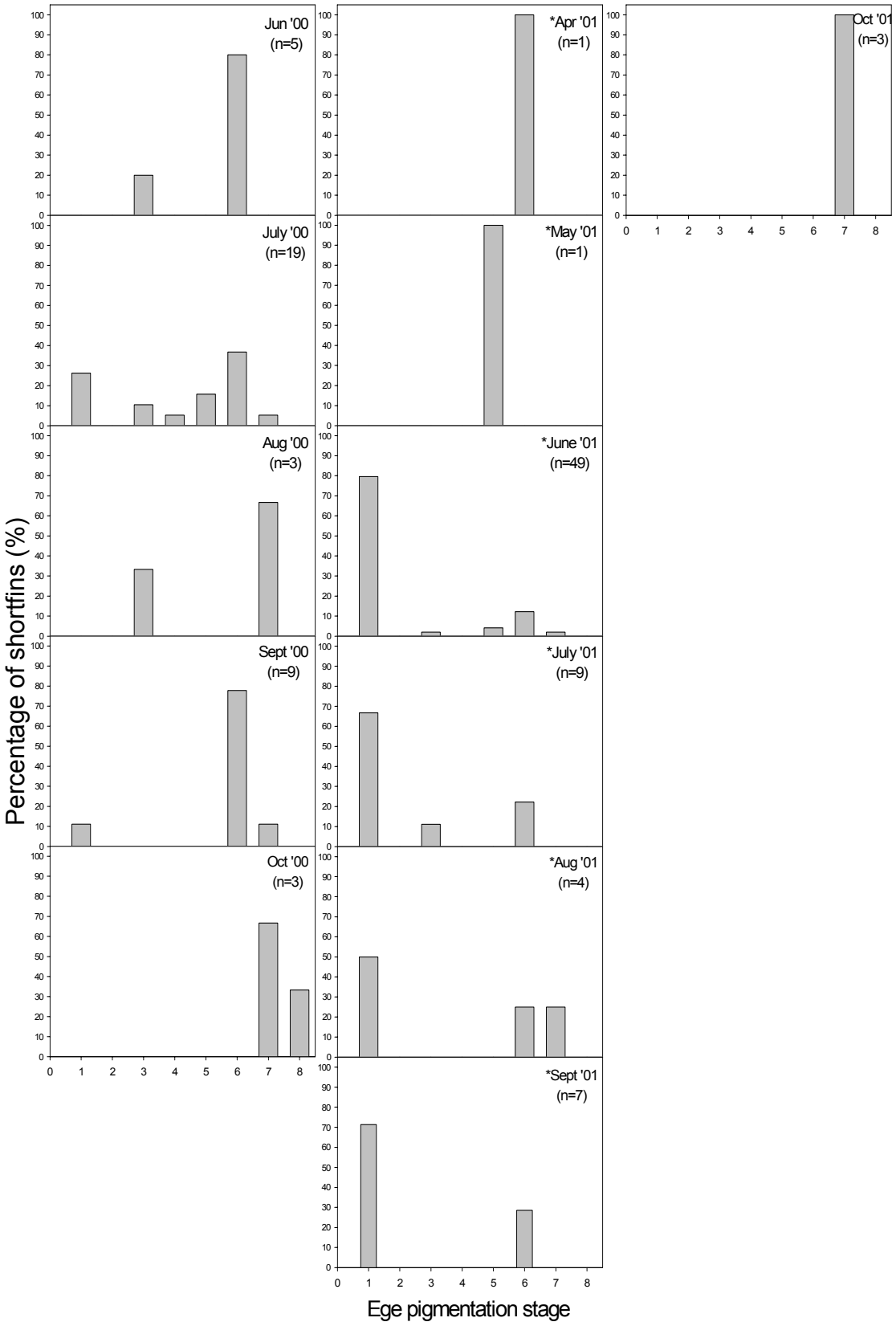


Fig. A1a – Proportion of pigmentation development of shortfins, according to Ege (1939), in each monthly Tweed R. sample, * indicates months of peak recruitment.

