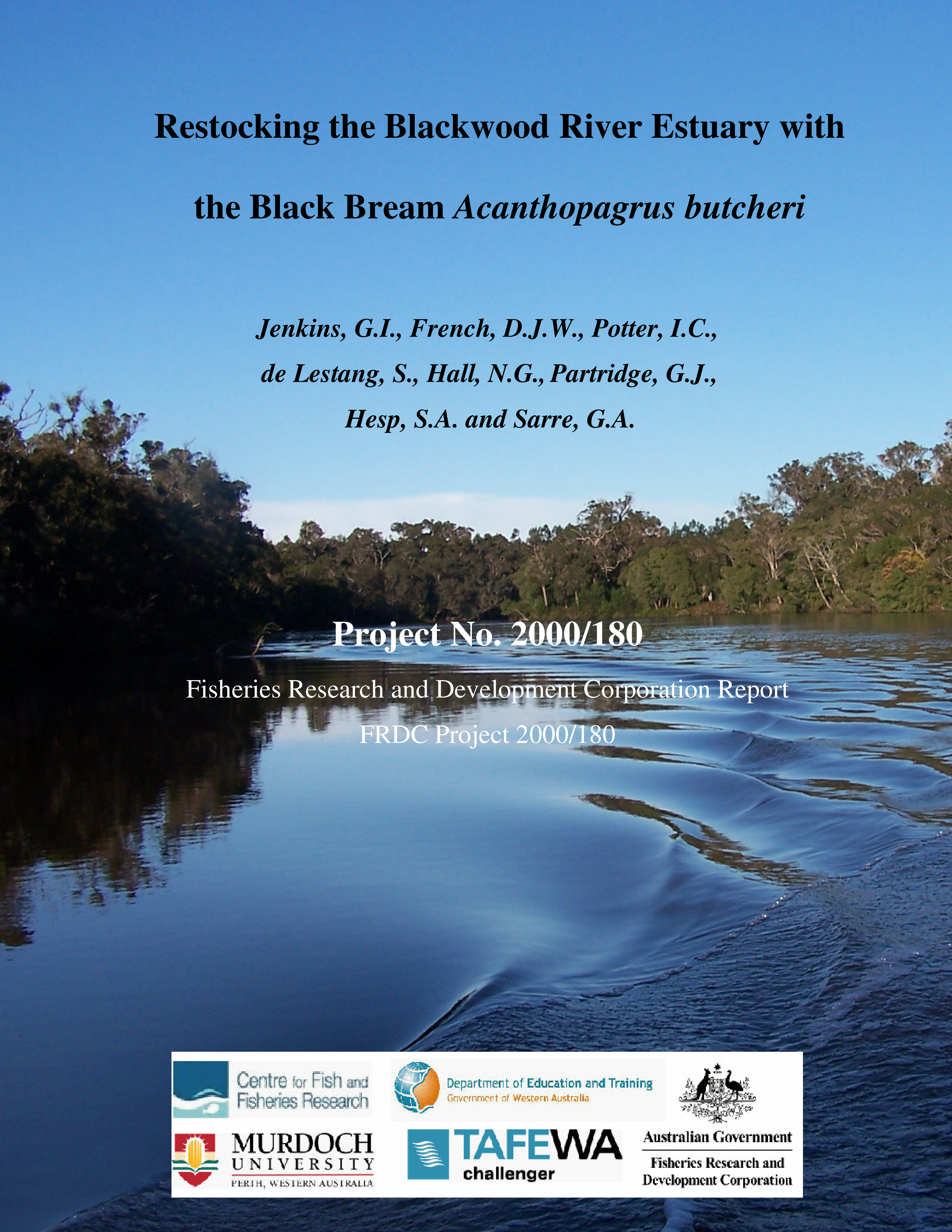

Restocking the Blackwood River Estuary with the Black Bream *Acanthopagrus butcheri*

Jenkins, G.I., French, D.J.W., Potter, I.C., de Lestang, S., Hall, N.G., Partridge, G.J., Hesp, S.A. and Sarre, G.A.

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Fisheries Research and Development Corporation

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2000/180	Restocking the Blackwood River Estuary with Black Bream *Acanthopagrus butcheri*

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OBJECTIVES:

1) Obtain baseline data on crucial biological parameters and catch statistics of Black Bream in the Blackwood River Estuary.
2) Collect mature Black Bream from the Blackwood River Estuary for use as brood stock to culture the juveniles of this species.
3) Identify the habitats (regions) important to Black Bream in the Blackwood River Estuary.
4) Compare the densities of Black Bream in important habitats in the Blackwood River Estuary with those in similar habitats within other systems
5) Introduce tagged, cultured juveniles of Black Bream into the Blackwood River Estuary.
6) Estimate the proportion of released Black Bream, which represent a known year class, among the total number of that age class, and thereby estimate the extent to which restocking has enhanced the population.
7) Obtain data on the biological parameters and catch statistics of Black Bream in the Blackwood River Estuary following restocking.
8) Evaluate the success of the restocking programme by comparing biological parameters and catch statistics prior to and after restocking.
9) Estimate the average cost to produce each fish that will survive to attain the minimum legal length and thus be available for exploitation.
10) Provide advice that can be used by management to develop plans to sustain the enhanced stock of Black Bream in the Blackwood River Estuary.

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

The results of this study show that hatchery-reared Black Bream can be used to enhance the stock of the population of this commercially and recreationally important species in the Blackwood River Estuary in which it has become depleted. An initial trial of different stains demonstrated that alizarin complexone was particularly effective for staining the otoliths (ear bones) of Black Bream. The mark on the otoliths, produced by this stain following immersion of hatchery-reared juveniles, was still visible to the naked eye 3.5 years later. Substantial numbers of the stocked Black Bream, which were introduced into the Blackwood River Estuary, were still living at the end of 3.5 years. On average, these individuals did not grow as rapidly as those in the wild population, and unlike the wild fish, not all stocked Black Bream attained maturity by 4 years of age. However, they still grew at a rate that was greater than that in some other estuaries and many did reach maturity by 4 years of age. The Black Bream is thus a particularly good candidate for restocking an estuary as it completes its life cycle within these systems in south-western Australia and consequently any stocked fish are unlikely to move into other estuaries in this region. The ease and relatively low cost of culture of Black Bream and its hardiness and restriction to its natal estuary make the restocking of Black Bream a feasible and economically-viable proposition. This study shows that restocking provides managers with a further and viable option for countering the effects of a decline in a stock of Black Bream in an estuary.

Increases in commercial and recreational fishing pressure in recent decades have led to over-exploitation of many valuable fish species. This problem in estuaries has sometimes been exacerbated by the detrimental effects that have occurred in these systems through adverse anthropogenic changes. Species whose entire life cycle is restricted to estuaries, such as the recreationally and commercially-important Black Bream in Western Australia, are especially vulnerable as their numbers are unlikely to be enhanced by recruitment from outside the estuary. The impact that fishing can exert on a species, such as Black Bream, which is restricted to estuaries, is demonstrated by the fact that the relative abundance of the older individuals of this species is less in estuaries subjected to a substantial amount of fishing than in those in which fishing is far more restricted (Sarre and Potter, 2000).

The sole commercial fisher and regular recreational fishers in the Blackwood River Estuary consider that the abundance of Black Bream in this system has declined in recent times. This view is supported by the fact that the numbers of Black Bream we obtained through extensive sampling in the Blackwood River Estuary were far lower than those caught during a detailed study of the fish fauna of this estuary in the 1970s (Lenanton, 1977). Widespread concern that the numbers of Black Bream had declined in the Blackwood River Estuary resulted in the initiation of a carefully-designed research program aimed at determining whether it would be feasible and economically worthwhile to stock Black Bream in this large and important estuary.

Our initial sampling demonstrated that Black Bream is largely found in the riverine component of the Blackwood River Estuary and particularly in its upper reaches. The abundance in those upper reaches is greatest in spring. As spawning occurs in this season, and the samples from these upper reaches contained many maturing, mature and spent Black Bream, it is concluded that *A. butcheri* migrates some distance upstream as it matures and spawns mainly in those upstream waters of the estuary in spring.

For our restocking research, 56 females and 50 males were collected from the Blackwood River Estuary to act as brood stock for producing juveniles for restocking this estuary. The otoliths (ear bones) of the cultured juveniles were tagged by immersion of the fish in a solution of alizarin complexone (ALC). The resultant pink stain on the otoliths of cultured Black Bream was still clearly visible more than three and a half years after the otoliths had been tagged with ALC. The cultured individuals were certified as disease free prior to release into the upper reaches of the Blackwood River Estuary in which Black Bream had previously been identified as being most abundant. The fish were introduced at several sites over a distance of *c*. 20 km.

The maximum total length and age of wild Black Bream caught during our study was 440 mm and 31 years. The latter age is the maximum yet recorded for any estuary in Western Australia. Analysis of our length-at-age data demonstrated that, on average, the individuals of the wild population of Black Bream reach total lengths of 143, 200, 244, and 279 mm at the end of their first, second, third, and fourth years of life, respectively. This represents a particularly rapid rate of growth for this species in a Western Australian estuary. The lengths of the stocked fish at ages 1 to 4 were 119, 182, 219 and 242 mm, respectively, which were less than those for wild fish of those ages. Comparisons of mean lengths at successive age intervals confirm that wild Black Bream grow more rapidly than stocked Black Bream. However, the growth of Black Bream stocked in the Blackwood River Estuary is still substantial, as is illustrated by the fact that it is greater than that of wild stocks of this species in some other Western Australian estuaries (Sarre and Potter, 2000).

The average length of the females and males of wild Black Bream at first maturity were 178 and 155 mm, respectively. After adjustment to take into account the fact that a number of the larger stocked Black Bream had not reached maturity, the average length of the females and males of stocked Black Bream at first maturity were 202 and 189 mm, respectively. Thus, stocked Black Bream do not typically reach maturity until they have attained a larger size than wild Black Bream. The majority of females (84%) and males (94%) of wild Black Bream attained maturity by the end of the second year of life, whereas only 75 and 54% of the females and males of stocked Black Bream had reached maturity by the end of their third year of life. Some stocked fish will require further years to reach maturity and some may never achieve maturity.

The stocked Black Bream, that were cultured and released in 2001 and 2002, survived well and comprised 75 and 92% of catches of Black Bream of the 2001 and 2002 year classes, respectively.

In summary, this study has demonstrated that the abundance of Black Bream in the Blackwood River Estuary was enhanced markedly through the introduction of restocking. cultured fish. The Black Bream is a particularly suitable candidate for restocking estuaries in south-western Australia as it is confined to these systems and thus any stocked fish are unlikely to migrate to other estuaries. The ease and relatively low cost estimated for culturing Black Bream, the hardiness of this species and its restriction to its natal estuary make the restocking of Black Bream an economically viable and valid proposition when a stock of this species has become highly depleted. Thus, such restocking provides a further tool for fisheries managers to use to sustain the stocks of Black Bream in Australian estuaries.

KEYWORDS: Black Bream, culture, tagging, restocking, estuaries, survival, biology of stocked *vs* wild fish, cost benefit of restocking

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Funding for this project was provided by the Fisheries Research and Development Corporation, Challenger TAFE and Murdoch University and, without which, this project could not have been undertaken.

1 GENERAL INTRODUCTION

1.1 BACKGROUND

The Black Bream *Acanthopagrus butcheri* is one of the most important recreational and commercial fish species in the estuaries of southern Australia (Lenanton and Potter, 1987; Kailola *et al.*, 1993). In south-western Australia, this sparid completes its life cycle in estuaries and can attain total lengths and weights of at least 475 mm and 2 kg and live for over 20 years (Potter and Hyndes, 1999; Sarre and Potter, 1999, 2000). However, the growth, age and size at maturity and dietary composition of *A. butcheri* vary greatly among estuaries in this region, reflecting, at least in part, marked differences in the environmental characteristics of those estuaries (Sarre and Potter, 1999, 2000; Sarre *et al.*, 2000; Partridge *et al.*, 2004; Chuwen *et al.*, 2006).

The genetic compositions of the populations of this species in the various estuaries of south-western Australia differ (Chaplin *et al.*, 1998). This provides strong circumstantial evidence that, although some *A. butcheri* are occasionally flushed out of estuaries during periods of heavy freshwater discharge, the population of Black Bream in any one estuary remains essentially discrete from those in other estuaries (Potter and Hyndes, 1999). As the populations of *A. butcheri* are confined to their natal estuaries, they are particularly susceptible to fishing pressure and any effects of overfishing cannot be ameliorated naturally by immigration of fish from other systems. The trends exhibited by commercial catch-per-unit effort data for *A. butcheri* in the Blackwood River Estuary between 1978 and 2002 oscillated greatly between years (Anon, 2004). This feature is considered by a commercial fisher, who has fished this estuary for over 30 years and has been the sole commercial fisher operating in this system since 1999, to reflect the fact that high catches are a function of those years in which freshwater discharge is particularly high (Trevor Price, pers. comm.). That commercial fisher has no doubt that the abundance of Black Bream has declined during the last 30 years, which is consistent with a comparison of the results of extensive sampling of the Blackwood River Estuary (Fig. 2.1) during 1973/74 and 1993/94 (*cf* Lenanton, 1977; Valesini *et al.*, 1997), and numerous anecdotal reports from recreational fishers. This contributed to the view that this species could have become overexploited in this system (Lenanton *et al.*, 1999). The fact that the proportion of *A. butcheri* older than 5 years was lower in the Swan River Estuary, 300 km to the north of the Blackwood River Estuary, than in far less heavily-fished estuaries (Sarre and Potter, 2000) also provides strong indications that fishing can have a marked detrimental effect on the age composition of a population of Black Bream.

The introduction of strong management measures in the Blackwood River Estuary, such as instituting closed seasons, closed areas and gear restrictions, coupled with a more conservative minimum legal size, might provide a relatively inexpensive approach to allowing the stock of Black Bream in this estuary to recover. However, there is no guarantee that such a recovery would occur through such management practices and any such recovery would be slow. Furthermore, such management regulations would be introduced in the absence of most of the critical types of biological data normally required to develop appropriate management plans. The absence of such data is particularly pertinent in the case of Black Bream, since the biological characteristics of this species vary so markedly among the different estuaries in south-western Australia (Sarre and Potter, 1999, 2000; Sarre *et al*., 2000).

Following the great interest shown by the recreational fishing community, and after consultation with the WA Recreational Fishing Advisory Committee, the Aquaculture Development Unit (ADU) of the WA Maritime Training Centre (WAMTC) initiated, in 1992, a program aimed at culturing Black Bream for restocking estuaries. As a result of this initiative, the ADU has been able to develop cost-effective and reliable protocols for culturing Black Bream under hatchery conditions. The view that such restocking had considerable potential was based on the fact that 12.6% of the 776 *A. butcheri*, which had been cultured and dart-tagged in the hatchery, and released at 14 months of age into the Swan River Estuary, were caught by recreational fishers in that estuary at intervals over the ensuing 30 months and before they had all become fully vulnerable to angling (Lenanton *et al.*, 1999; Dibden *et al.*, 2000).

The restocking of the Blackwood River Estuary with large numbers of juvenile Black Bream could lead to the successful rehabilitation of the stock of this species in this estuary, especially if this restocking was accompanied by more stringent management policies. However, it would have been counterproductive to introduce more severe management measures prior to restocking, because, at that stage, such measures would have been received adversely by recreational and commercial fishers and the local community and thereby reduced the potential for the cooperation required to assess the status of the fishery prior to any restocking.

The restocking of Black Bream in the Blackwood River Estuary offers an excellent opportunity to assess the degree to which a targeted and depleted stock, which is confined to estuaries, can be rehabilitated. However, such an assessment cannot be successful without first obtaining sound information on the biological status of the stock prior to the restocking program and subsequently monitoring the progress of the Black Bream stock in that estuary. Protocols for developing, appraising and managing marine stock enhancement were proposed by Blankenship and Leber (1995) and endorsed by Taylor *et al.* (2005a) for estuarine environments.

1.2 NEED

There was an urgent need to rehabilitate and then maintain the stock of Black Bream in the Blackwood River Estuary and for the stock subsequently to be sustained at a higher level than at the commencement of the project. It was important, however, that any restocking used Black Bream cultured from brood stock obtained from the Blackwood River Estuary so that they had the same genetic characteristics as those of the wild population. It was also important to confirm subsequently that the released Black Bream survived and made a significant contribution to the fishable stock in that estuary.

The collection of baseline data on the stock of Black Bream in the Blackwood River Estuary was required to facilitate comparisons between the status and biological characteristics of hatchery-reared Black Bream released into the Blackwood River Estuary with those of the wild population in that estuary and in other estuaries.

Fisheries managers need to ensure that the Black Bream fishery is regulated so that the enhanced stock is sustained. The development of management plans requires a sound understanding of key biological parameters, *i.e.* age, growth and reproductive biology, prior to and after restocking. Information is also required on the catch rates and size compositions of recreational and commercial catches of Black Bream before and after restocking.

1.3 OBJECTIVES

1) Obtain baseline data on crucial biological parameters and catch statistics of Black Bream in the Blackwood River Estuary.
2) Collect mature Black Bream from the Blackwood River Estuary for use as brood stock to culture the juveniles of this species.
3) Identify the habitats (regions) important to Black Bream in the Blackwood River Estuary.
4) Compare the densities of Black Bream in important habitats within the Blackwood River Estuary with those in similar habitats within other systems

5) Introduce tagged, cultured juveniles of Black Bream into the Blackwood River Estuary.

6) Estimate the proportion of released Black Bream, which represent a known year class, among the total number of that age class, and thereby estimate the extent to which restocking has enhanced the population.

7) Obtain data on the biological parameters and catch statistics of Black Bream in the Blackwood River Estuary following restocking.

8) Evaluate the success of the restocking programme by comparing biological parameters and catch statistics prior to and after restocking.

9) Estimate the average cost to produce each fish that will survive to attain the minimum legal length for retention and thus be available for exploitation.

10) Provide advice that can be used by management to develop plans to sustain the enhanced stock of Black Bream in the Blackwood River Estuary.

2 GENERAL MATERIALS AND METHODS

2.1 COMMERCIAL CATCH STATISTICS

The commercial catch statistics for Black Bream in the Blackwood River Estuary have recently been published (Anon, 2004). This report provides the catches in the estuary for each season of each year between 1978 and 2002 and the commercial catch per unit effort (CPUE) for each of those years.

2.2 CULTURE, TAGGING AND RELEASE OF BLACK BREAM

2.2.1 *Collection of brood stock*

The Molloy Island Group nominated Mr Andrew Kikeros of Fish Unlimited as its representative in the restocking activities associated with this project. Mr Kikeros is an aquaculture and rural consultant with training in marine finfish culture and, under the supervision of ADU staff, has undertaken, since July 2000, a range of fish culture activities at the WAMTC. These included the collection of brood stock and training in larviculture and intensive fish husbandry. Such activities represented the first stage of the hatchery technology transfer as identified as a milestone in the project proposal.

Three sampling trips were undertaken to the Blackwood River Estuary to collect the brood stock required for culturing Black Bream. These trips were planned in conjunction with local recreational fishers and conducted during school holidays and long-weekends to take advantage of the presence of large numbers of recreational line fishers. Despite the substantial effort invested, only five potential brood stock individuals were obtained during the three sampling trips. This low number of fish indicates that the number of Black Bream present in the Blackwood River system is low, which is consistent with the relatively small number of Black Bream caught during field sampling in the first two years of the study (see Chapter 4).

A number of meetings were subsequently held between Murdoch University, the WA Department of Fisheries (DOF) and ADU staff to discuss ways of overcoming the problems of obtaining mature-sized Black Bream. This group agreed that approximately 100 brood stock were required for this project and reaffirmed that it would be preferable for them to be obtained from the Blackwood River Estuary stock. Thus, it was decided that, together with the willing support of Mr Trevor Price, the local professional fisher for Black Bream, a concerted effort should be made in July to September 2001 to collect *c.* 100 brood stock. Although it was anticipated that the requisite number of fish would be collected by the professional fisher, his use of gill netting to catch these fish would inevitably inflict damage on some of the fish. To overcome this problem, it was agreed that the fish would be transported to the ADU's Fremantle facilities, where they could be cared for and administered prophylactic and other treatments.

With the assistance of Mr Trevor Price, 106 mature-sized Black Bream were caught in the Blackwood River Estuary in August and September 2001 using gill nets. These fish, many of which had suffered external damage during capture, were transported to the ADU hatchery at Fremantle using established techniques and then administered treatment for their injuries (see Jenkins *et al.*, 1999; Partridge *et al.* 2003).

2.2.2 *Culture of Black Bream juveniles*

It was originally intended to culture juvenile Black Bream at a site on Molloy Island, but for several reasons it became apparent in 2001 that the juveniles had to be cultured at the ADU in Fremantle. First, and very importantly, the discharge rate in the Blackwood River Estuary was still atypically very high at the time of spawning and thus the salinities were less than optimal for using the proposed hatchery location on Molloy Island as a site for culturing Black Bream. Second, it was considered essential to locate the brood stock at Fremantle in order to facilitate their optimal treatment for the injuries that some fish would inevitably receive on their capture by gill nets.

To ensure that, from a genetic point of view, a relatively large number of brood stock would contribute to the cultured juvenile fish used for restocking, the following procedures were applied. After a period of rehabilitation at the ADU, the Black Bream brood stock was transferred to a semi-recirculating tank of 40,000 L that was maintained at an ambient water temperature of 19°C. After an acclimation period of 20 days, the water temperature was slowly increased every second day by 0.5°C until it had reached 21.5°C. The fish began spawning naturally at this temperature in the first week of November.