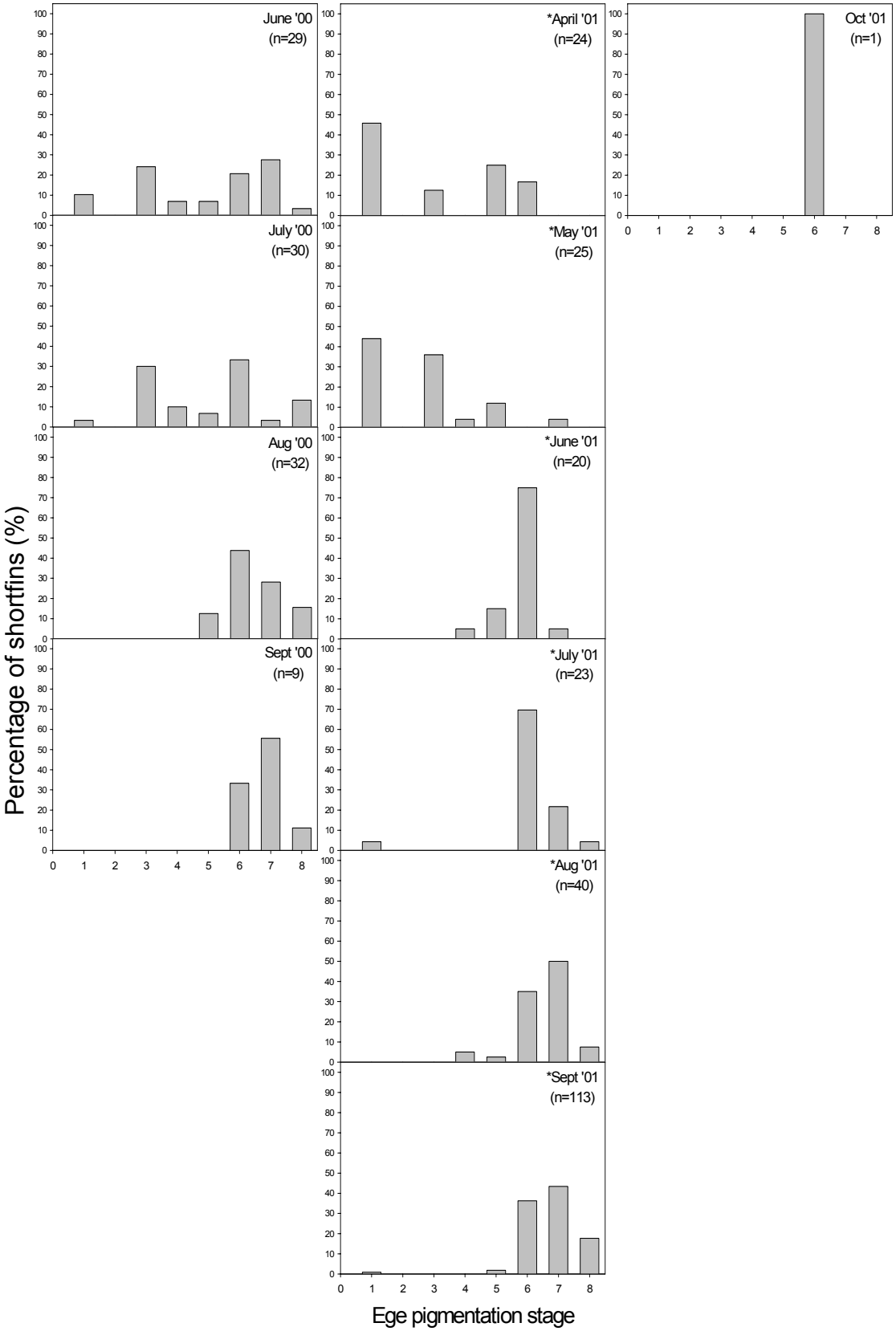
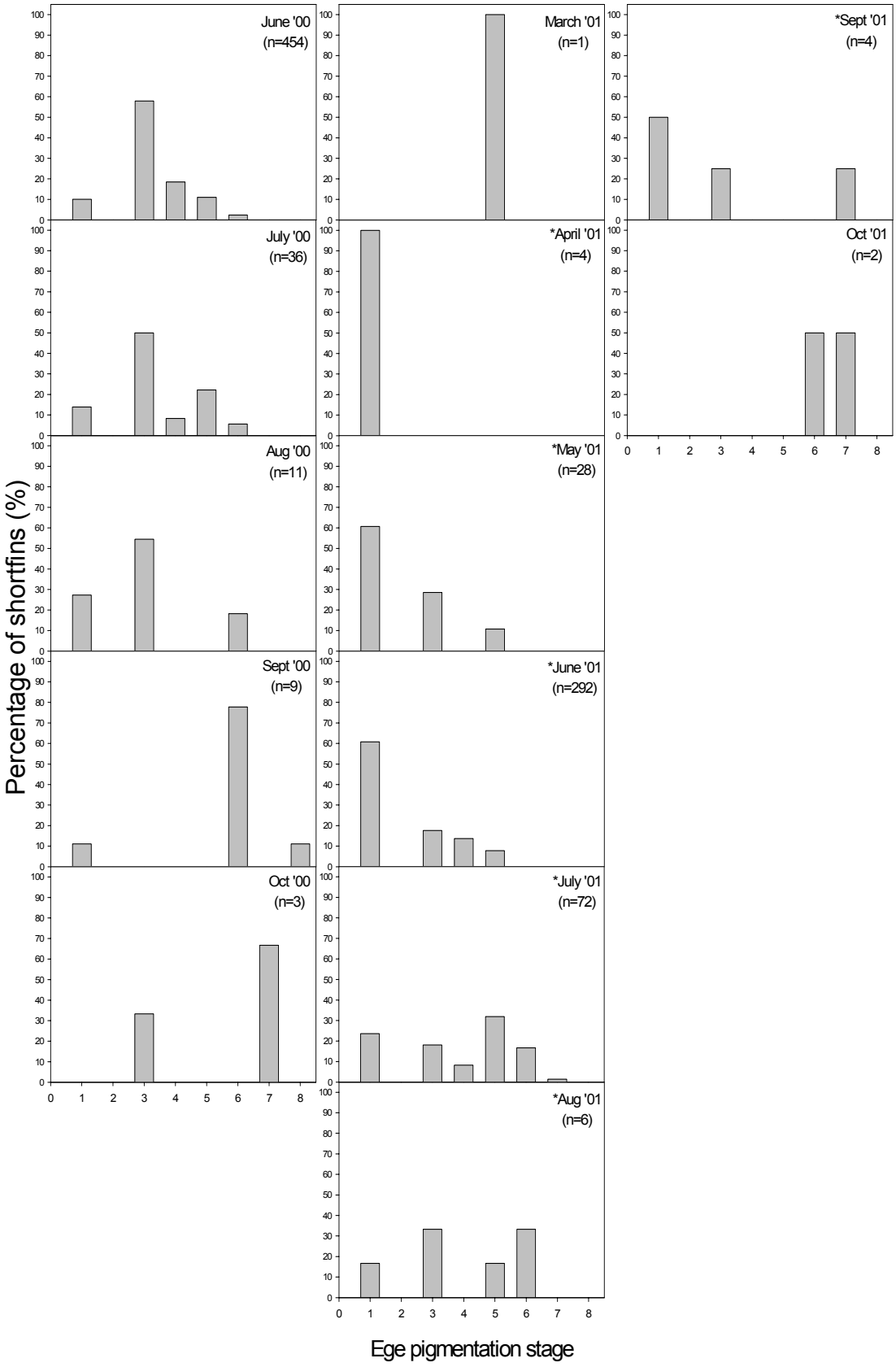


Fig. A1b – Proportion of pigmentation development of shortfins, according to Ege (1939), in each monthly Lansdowne R. sample. * indicates months of peak recruitment.



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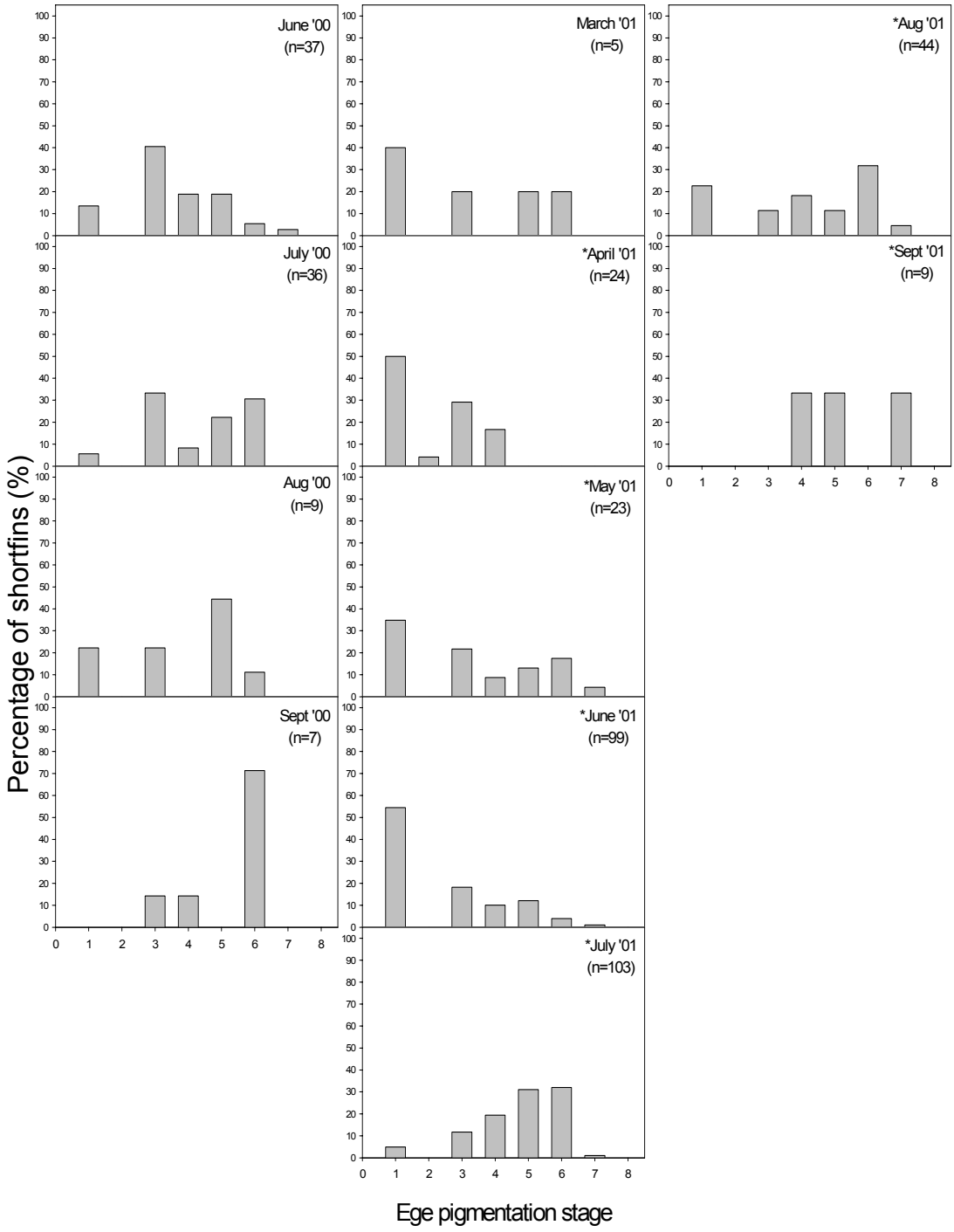

Fig. A1d – Proportion of pigmentation development of shortfins, according to Ege (1939), in each monthly Mullet Ck. sample. * indicates months of peak recruitment.

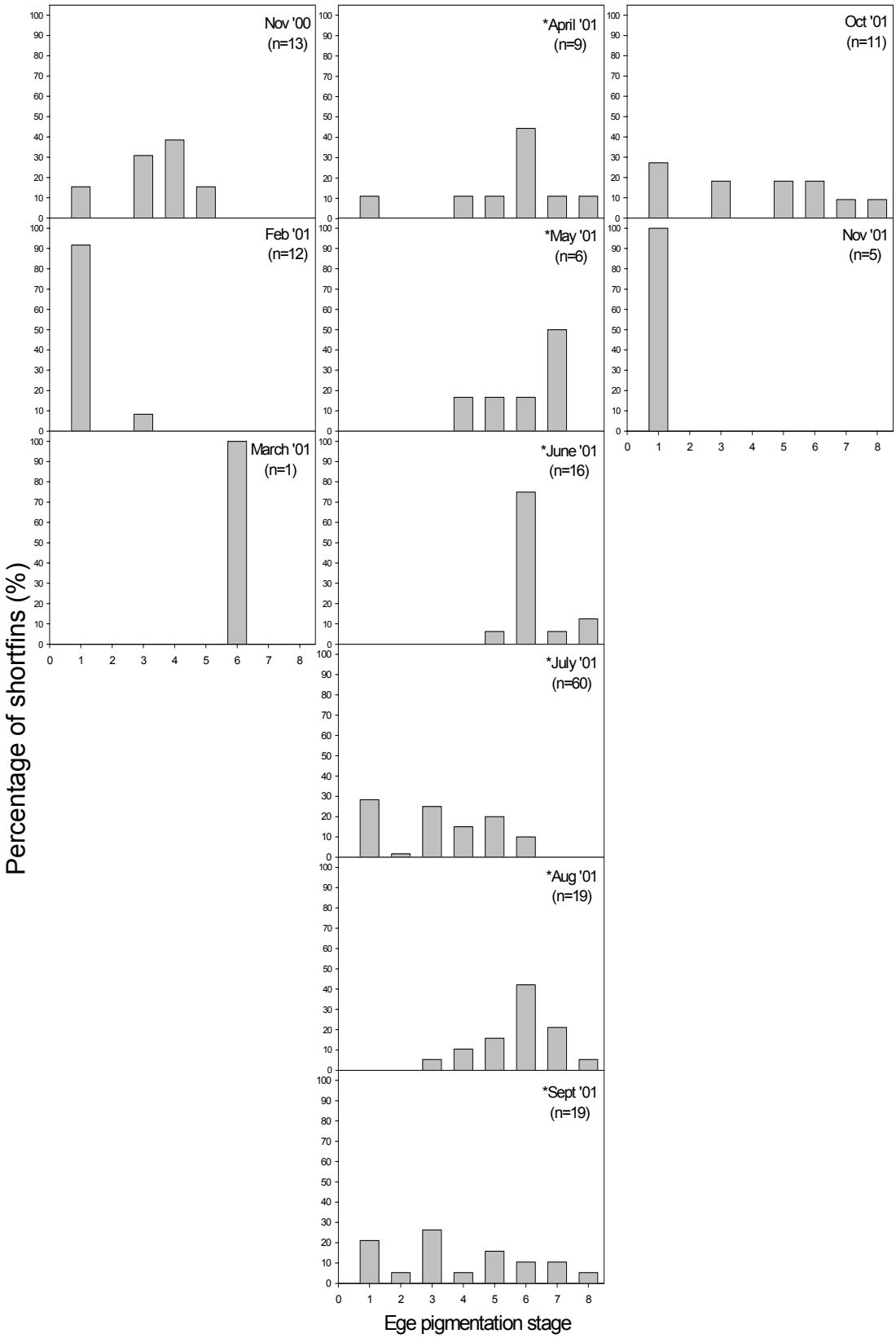

Fig. A1e – Proportion of pigmentation development of shortfins, according to Ege (1939), in each monthly Croobyar Ck. sample. * indicates months of peak recruitment.

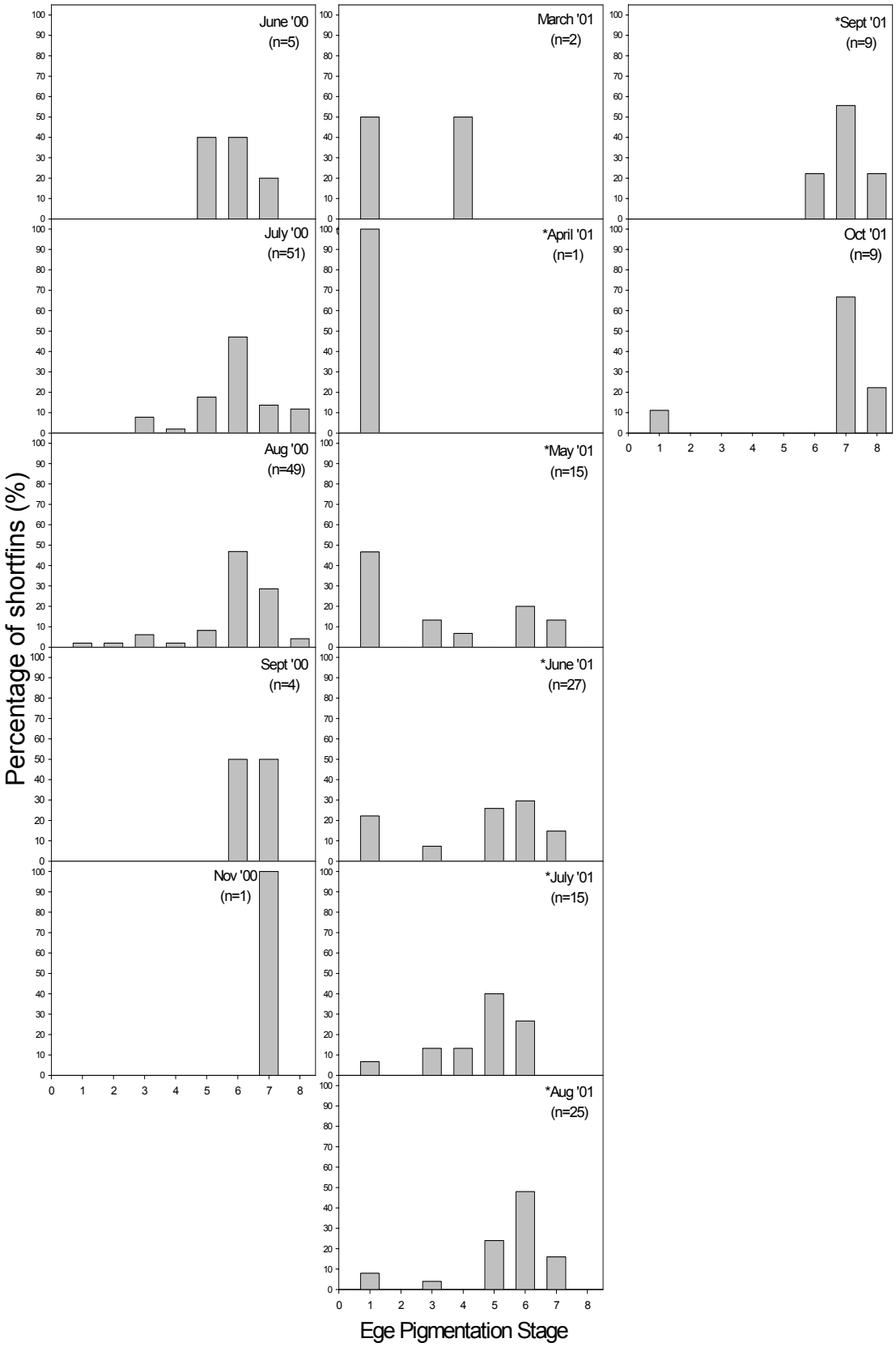

Fig. A1f – Proportion of pigmentation development of shortfins, according to Ege (1939), in each monthly Boggy Ck. sample. * indicates months of peak recruitment.