In early November 2001, the water level in the tank was lowered and fish were collected in tubs, anaesthetised in a bath containing 20 ppm AQUI-S® and sexed. Fifty males, identified as running-ripe by gently squeezing their abdominal cavity to confirm that they extruded sperm, were transferred to a second 40,000 L tank. Females were identified and their stage of egg development was assessed by ovarian biopsy using an endometrial biopsy cannula. Each female was then weighed and given an intra-peritoneal injection of Ovaprim®, with the dose rate being adjusted to facilitate the continued development of eggs and their subsequent ovulation. Female Black Bream possessing vitellogenic oocytes were given a dose of Ovaprim® equivalent to 0.25 mL kg^{-1} body weight, while those with hydrating oocytes were given a dose equivalent to 0.1 mL kg^{-1} body weight.

Females were randomly distributed into two 2,500 L tanks to facilitate their ready capture and stripping once ovulation had occurred. 52 of the 56 females contained vitellogenic eggs, while the other four possessed hydrating eggs. Forty-eight hours after hormone administration, the fish were stripped in the following manner. First, the tank containing the male fish was drained and the fish anaesthetised. After their ventral body surface had been washed with fresh water and dried with a cloth, a 3 mL syringe was inserted through the sperm duct into the testes and the sperm thus collected immediately stored on ice. Stripped males were then returned to the original 40,000 L tank.

Ovulated eggs were stripped from individual females into a 2 L bucket. Sperm from five randomly-selected males were added to each batch of stripped eggs (*c*. 1 mL sperm L^{-1} of eggs) and thoroughly mixed to ensure that the sperm was well distributed amongst the eggs. Seawater was then added to initiate sperm motility and the mixture left to stand for 5 min to allow fertilisation to occur. The fertilised eggs were transferred on to a wet 300 µm screen and rinsed for a further 5 min to remove excess sperm. They were then transferred into a graduated cylinder with a 300 µm mesh at its base to determine the volume of eggs obtained from each female. A 0.3 mL subsample of these eggs was taken with a micropipette and transferred to a 1 L beaker to assess the viability of the eggs in each batch. The remaining eggs were transferred to a 100 L egg-counting cone.

The 31 females that were successfully stripped yielded a large number of eggs, the viability of which was determined in a 100 L counting cone. First, a total egg count was obtained by vigorously aerating the water to ensure that the eggs were randomly mixed. Five subsamples of 5 mL were dispensed on to a taut piece of 500 µm mesh. The eggs on each of those meshes were counted and their average number was used, in conjunction with the volume of the tank, to estimate the total number of eggs that were stripped. This value was estimated to be 2.6×10^6. The air was then removed from the counting cone and the eggs left to stand for 20 min. The poor quality, infertile eggs sank to the bottom of the cones, whereas the good quality, fertile eggs floated to the surface. The sunken eggs were removed by draining the bottom of the counting cone. The tank was then topped back up to 100 L and again vigorously aerated.

The counting procedure described above was then repeated to determine the total number of viable eggs, which was expressed as a percentage of the total egg count. Viabilities ranged from 0 to 100%, with an average of 51%.

Although the remaining 25 female fish could not be stripped, as their eggs were not fully hydrated, cannulation revealed that their oocytes were undergoing hydration. These females, together with those that had been successfully stripped, were returned to the original 40,000 L tank with the males. Later that evening, 2.3×10^6 naturally-released eggs, with a viability of 69%, were released into the tank and collected on the following morning. As the 31 stripped fish were stripped of all ovulated eggs, it is assumed that the naturally-released eggs were obtained from among those 25 females whose eggs were hydrating at the time of stripping.

On Day 2 (where Day 0 is the day of hatch), culture tanks were stocked with larvae. A total of 22,000 larvae survived to Day 2 from the females that were stripped (1.8% of the viable eggs obtained) and these were stocked into a 5,000 L larval rearing tank. Twenty-three of the 31 stripped females contributed larvae.

Survival to Day 2 of the naturally-spawned, induced fish was 30%. A separate 5,000 L larval rearing tank was stocked with 100,000 of these larvae. An additional 68,000 larvae from this batch were added to the 22,000 larvae derived by stripping which increased the total number of larvae in this tank to 90,000. The larvae were reared under a semi-intensive, green-water regime as described by Partridge *et al.* (2003). In the following year, fertilised eggs, obtained at the ADU from natural spawning of the same brood stock as used in 2001, were subsequently reared as larvae and juveniles in the manner described by Partridge *et al.* (2003). The survival in 2002, from hatch to day 35, was *c*. 80%.

2.2.3 *Tagging*

A number of options for tagging stocked fish in a way that would allow their subsequent identification were considered. The possibility of marking juvenile fish individually (*i.e.* using elastomer inserts, coded wire tags *etc.*) was ruled out as they were too time consuming and costly for a one-off restocking project. Previous experience of the staff at the ADU had also demonstrated that the marks produced on the otoliths of Black Bream by oxytetracycline fade considerably after six months, which was too brief a time frame to fulfil the aims of the restocking project.

For the above reasons, trials were undertaken to evaluate the suitability of other otolith-staining chemicals. Following a literature survey and discussions with Dr R. Farragher and Mr R. Winstanley of NSW Fisheries and Dr B Ingram of the Marine and Freshwater Institute of Victoria, small quantities of other otolith-staining compounds were purchased by the ADU. Once juvenile Black Bream became available for tagging, a series of small-scale experiments were undertaken to test whether the marks made by these chemicals would be suitable for marking juvenile Black Bream. In initial small-scale trials, alizarin red and alizarin complexone (ALC) both produced intense marks on the otoliths of Black Bream. The former was initially chosen for use because it was far cheaper. However, for a still unresolved reason, it was not possible consistently to mark all of the otoliths when large numbers of Black Bream were placed in a tank containing alizarin red. In contrast, ALC did stain all of the otoliths under these type of conditions and was thus suitable for such mass-marking of the otoliths of Black Bream. For staining their otoliths, Black Bream (0.6 g in weight) were immersed for 24 h in water containing 30 mg L^{-1} of ALC and at the equivalent of 5 g of fish L^{-1}. As a pH of 6.5 was found to produce a better mark on otoliths than those of 7.5 and 8.5, the pH of the bathing solution was reduced to 6.5 using phosphoric acid. Because the trials of these chemicals took longer than expected to produce an acceptable outcome, the release of the first batch of fish marked with ALC was delayed by several months beyond its planned date in 2002.

At the time of restocking in 2002, the amount of ALC we had purchased was found to be sufficient for marking only 32,700 juveniles. These individuals were released in May 2002. A sufficient quantity of ALC was borrowed from NSW Fisheries (Dr Andrew Sanger) to mark and release a further 37,300 juvenile Black Bream in July. The quantity of ALC required to mark juveniles for release in 2003, and replace that supplied by the NSW Fisheries, was ordered from the USA. Due to the very high costs of ALC, additional funding was granted by the FRDC. The Centre for Fish and Fisheries Research at Murdoch University also provided some additional funds for the chemicals and the ADU accepted the considerable additional costs required to ensure that the restocking programme was not compromised.

2.2.4 *Black Bream release strategies*

A number of potential release sites in the Blackwood River Estuary were identified for the restocking project during the sampling surveys undertaken in 2000 to 2002. Three of these sites were selected as most appropriate as they were located close to extensive areas comprising the types of habitat that act as nursery areas for Black Bream and which were accessible for transporting the otolith-tagged juvenile Black Bream into those sites by truck.

2.2.5 *Disease minimisation strategy*

Western Australian legislation requires that aquaculture hatcheries are tested for disease and that hatchery-produced fish are certified prior to their exit from the hatchery. The ADU has been operating for over 15 years and its staff has worked closely with the Fish Health section of DOF over this time. The ADU hatchery has an excellent reputation for producing disease-free fish, without the use of antibiotics. The ongoing certification of disease-free fish from this hatchery is an integral part of minimising the risk of introducing diseases into the natural environment through the restocking of Black Bream.

A sample of the Black Bream juveniles produced in this project were sent to the DOF Fish Health Laboratory and achieved disease-free certification.

2.2.6 *Release of otolith-tagged Black Bream*

During 2002, two batches of *c.* 35,000 juvenile Black Bream, derived from hatchery fertilization in November 2001, were transported to the Blackwood River Estuary where they were divided into groups and released in three well-spaced regions of the upper estuary that constituted typical nursery habitats for this species. The first and second batches were released in early May and early July, respectively. A further 150,000 otolith-marked juvenile Black Bream, derived from hatchery fertilization in October 2002, were released at the same three sites between February and May 2003.

Release of externally-tagged Black Bream, 2003

1217 juvenile Black Bream, cultured as part of the 2001 Blackwood cohort (see Chapter 3.2.2) and reared in tanks at the ADU, were tagged externally with t-bar tags at an age of 18 months. To ensure all tags were retained, the fish were maintained for a period of one week in the tanks at the ADU before they were transported to the Blackwood River Estuary in early April 2003 and released into the upper estuary. The 102 surviving brood stock were also t-bar tagged at the ADU and released into the upper estuary.

2.3 SAMPLING REGIME

The Blackwood River, whose mouth is located at 34° 20' S 115° 10' E (Fig. 2.1), drains an area of *c*. 20 500 km^2 (Hodgkin, 1976; Morgan *et al.*, 2003). The estuary of this river remains permanently open to the ocean and extends 42 km in an upstream direction (Hodgkin, 1976). It comprises a short entrance channel (lower estuary), a basin with an area of 8.5 km^2 (middle estuary) and the lower reaches of the Blackwood River (upper estuary) (Fig. 2.1). As in other permanently-open estuaries in south-western Australia, the upper estuary of this system is the region mainly occupied by *A. butcheri* for much of the year and where most of the angling for this species is focussed (Lenanton, 1977; Sarre, 1999).

Sunken composite gill nets were used to sample *A. butcheri* in the upper Blackwood River Estuary at two or three monthly intervals between December 2002 and November 2005. Sampling was undertaken at the three of the nine sites which, during bimonthly sampling over the previous two years to obtain initial data for the wild stock, had yielded most Black Bream (Fig. 2.1). These sites were located over a length of *c.* 15 km of the upper estuary. The gill nets comprised six 20 m long and 2 m high panels, each containing a different mesh size, *i.e.* 38, 51, 63, 76, 89 or 114 mm. The nets were set at dusk perpendicular to the bank and retrieved at first light on the following morning. The total number of fish caught in each gill net was converted to a catch rate, *i.e.* number of individuals 12 h^{-1} of netting.

On each sampling occasion, water temperature and salinity were recorded at the surface and bottom of the water column at each sampling site using a Yellow Springs International conductivity and temperature meter model 30/25 FT.

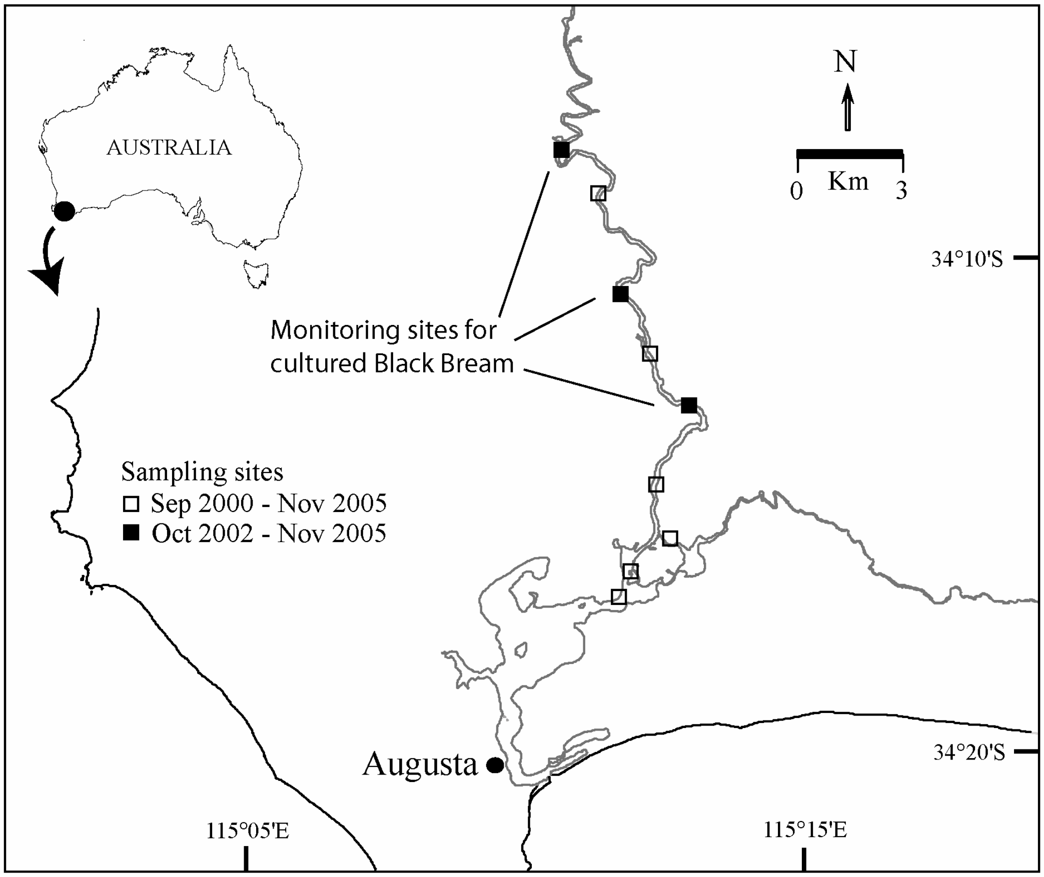

Figure 2.1 Map of the Blackwood River Estuary showing sampling sites for *Acanthopagrus butcheri*.

2.4 AGE AND GROWTH

The total length (TL) and wet weight of each fish caught between December 2000 and October 2005 were recorded to the nearest 1 mm and 1 g, respectively. The two sagittal otoliths of each fish were removed, cleaned, dried and stored. Otoliths were subsequently viewed against a white background under a dissecting microscope and identified as either marked with ALC or unmarked. They were then immersed in methyl salicylate and examined against a black background using reflected light and a dissecting microscope. Otoliths with more than six opaque zones, and thus those in which their opaque zones were not as readily distinguishable as in those with fewer zones, were mounted and embedded in clear epoxy resin and cut into *c*. 0.3 mm transverse sections using an Isomet low-speed diamond saw (Sarre and Potter, 2000). Sections were ground on carborundum paper (400 grade) and mounted on glass slides using DePX mounting adhesive. Sectioned otoliths were placed on a black surface and examined microscopically under reflected light using a dissecting microscope. The number of opaque zones (annuli) on each whole and each sectioned otolith were counted.

The estimated middle of the spawning period, determined from the trends exhibited by gonadosomatic indices and the development of the ovaries and testes throughout the year (see Results), was used as the birth date of Black Bream in the Blackwood River Estuary (November 1). The age of each fish on its date of capture was determined using a combination of the number of opaque zones in its otoliths, the time of year when those zones become delineated and the above birth date.

Using non-linear regression in the Statistical Package for the Social Sciences (SPSS Inc., 2004), von Bertalanffy growth curves were fitted separately to the lengths at age of females and males of wild *A. butcheri*. The lengths at age of juvenile fish, which were unable to be sexed, were randomly allocated and equally distributed to the female and male data sets. The von Bertalanffy growth equation is;

$$L_t = L_\infty \left(1 - \exp^{-k(t-t_0)}\right),$$

where L_t is the predicted total length at age t (years), L_∞ is the mean asymptotic length predicted by the equation, k is the growth coefficient ($year^{-1}$) and t_0 is the hypothetical age (years) at which fish would have zero length.

The growth curves derived for the females and males of wild *A. butcheri* were compared using a likelihood-ratio test to determine whether the growth of the two sexes differed significantly (Cerrato, 1990). As the growth of females and males did not differ significantly ($P < 0.05$), the lengths at age of individuals of both sexes of wild fish were pooled to produce a single growth curve (see Results). The above statistical procedure was also adopted to test whether the growth curve for stocked *A. butcheri* (max. age = 4 years) differed significantly from the growth curve that was derived for wild stock but which was now restricted to using the lengths at age of fish within the same age range.

2.5 REPRODUCTIVE BIOLOGY

The gonads of each fish were removed and, when identifiable under a dissecting microscope as either ovary or testis, were weighed to the nearest 0.01 g. On the basis of its macroscopic characteristics, each pair of gonads was assigned to one of the following maturity stages, adapted from the scheme of Laevastu (1965), *i.e.* I/II = immature/resting, III/IV = developing/maturing, V/VI = mature/spawning, VII/VIII = spent/recovering.

The lengths at which 50% of the females and males of both wild and stocked fish attained maturity during the main part of the spawning period (October to December) were determined by Markov Chain Monte Carlo (MCMC) analysis using WinBUGS software (Bayesian Inference using Gibbs sampling for Windows, version 1.4.1). For this purpose, mature fish were defined as those possessing gonads at stages V-VIII, since such fish were either mature, in spawning condition or had recently spawned (Sarre and

Potter, 1999). The probability, P, that a fish at a specified length had attained maturity was described by the logistic equation

$P = P_{\max} / \{1 + \exp[-\log_e(19)(L - L_{50\%\mathrm{Pmax}}) / (L_{95\%\mathrm{Pmax}} - L_{50\%\mathrm{Pmax}})]\}$, where $P_{\max}$ (≤ 1) is the maximum proportion of mature fish, L is the total length of the fish in mm and $L_{50\%\mathrm{Pmax}}$ and $L_{95\%\mathrm{Pmax}}$ are the lengths in mm by which 50 and 95% of $P_{\max}$ of the fish have attained maturity, respectively. The equation was reparameterised to use the parameters $P_{\max}$, L_{50}, and $L_{95\%\mathrm{Pmax}}$, where L_{50} is the length at which 50% of the fish are expected to have become mature, and

$L_{50\%\mathrm{Pmax}} = \dfrac{L_{50} + L_{95\%\mathrm{Pmax}} \log_e(2P_{\max} - 1) / \log_e(19)}{1 + \log_e(2P_{\max} - 1) / \log_e(19)}$. L_{50} and $L_{95\%\mathrm{Pmax}}$ were assumed to have noninformative normal prior probability distributions, while $P_{\max}$ was assumed to exceed 0.5 and to possess a noninformative normal prior bounded by 0.5 and 1. Following confirmation that convergence appeared to have been attained and, after deleting data for an appropriate "burn-in" period, the point estimates and 95% confidence intervals for each parameter, and of the probabilities of fish being mature at a range of specified lengths, were taken as the medians and 2.5 and 97.5 percentiles of the estimates produced by WinBUGS, respectively.

The gonadosomatic index (GSI) of each female and male wild and stocked fish, whose length was $\geq L_{50}$ for the corresponding sex for wild and stocked fish at maturity, respectively, was calculated from the equation $W_1/W_2 \times 100$, where W_1 = wet weight of the gonads and W_2 = wet weight of the fish, both recorded in g.

The mean weights of stage V/VI gonads at a constant total length for female and male wild and stocked fish were determined for two months during the "peak" period of spawning, *i.e.* October and November, using analysis of covariance (ANCOVA) in SPSS. For this analysis, the natural logarithm of the gonad weight was used as the dependent variable, the categories “wild” or “stocked” as the fixed factor and the natural logarithm of total length as the covariate. The common constant total length for female and male wild and stocked fish was determined by the ANCOVA procedure in SPSS.

2.6 MORTALITY

The point estimate and associated 95% confidence intervals for the instantaneous coefficient of natural mortality, *M*, for wild Black Bream were estimated by using SPSS to refit Hoenig’s (1983) empirical equation for fish and inserting into that equation the maximum age of 31 years recorded in the present study. The Hoenig equation describes the relationship between maximum age and *M* for 82 fish stocks (listed in Hoenig, 1982). Although Hoenig’s equation was developed for use with lightly-fished stocks, and Black Bream is targeted by fishers in the Blackwood River Estuary, it is relevant that the above maximum age recorded for *A. butcheri* in the Blackwood River Estuary was the greatest yet recorded for this species in Western Australia. Although Hoenig’s equation is still not ideal, it provides, in view of the data that exists, the best available estimate for *M*. Note that the resulting estimate of *M* represents the average natural mortality experienced by Black Bream throughout most of their life, but that juvenile fish would be expected to experience a much greater natural mortality.

M was next estimated for stocked fish during the 12 months following their release into the Blackwood River Estuary at an age of *c*. 6 months. For this purpose, 1217 juvenile Black Bream, that were produced as part of the culturing process in 2001 and retained in the hatchery (see Chapter 3.2.2), were tagged with t-bar anchor tags at 18 months of age. They were transported to the Blackwood River Estuary in early April 2003, released into the upper estuary and given one month to disperse before attempts were made to sample them on two occasions in the following month (see Chapter 2.1). The natural mortality experienced by stocked fish during their first year of release into the Blackwood River Estuary was calculated using the ratio of otolith-tagged to t-bar tagged Black Bream, which were caught during the two sampling occasions undertaken one month after the initial release of the t-bar tagged fish into the Blackwood River Estuary (see Chapter 3.2.6).