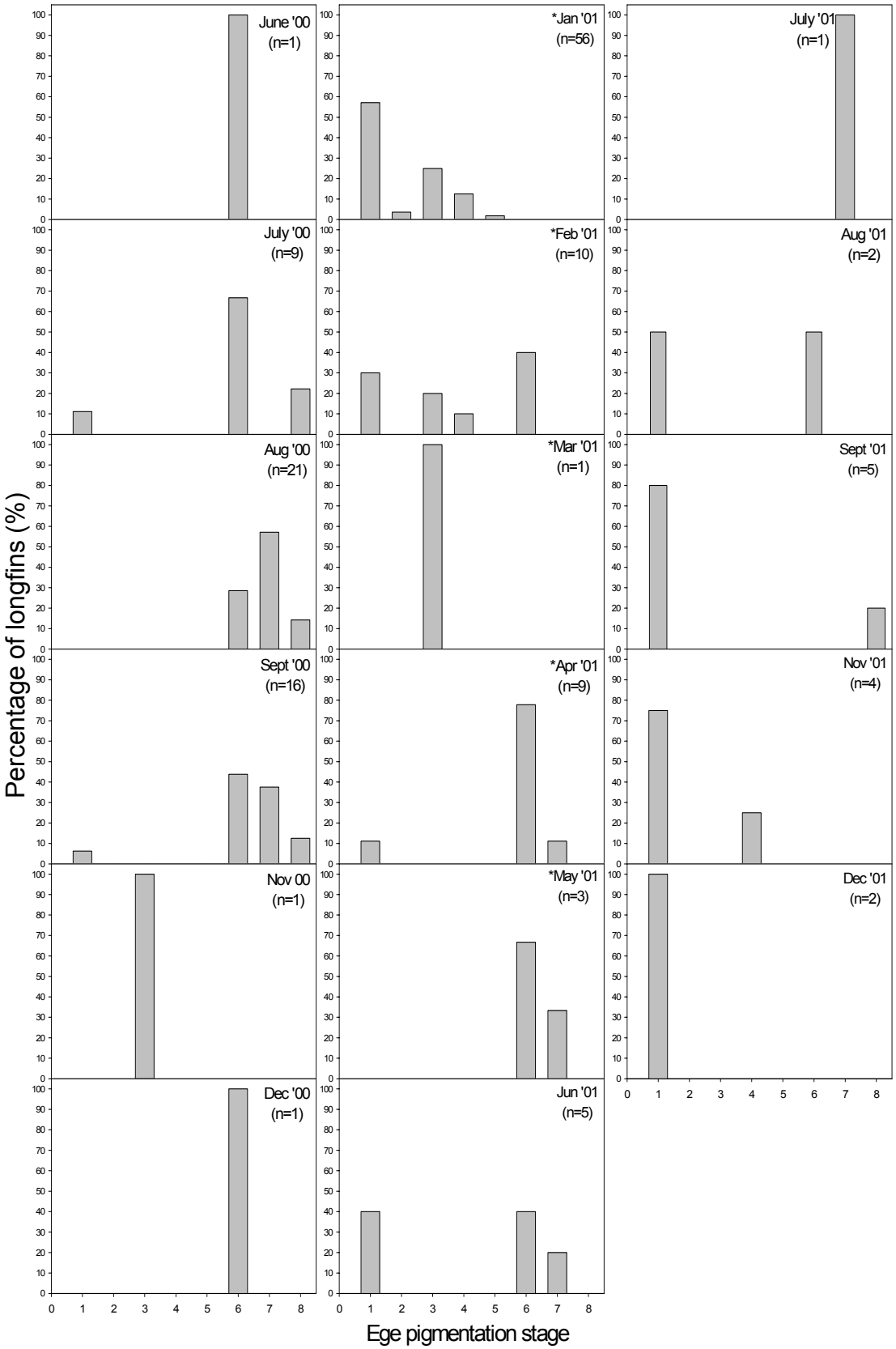

Fig. A1g – Proportion of pigmentation development of longfins, according to Ege (1939), in each monthly Tweed R. sample. * indicates months of peak recruitment.

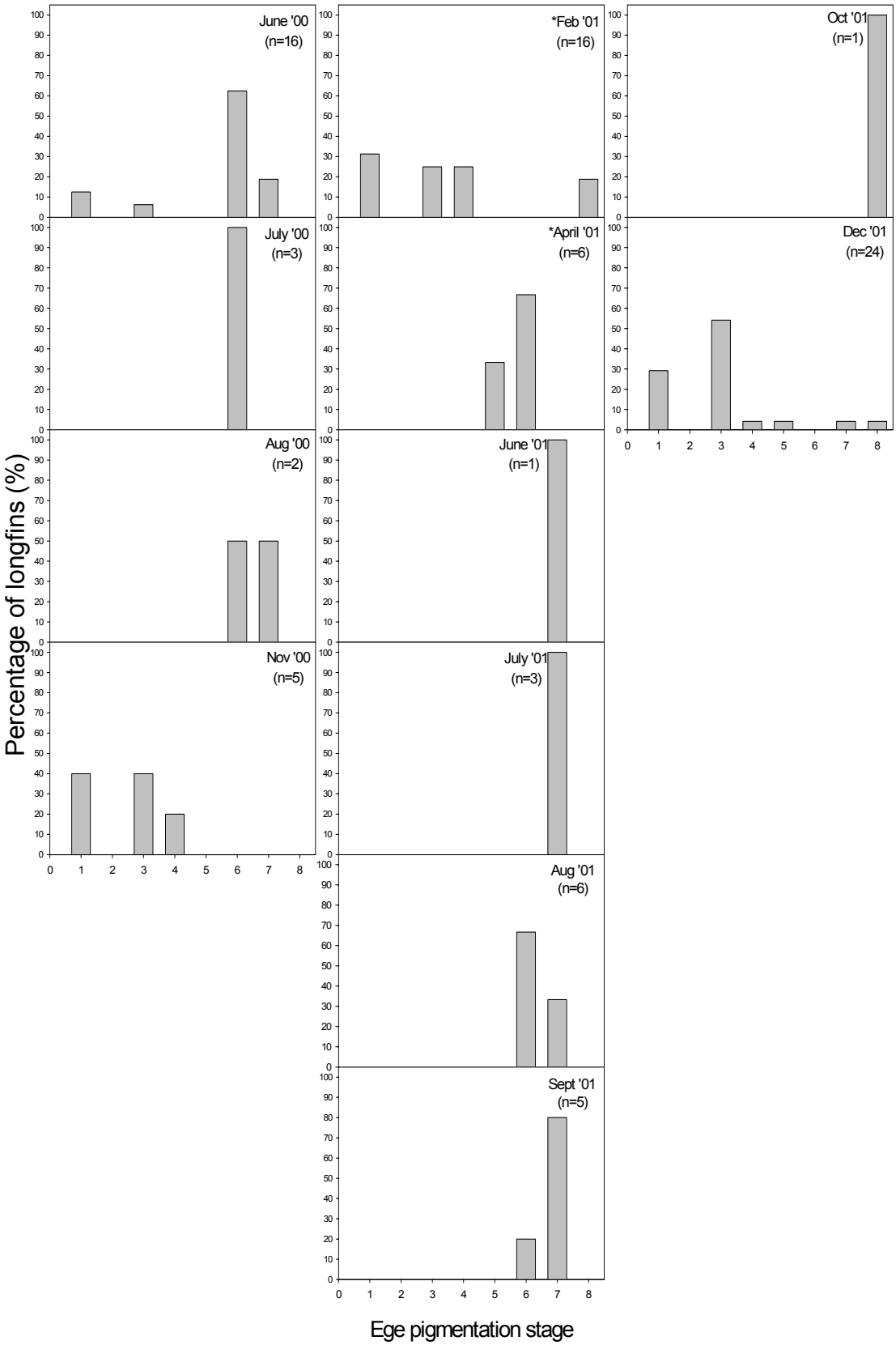

Fig. A1h – Proportion of pigmentation development of longfins, according to Ege (1939), in each monthly Lansdowne R. sample. * indicates months of peak recruitment.