Our method used for estimating *M* was based, as follows, on the Lincoln-Petersen method (Tanner, 1978). The number of otolith-marked fish in the system at the time of release of the fish with the t-bar tags, N_{os}, was estimated as $N_{os} = (N_{er} / N_{ec})N_{oc}$, where N_{er} is the number of t-bar tagged fish released, N_{ec} is the number of t-bar tagged fish caught and N_{oc} is the number of otolith-tagged fish caught. Accordingly, the fraction of the number of otolith-tagged fish initially released in 2002, and which had survived to this time was N_{os} / N_{ir}, where N_{ir} is the number of otolith-tagged fish initially released in 2002. Thus, the proportion of the fish that had died in this period was $1 - N_{os} / N_{ir}$. If *T* is the period between the initial release of the otolith-marked fish and the subsequent release of the fish with t-bar tags, the instantaneous rate of *M* of the stocked, juvenile fish may be calculated as $M = -\frac{\log_e [N_{os} / N_{ir}]}{T}$. This method assumes that (1) t-bar tagged Black Bream became distributed within the upper estuary in the month after their release, (2) mortality was the same for tagged and untagged Black Bream in the estuary during this period, (3) no tags were lost from t-bar tagged bream and (4) that all Black Bream were equally vulnerable to capture in the upper estuary.

2.7 YIELD AND EGG PER RECRUIT AND SPAWNING POTENTIAL RATIO

The yield per age-0 recruit (YPR) was calculated assuming knife-edge recruitment to the fishery at the age of full recruitment to the exploited stock t_c, constant total mortality for fully-recruited fish and a maximum age of 60 years. Yield per recruit was calculated as:

$YPR = \sum_{a=0}^{60} \frac{F_a}{Z_a}[1 - \exp(-Z_a)]N_a W_a$, where the proportion of age 0 recruits surviving to age *a* is $N_a = \exp\left[-\sum_{t=0}^{a-1} Z_t\right]$, the total mortality experienced by fish of age *a* is $Z_a = M + F_a$, and the fishing mortality of those fish is $F_a = 0$ for $a<t_c$ otherwise $F_a = F$. W_a, the total body weight at age *a*, was determined from the predicted length at age determined using the von Bertalanffy growth curve for wild fish and employing the total body weight (g) to length (mm TL) relationship calculated for this species. The values for the level of fishing mortality that maximises YPR, F_{max}, and that at which the derivative of YPR with respect to *F* is one tenth of that at the origin, $F_{0.1}$, were estimated numerically in Excel™. These two values were used as biological reference points for fishing mortality.

Eggs per age-0 recruit, E/R, were determined from the equation $E/R = \sum_{a=1}^{60} p_{female} E_a N_a$, where p_{female} is the proportion of recruits that are female, *i.e.* 0.5, E_a is the estimated total annual fecundity at age *a* and was determined using the relationship recorded by Sarre and Potter (1999) for total annual fecundity *vs* total length of *A. butcheri* in the Swan River Estuary and the von Bertalanffy growth curve derived in our study for wild female Black Bream. The spawning potential ratio (SPR) was calculated by dividing the value for E/R for each fishing mortality by the corresponding value of E/R calculated for the unfished stock (Goodyear, 1993). The value for fishing mortality $F_{40\%SPR}$ that results in a SPR of 40% was calculated as a biological reference point.

2.8 CATCH RATES

A three-way ANOVA was used to determine whether the catch rates of Black Bream varied among regions, seasons or gill net mesh size between winter 2001 and autumn 2002. Prior to each ANOVA, the data for each dependent variable at each site on each sampling occasion were plotted as $\log_{10}(n+1)$ of their mean against their standard deviation to determine the type of transformation required to satisfy best the assumptions of constant variance and normality for this analysis (Clarke and Gorley, 2001). This demonstrated that a $\log_{10}$ transformation was the most appropriate. If the ANOVA demonstrated that there was a significant difference between the means for a variable or there was a significant interaction, the Student-Newman-Kuel (SNK) test was employed to determine the basis of those differences. In cases where there was a significant interaction and multiple comparisons were thus required, the significance level was adjusted using the Bonferroni correction (Underwood, 1997).

To determine whether restocking had affected catch rates, the mean catch rates of wild and stocked fish in each season between 2004 and 2005 were compared using t-tests, using a Bonferroni correction.

2.9 COST PER RECRUIT TO THE FISHERY

The number of stocked fish predicted to enter the fishery, *i.e.* attain the minimum legal length for retention (MLL) of 250 mm TL, was calculated using the following two assumptions. (1) All stocked fish were released into the Blackwood River Estuary at 0.5 years of age and, for the first 12 months after their release, experienced the natural mortality, M, calculated using the data from the release of fish with t-bar tags. (2) During the period taken to reach the MLL, determined from the von Bertalanffy growth equation calculated for stocked fish, the level of mortality experienced was regarded as the value of M determined for wild fish using Hoenig's (1983) equation for fish. Thus, the cost per recruit of stocked fish to the exploited stock, C / R, was determined by dividing the total cost of the project, *i.e.* capturing the brood stock, producing, marking and releasing the juvenile Black Bream and monitoring both the wild and stocked Black Bream, by the estimated number of fish surviving to reach the MLL. C / R was also estimated for a number of scenarios, both including and excluding the various costs associated with marking the fish to be stocked and monitoring both wild and stocked assemblages.

3 BIOLOGY OF WILD AND RESTOCKED FISH

3.1 INTRODUCTION

Justification of restocking a species in its natural environment requires evidence that the stocks of that species in that environment have been significantly diminished (Blankenship and Leber, 1995; Taylor *et al*., 2005). Although numerous *A. butcheri* were caught in the basin of the Blackwood River Estuary in the mid-1970s (Lenanton, 1977), none was caught by Valesini *et al*. (1997) in that region of the estuary 20 years later. However, Lenanton (1977) did catch large numbers of Black Bream in the saline lower reaches of the tributary (Blackwood River) of this estuary during the winter period. This region, subsequently referred to as the upper estuary, was not sampled by Valesini *et al*. (1997). There has been no published work on the Black Bream in that riverine part of the Blackwood River Estuary since the study of Lenanton (1977). However, from other studies in Western Australian estuaries, and anecdotal evidence from the sole commercial fisher operating in this estuary, Black Bream are likely to be concentrated in the upper estuary, particularly during the dry summer and autumn periods (Loneragan *et al*., 1989, Potter and Hyndes, 1994, Young *et al*., 1997, Sarre, 1999).

A number of studies have focused on elucidating the size and age compositions, growth, reproductive biology and diets of *A. butcheri* in various estuaries (Morrison *et al.,* 1998, Sarre and Potter, 1999, 2000; Sarre *et al*., 2000 Haddy and Pankhurst, 1998, Partridge *et al*., 2003). Studies in Western Australia have shown that the age compositions, growth and diets of Black Bream in the various estuaries of this state vary markedly (Sarre and Potter, 1999, Sarre and Potter, 2000, Sarre *et al*., 2000). In addition, allozyme studies have demonstrated that the populations of Black Bream in the different estuaries of Western Australia are genetically distinct, which is consistent with the view that *A. butcheri* complete their life cycle in their natal estuary (Chaplin *et al.*, 1998).

However, the growth rates of fish cultured from brood stocks from two different types of estuary, in which the pattern of growth was very different, were very similar (Partridge *et al.*, 2004). Thus, variations in growth between the wild stocks in these estuaries are apparently due to one or more of the pronounced environmental differences in those estuaries than to genetic differences in the populations. Although genetic differences may have little influence on the biological characteristics of *A. butcheri* in different estuaries, it would still be advisable, if it was decided to stock this species in an estuary, to use individuals cultured from brood stock collected from that same estuary. Once restocking has been undertaken, it is also desirable to compare the biological characteristics of the stocked individuals with that of the wild stock to ascertain whether they are similar.

The aims of this part of the study were as follows.

1) Obtain baseline data on crucial biological parameters and catch statistics of Black Bream in the Blackwood River Estuary.
2) Identify the habitats (regions) important to Black Bream in the Blackwood River Estuary.
3) Compare the densities of Black Bream in important habitats within the Blackwood River Estuary with those in similar habitats in other systems
4) Estimate the proportion of released Black Bream, which represent a known year class, amongst the total number of that age class, and thereby estimate the extent to which restocking has enhanced the numbers of that year class.
5) Obtain data on the biological parameters and catch statistics of Black Bream in the Blackwood River Estuary following restocking.
6) Evaluate the success of the restocking programme by comparing biological parameters and catch statistics prior to and after restocking.

7) Estimate the average cost to produce each fish that will survive to attain the minimum legal length for retention and thus be available for exploitation.

8) Provide advice that can be used by managers to develop plans to sustain the enhanced stock of Black Bream in the Blackwood River Estuary.

The above aims are in accord with the protocols proposed by Taylor *et al.* (2005a) for enhancing the stocks of estuarine fish species.

3.2 MATERIALS AND METHODS

See Chapter 2.0

3.3 RESULTS

3.3.1 *Commercial catch statistics*

In each year since 1983, the vast majority, or all of the catches, were taken in winter except in the occasional year when substantial catches were taken in spring. The annual catches ranged from less than 100 kg in 1996 and 1998, to over 2000 kg in 1982 and 1983 (Anon, 2004). The CPUE, expressed as kg boat day^{-1}, ranged from very low values in 1991, 1996 and 1998, to over 25 in 1986, 2000 and 2002. However, caution should be exercised in drawing conclusions from these catch statistics as the number of fishers has declined to just one in recent years and there is considerable noise in the data.

3.3.2 *Water temperature and salinity*

Water temperatures at the surface and bottom of the water column, derived using data for all sampling sites within the upper Blackwood River Estuary underwent pronounced and consistent seasonal changes (Fig. 3.1a). Thus, in surface waters, the minimum mean monthly temperatures rose rapidly from *c*. 12°C in winter or early spring to reach their maximum of *c*. 22 to 26 °C in summer and early autumn and then declined precipitously.