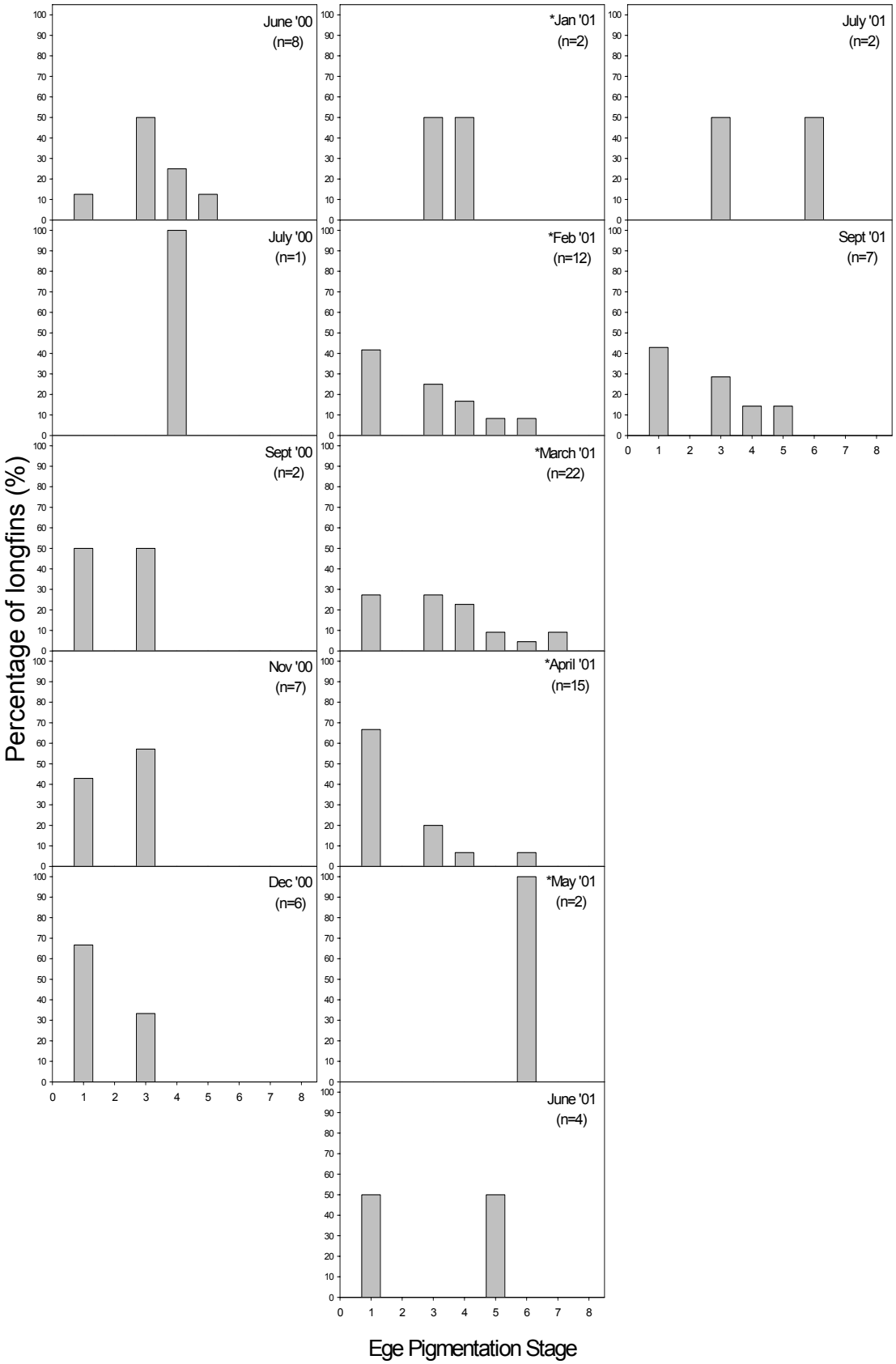

Fig. A1i – Proportion of pigmentation development of longfins, according to Ege (1939), in each monthly Audley sample. * indicates months of peak recruitment.

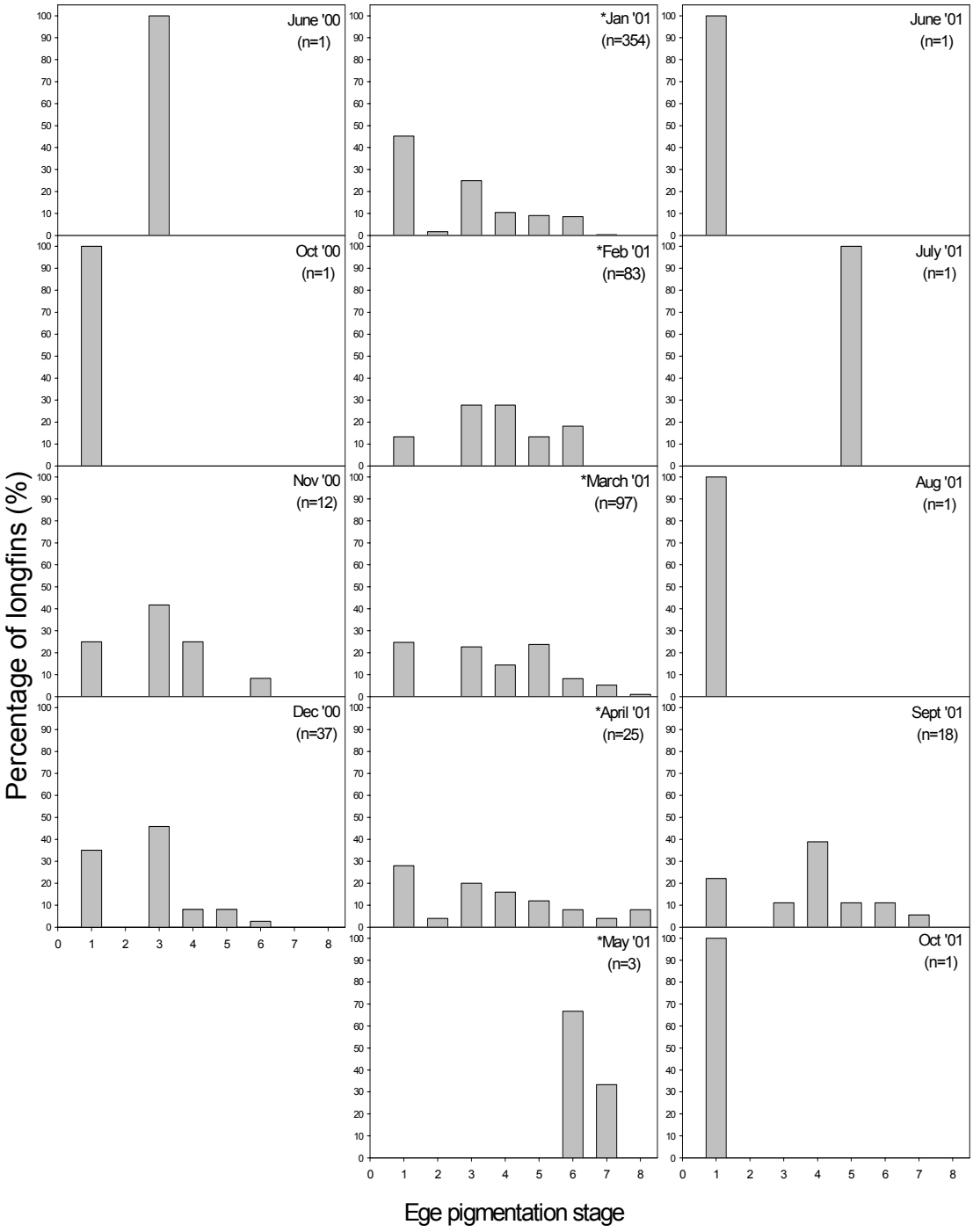

Fig. A1j – Proportion of pigmentation development of longfins, according to Ege (1939), in each monthly Mullet Ck. sample. * indicates months of peak recruitment.

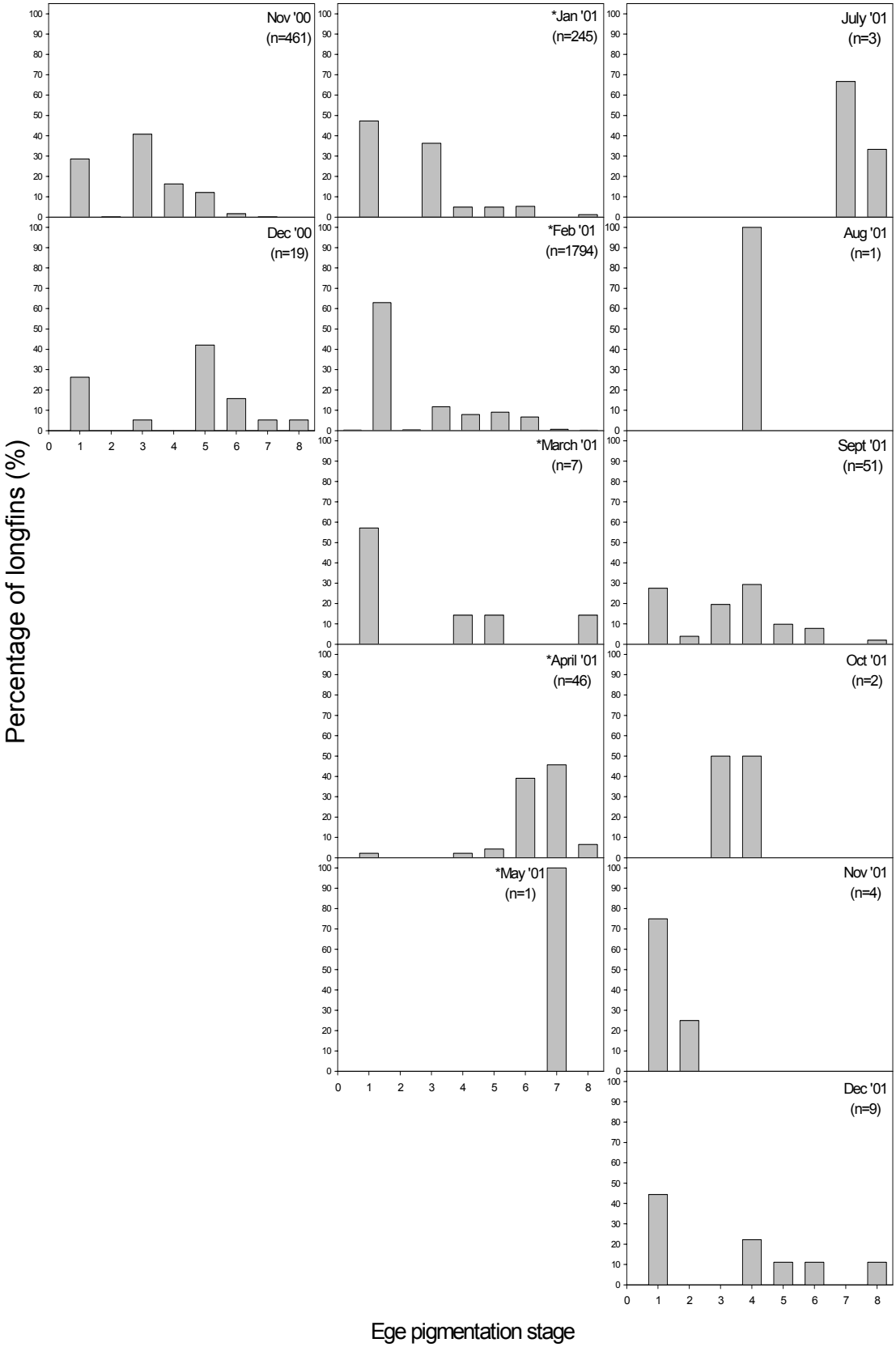

Fig. A1k – Proportion of pigmentation development of longfins, according to Ege (1939), in each monthly Croobyar Ck. sample. * indicates months of peak recruitment.