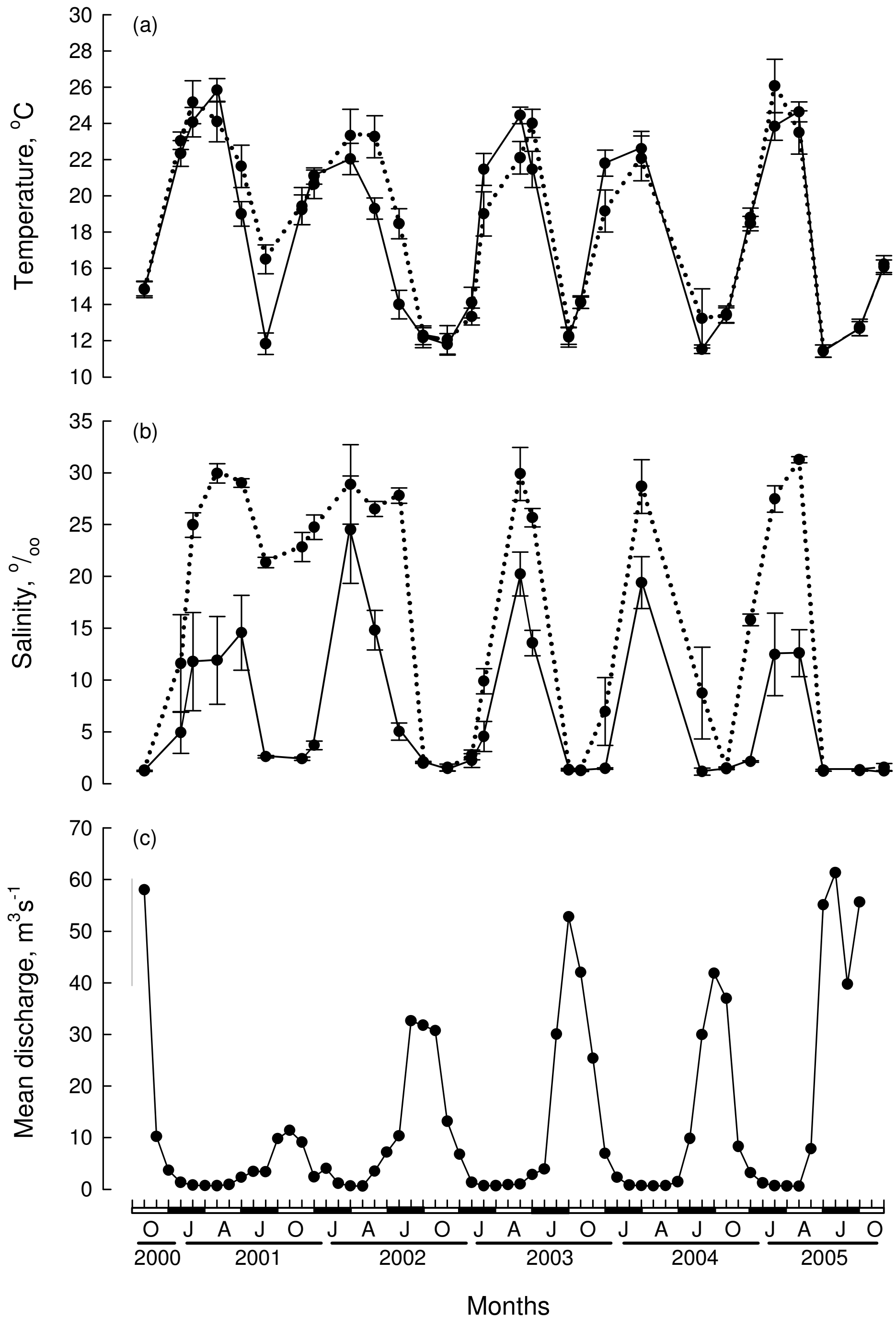

Figure 3.1 Mean monthly water temperatures and salinities ± 1 SE at the surface (solid line) and bottom (dotted line) of the water column at sampling sites in the upper Blackwood River Estuary. Mean monthly discharges recorded for the upper estuary of the Blackwood River (courtesy of Department of Environment) are also shown. Open rectangles refer to spring and autumn months and closed rectangles to summer and winter months.

A small thermocline sometimes formed, with the maximum difference of *c*. 5 °C between surface and bottom waters being recorded in the winter of 2001 (Fig. 3.1a).

Salinities at the top of the water column also underwent marked seasonal changes (Fig. 3.1b). However, while the mean monthly salinities at the top of the water column always declined to < 3 at some time in each year, the mean monthly salinities at the bottom of the water column in winter varied greatly among years, ranging from *c.* 1 in the winter of 2005 to *c.* 21 in 2001 (Fig. 3.1b). Furthermore, haloclines were present in most months of the year and were most pronounced in the late winter and early spring of 2001, when discharge was relatively low (Fig. 3.1c). During that period, salinities at the top of the water column were only 2.5 compared with 21-25 at the bottom (Fig. 3.1b).

3.3.3 *Habitat*

Seine net sampling of nine sites at regular intervals during 2000, 2001 and 2002 yielded a total of only 33 Black Bream.

ANOVA demonstrated that, within the upper estuary, the catch rates of *A. butcheri* differed significantly among regions and seasons and that there was a significant interaction between these two variables (Table 3.1). However, the mean squares were far greater for region and season than for the interaction. The mean catch rates were greatest in the upper regions of the upper estuary in each season, and were greatest in spring, followed by winter in both the upper and middle regions (Fig. 3.2). The mean seasonal catch rates were always low in the lower region of the upper estuary. The region × season interaction was due, in part, to the fact that the mean catch rates in the lower region were greater than in the middle region in summer, whereas the reverse was the case in the other three seasons of the year (Fig. 3.2).

Table 3.1 Mean squares and significance levels for three-way ANOVAs of the mean catch rates of native *Acanthopagrus butcheri* in the Blackwood River Estuary in each season between winter 2001 and autumn 2002.

	Main effects			Interactions				
	Region (R)	Season (S)	Mesh (M)	RxS	RxM	SxM	RxSxM	Residual
	DF (2)	(3)	(2)	(6)	(4)	(6)	(12)	(180)
Mean catch rate	1.020***	1.262***	0.104	0.275**	0.046	0.172	0.092	0.092

DF, degrees of freedom; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

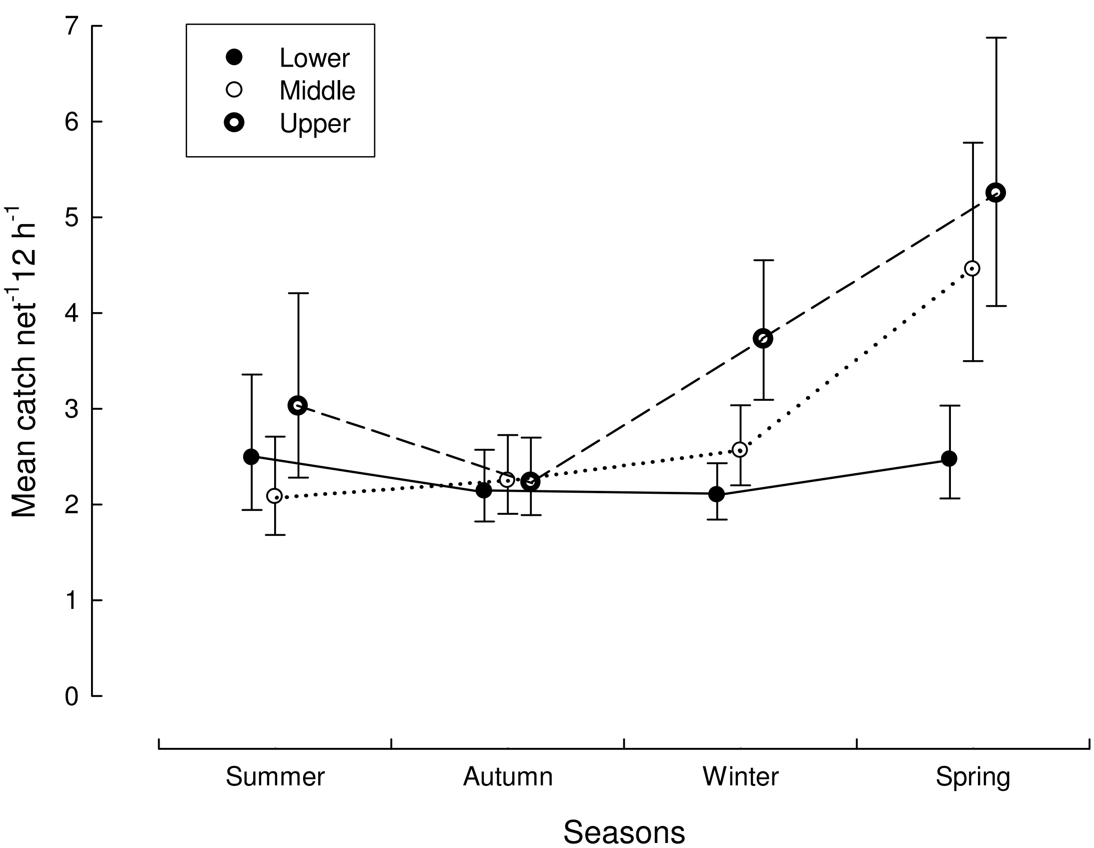

Figure 3.2 Mean catch rates $\pm$ 95% confidence intervals of *Acanthopagrus butcheri* in gill nets set for 12h^{-1} at sites in the lower, middle and upper regions of the upper Blackwood River Estuary between 2001 and 2002.

3.3.4 *Length and age compositions and growth*

The lengths of wild fish in 2000 ranged from 100 to 430 mm, with the majority lying between 100 and 190 mm (Fig. 3.3). The proportion of wild fish in the latter length range declined markedly in 2001 and 2002, in which years the majority of fish were greater than 170 mm in length and ranged up to 440 mm in length. In 2005, the lengths of virtually all wild fish were > 220 mm and the majority lay between 240 and 310 mm (Fig. 3.3).

Stocked fish were first caught in 2002, at which year their lengths ranged from 90 to 110 mm (Fig. 3.3). The distribution of the lengths of stocked fish was markedly bimodal in 2003, with the modal length class of the 2002 year class lying at 100 to 109 mm and that of the 2001 year class lying at 170 to 179 mm. The distribution of the stocked fish in 2004 was skewed to the left, with a well-defined modal length class of 160 to 169 mm, and in 2005 the modal length class had increased to 200 to 209 mm (Fig. 3.3).