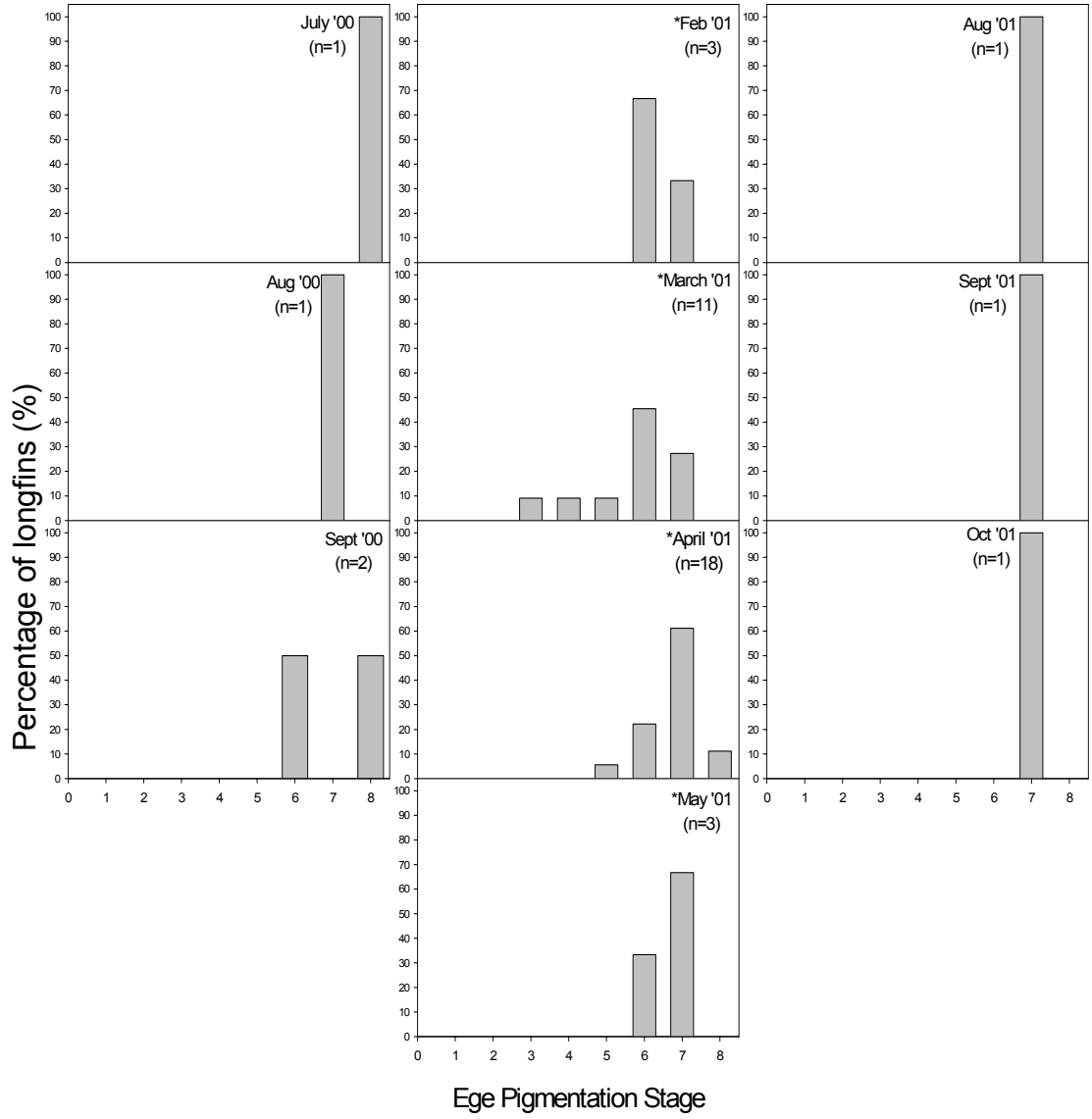

Fig. A1l – Proportion of pigmentation development of longfins, according to Ege (1939), in each monthly Boggy Ck. sample. * indicates months of peak recruitment.

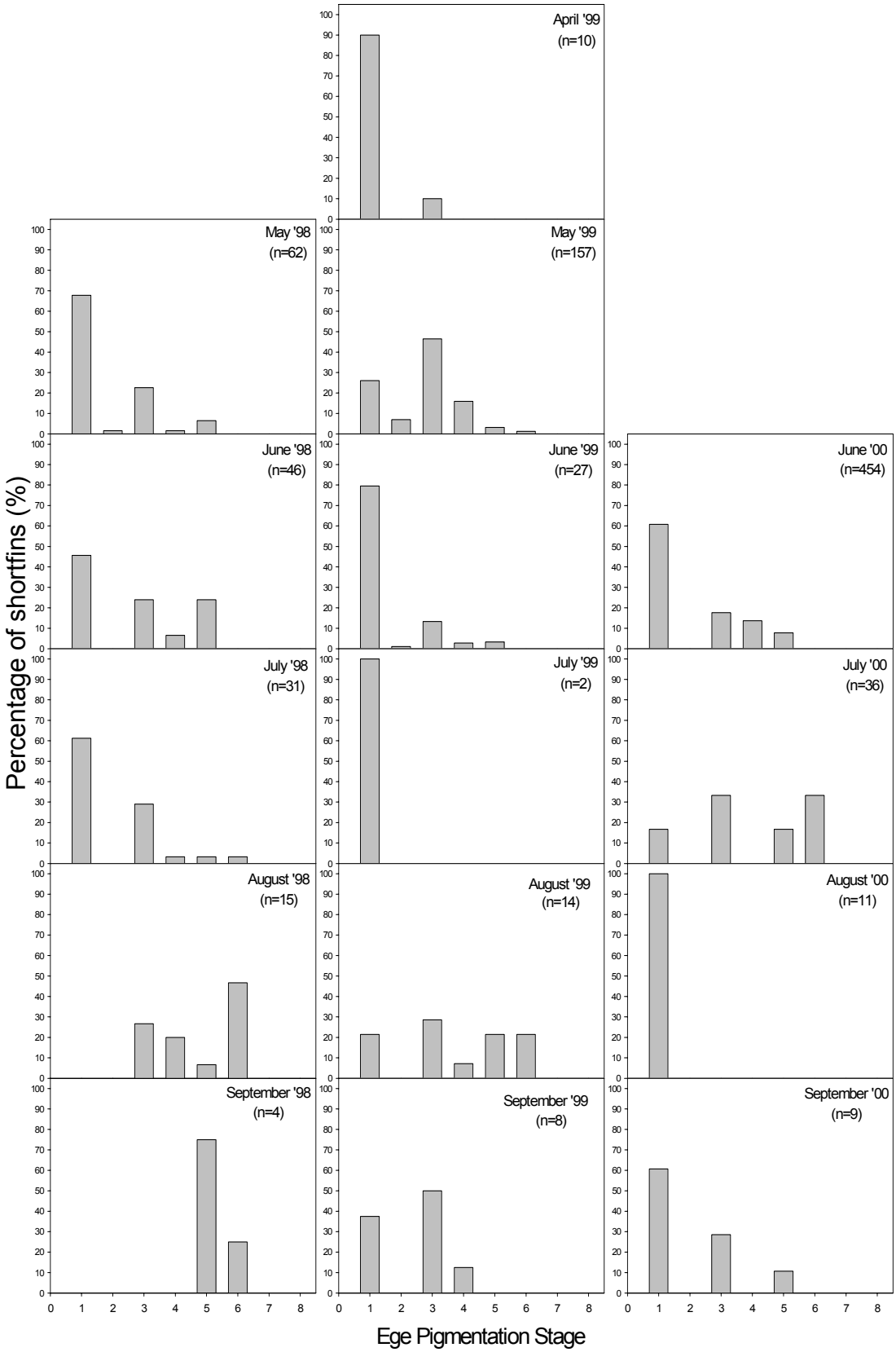

Fig. A1m – Proportion of pigmentation development of shortfins, according to Ege (1939), in each monthly Audley sample over 5 years.

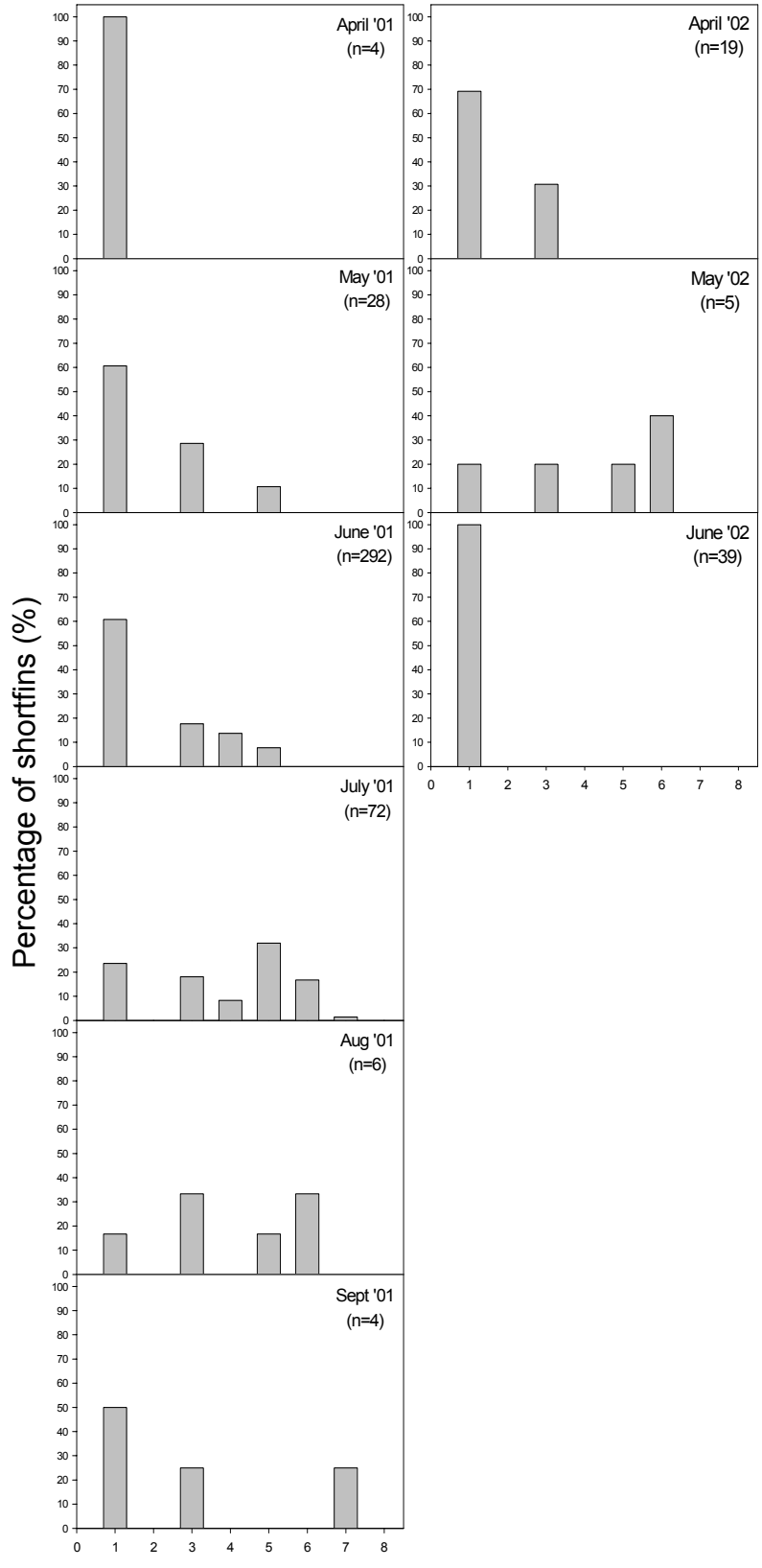

Fig. A1m continued – Proportion of pigmentation development of shortfins, according to Ege (1939), in each monthly Audley sample over 5 years.

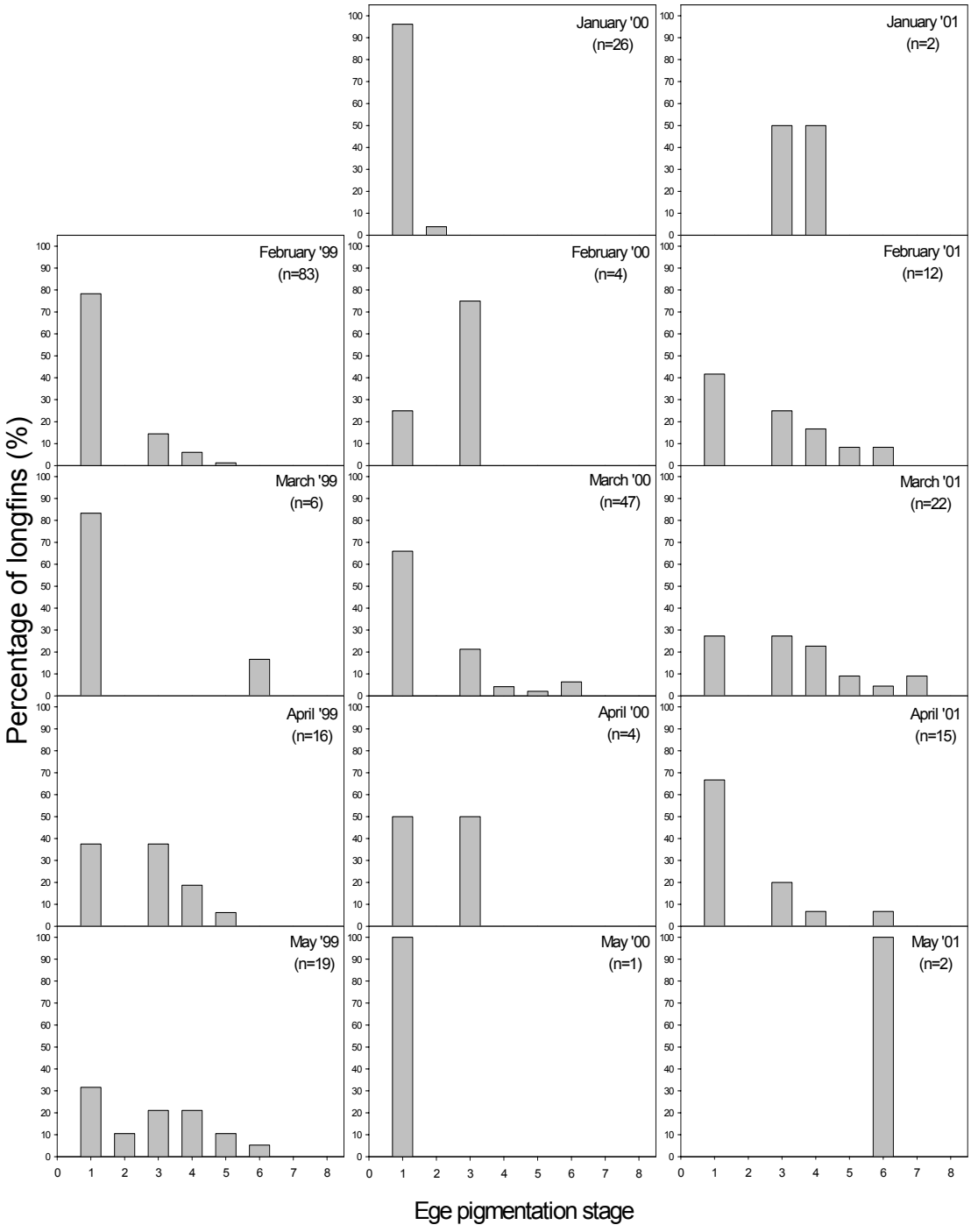

Fig. A1n – Proportion of pigmentation development of longfins, according to Ege (1939), in each monthly Audley sample over 4 years.

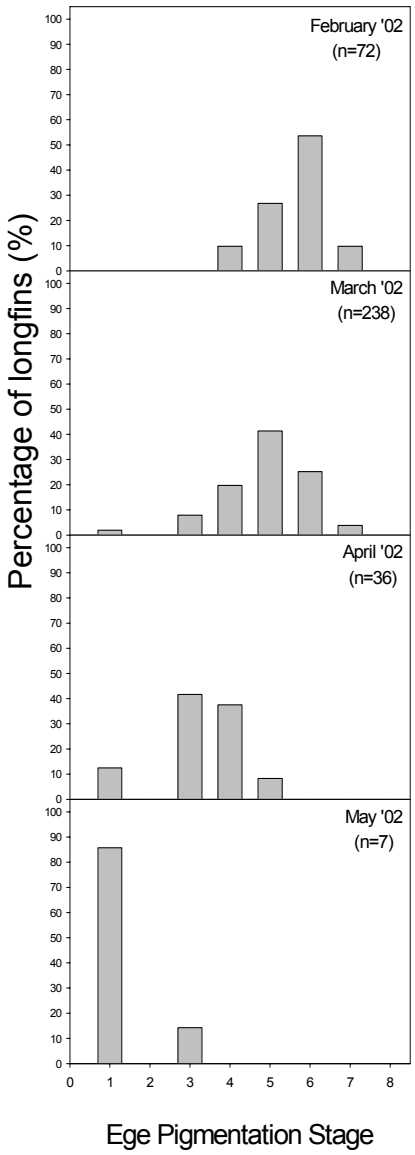

Fig. A1n continued – Proportion of pigmentation development of longfins, according to Ege (1939), in each monthly Audley sample over 4 years.