As the von Bertalanffy growth curves of females and males of wild *A. butcheri* did not differ significantly ($P > 0.05$), the lengths at age of both sexes were pooled and used to produce a single von Bertalanffy growth curve (Fig. 3.4). The data in Figure 3.4a show that, while the 1025 wild fish caught during the present study ranged from a few months to 31 years in age, the vast majority were < 10 years in age. The von Bertalanffy growth curve provided a good fit to the lengths at age of wild *A. butcheri* (Fig. 3.4a), as is demonstrated by the high value of 0.861 for the coefficient of determination for that growth curve (Table 3.2). Sampling of the Blackwood River Estuary at regular intervals yielded stocked individuals up to 4 years old (Fig. 3.4b), reflecting the fact that the period between the culture of the first group of fish used for restocking and the last time of

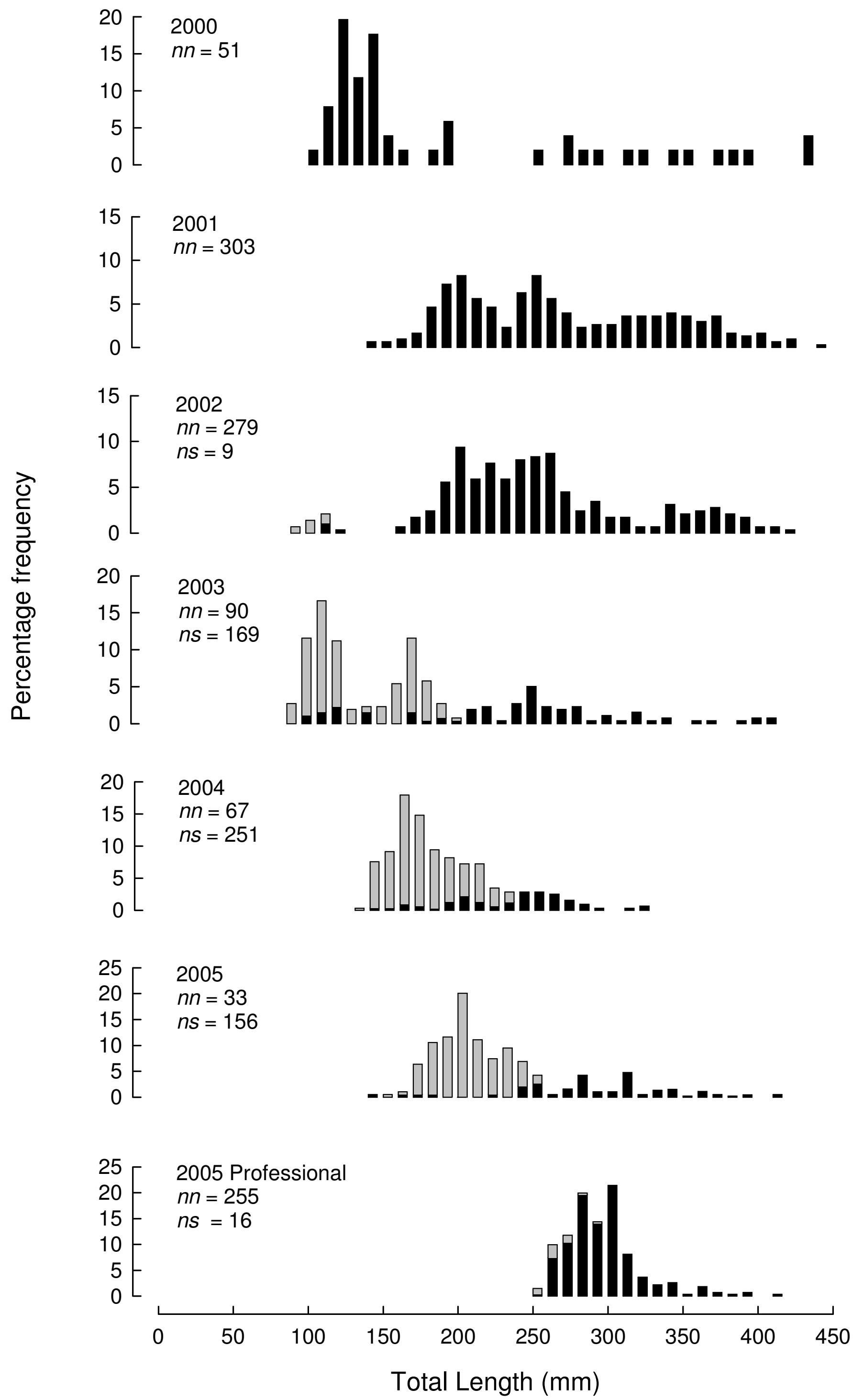

Figure 3.3 Length-frequency distributions for wild and stocked *Acanthopagrus butcheri* in our gill nets set in the Blackwood River Estuary between 2000 and 2005 and also in those of a commercial gill net fisher in 2005. Black bars = wild fish, grey bars = stocked fish, *nn* = number of wild fish, *ns* = number of restocked fish.

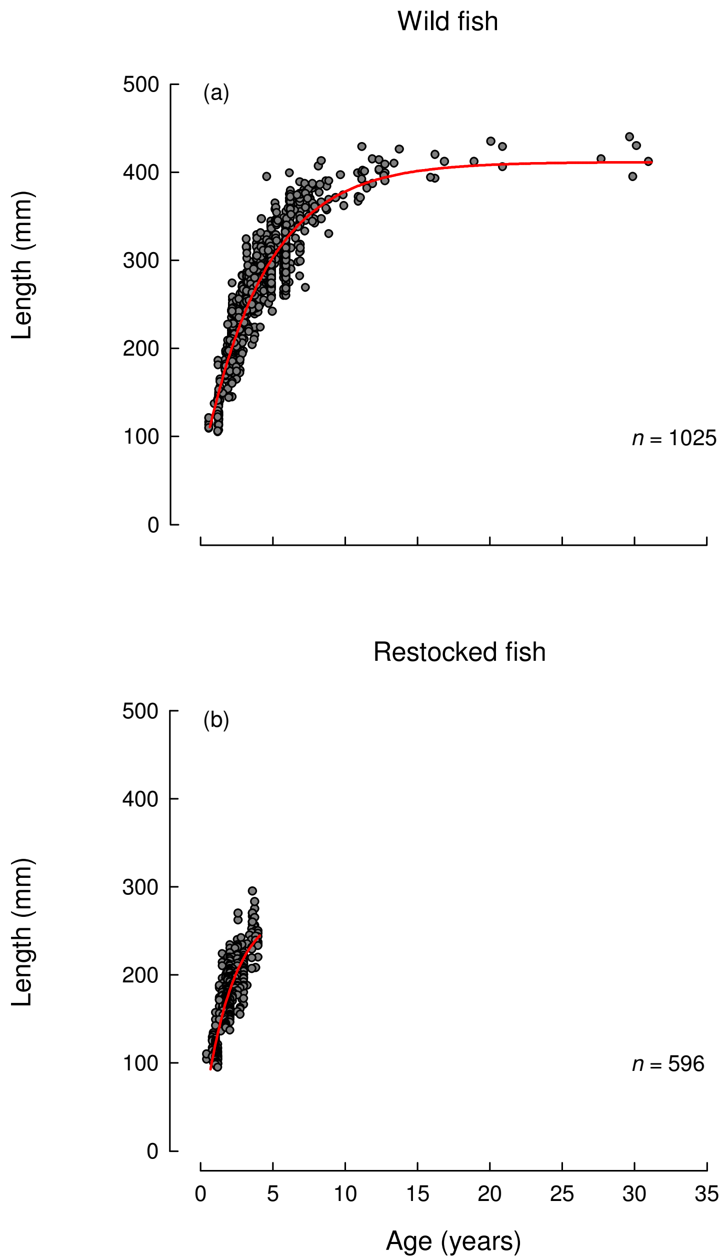

Figure 3.4 von Bertalanffy growth curves fitted to the lengths at age of (a) wild and (b) restocked *Acanthopagrus butcheri* in the Blackwood River Estuary. *n* = sample size.

sampling was four years. The maximum length obtained by older individuals was *c.* 300 mm.

Although a comparison of the von Bertalanffy growth curves for stocked fish and those for wild fish < 4 years strongly indicate that, by four years, wild fish had attained a greater length (Table 3.2, Fig. 3.5), the values for t_0 for the two groups differed. Thus, the mean lengths at age were calculated for fish in both groups at each sequential half year interval from the commencement of the second half of the first year of life (Fig. 3.6). The mean lengths at age of wild and stocked fish progressively diverged with increasing age and, at each age interval above 1.5 years, was significantly different ($P < 0.05$ or 0.01 or 0.001), apart from those for the 3-3.49 age interval for which only three stocked fish were caught.

Figure 3.7 compares the growth rates of females and males of wild Black Bream with those of their corresponding sex in the Swan River Estuary and in the Nornalup-Walpole Estuary. Note that the growth curves for females and males were kept separate for these comparisons as the growth curves of the two sexes were significantly different ($P < 0.05$) in the latter two estuaries (Sarre and Potter, 2000). The Swan River Estuary was chosen for comparison as it is permanently open and located to the north of the Blackwood River Estuary on the lower west coast of Australia and because its Black Bream population is likewise subjected to substantial fishing pressure. The Nornalup-Walpole Estuary was selected because it is permanently open and located to the east of the Blackwood River Estuary on the south coast of Western Australia. The data shown in Figure 3.7 illustrate very clearly the extent to which growth of Black Bream can vary among estuaries. Thus, for example, after three years, the females had reached 267 and 246 mm in the Swan and Blackwood estuaries, respectively, but only 161 mm in the

Table 3.2 von Bertalanffy growth parameters, including upper and lower 95% confidence limits, derived from lengths at ages of both wild and restocked *Acanthopagrus butcheri* from the Blackwood River Estuary. n = sample size.

	L_∞ (mm)	k (year^{-1})	t_0 (years)	R^2	n
Wild *A. butcheri* (all fish)					
Estimate	**412**	**0.23**	**-0.83**	0.861	1025
Upper	402	0.25	-0.68		
Lower	406	0.22	-0.98		
Wild *A. butcheri* (≤4 years)					
Estimate	**406**	**0.27**	**-0.50**	0.764	604
Upper	470	0.36	-0.23		
Lower	342	0.18	-0.77		
Restocked *A. butcheri*					
Estimate	**280**	**0.49**	**-0.14**	0.729	596
Upper	304	0.58	0.03		
Lower	257	0.37	-0.31		

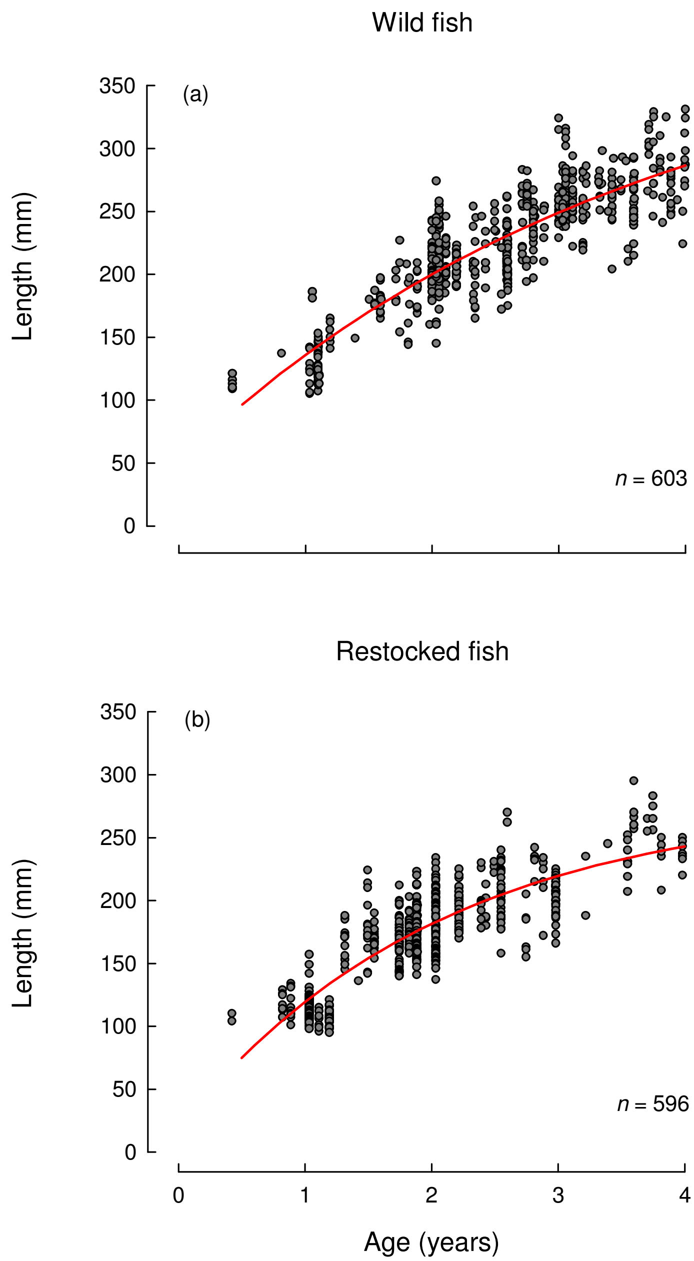

Figure 3.5 von Bertalanffy growth curves fitted to the lengths at age of wild and restocked *Acanthopagrus butcheri* ≤ 4 years of age in the Blackwood River Estuary. *n* = sample size.

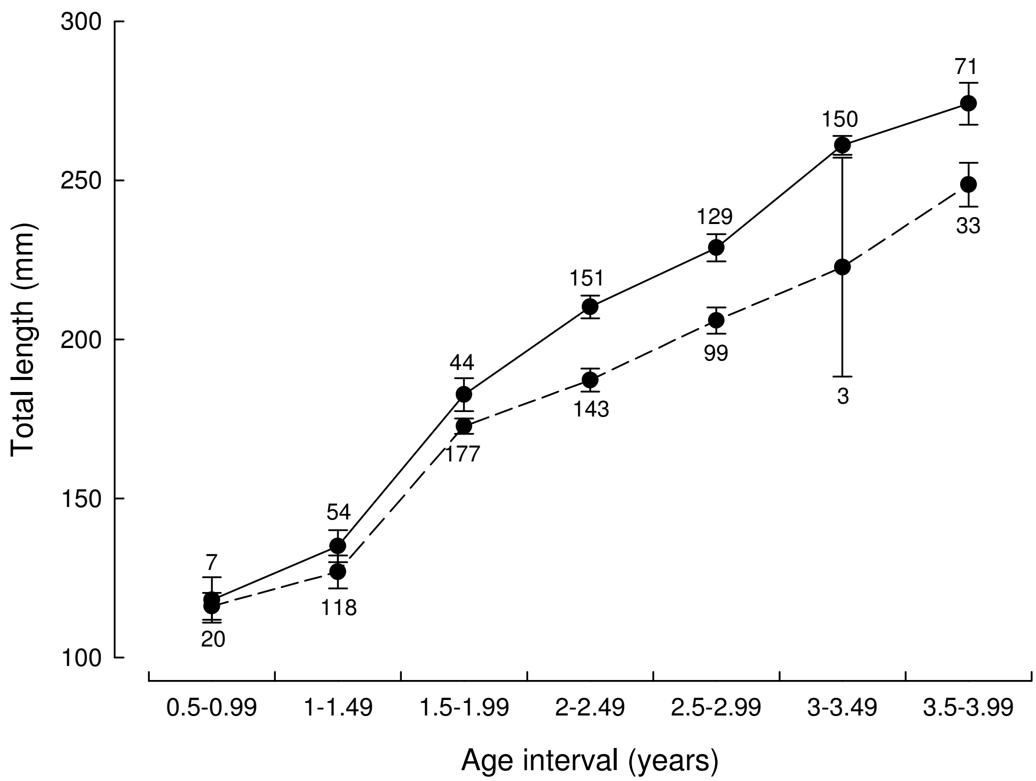

Figure 3.6 Mean lengths ± 95% confidence intervals of different age intervals of wild (solid line) and restocked (dashed line) *Acanthopagrus butcheri*. Numbers refer to sample sizes. N.B. Numbers of older wild fish are greater than those of restocked fish because older wild fish were present in all years.

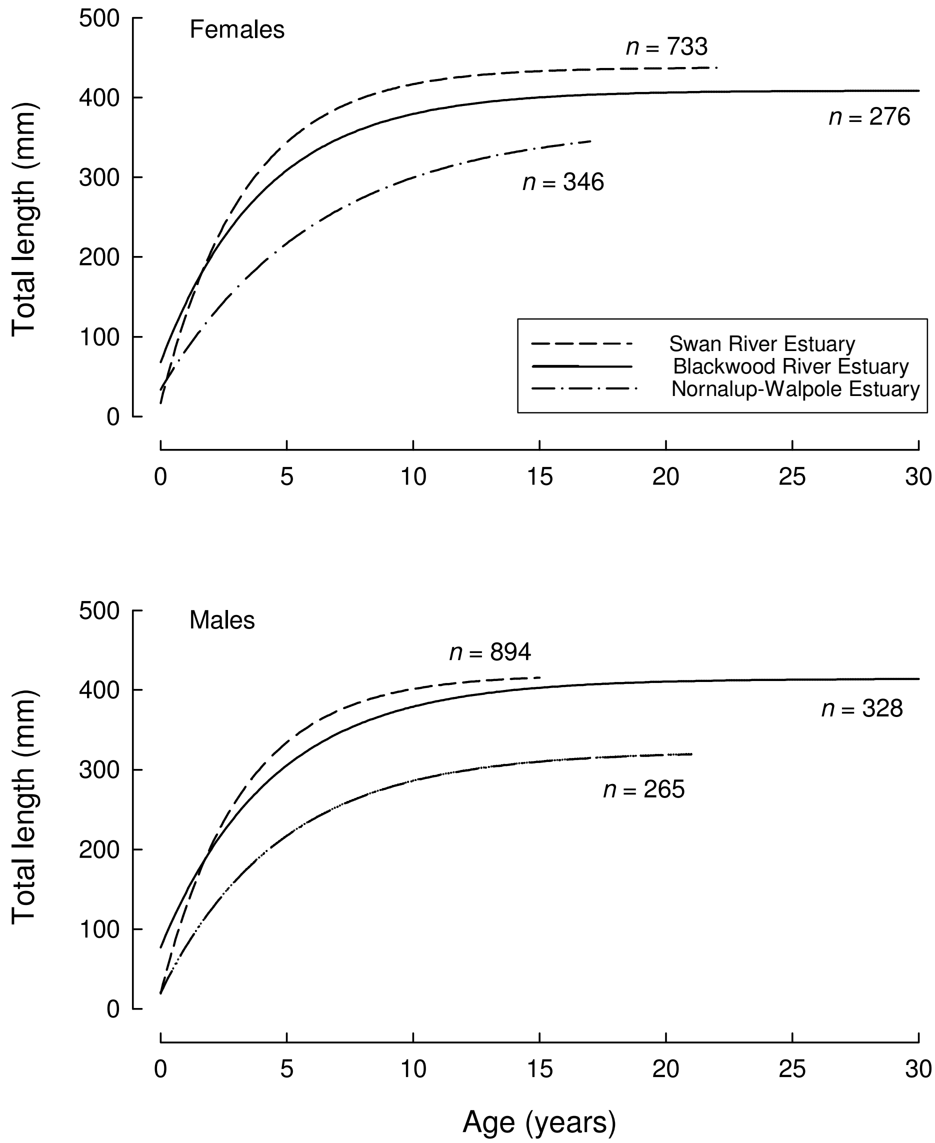

Figure 3.7 von Bertalanffy growth curves fitted to lengths at age of female and male *Acanthopagrus butcheri* from the Blackwood River, Swan River and Nornalup-Walpole Estuaries. Data for the Blackwood River Estuary were restricted to those for the wild stock and the Swan River and Nornalup-Walpole estuaries were obtained from Sarre and Potter (2000). *n* = sample size.

Nornalup-Walpole Estuary, and the L_{∞}, particularly for males, was considerably less in the Nornalup-Walpole Estuary than in the other two estuaries (Table 3.3).

3.3.5 *Reproduction*

The mean monthly GSIs of the females of wild Black Bream, derived from data for the full length and age range, rose sharply from 1.1 in June to 6.3 in October, and then underwent a precipitous decline to 1.1 in February and remained at a similarly low level in the following months (Fig. 3.8). The mean monthly GSIs of males exhibited very similar trends and reached a similar maximum to that of females in October. Although the mean monthly GSIs of the females and males of stocked Black Bream followed a similar overall trend to those of the corresponding sexes of wild fish, their maxima of only 3.4 to 3.6 were far less than the values of over 6.3 to 6.7 recorded for wild fish. Furthermore, in the case of both the females and males, the prevalence of Black Bream with stage V/VI gonads was far greater among the wild than stocked fish (Fig. 3.8). It is important to recognise, however, that the mean weights of stage V/VI gonads of female stocked and wild *A. butcheri*, calculated for fish of a constant total length in both October and November, *i.e.* during the middle of the spawning period, were not significantly different ($P > 0.05$) in either of these months. This was also the same for stocked and wild males ($P > 0.05$). Thus, the weights of mature gonads of stocked and wild fish were similar when the relationship between gonad weight and body length were taken into account.

All wild female fish that were caught in February and March and were $\geq L_{50}$ at maturity possessed gonads at either stages I/II (virgin/resting) or VII/VIII (spent/recovering) (Fig. 3.9). Females with stage III/IV gonads were first caught in April, and their prevalence started to decline after September and they were not found in

Table 3.3 von Bertalanffy growth parameters, including upper and lower 95% confidence limits, derived from lengths at ages of wild *Acanthopagrus butcheri* from the Blackwood River, Swan River and Nornalup-Walpole estuaries.

	L_∞ (mm)	k (year^{-1})	t_0 (years)	R^2	N
Blackwood River Estuary					
Female					
Estimate	409	0.24	-0.74	0.86	460
Upper	423	0.27	-0.53		
Lower	394	0.21	-0.95		
Male					
Estimate	414	0.23	-0.91	0.86	566
Upper	427	0.25	-0.69		
Lower	401	0.20	-1.12		
Swan River Estuary					
Female					
Estimate	438	0.30	-0.13	0.94	733
Upper	450	0.31	-0.10		
Lower	426	0.28	-0.17		
Male					
Estimate	419	0.31	-0.15	0.94	894
Upper	428	0.32	-0.11		
Lower	411	0.29	-0.19		
Nornalup-Walpole Estuary					
Female					
Estimate	367	0.16	-0.60	0.91	346
Upper	382	0.18	-0.43		
Lower	353	0.14	-0.88		
Female					
Estimate	323	0.21	-0.31	0.90	265
Upper	335	0.24	-0.08		
Lower	312	0.19	-0.28		

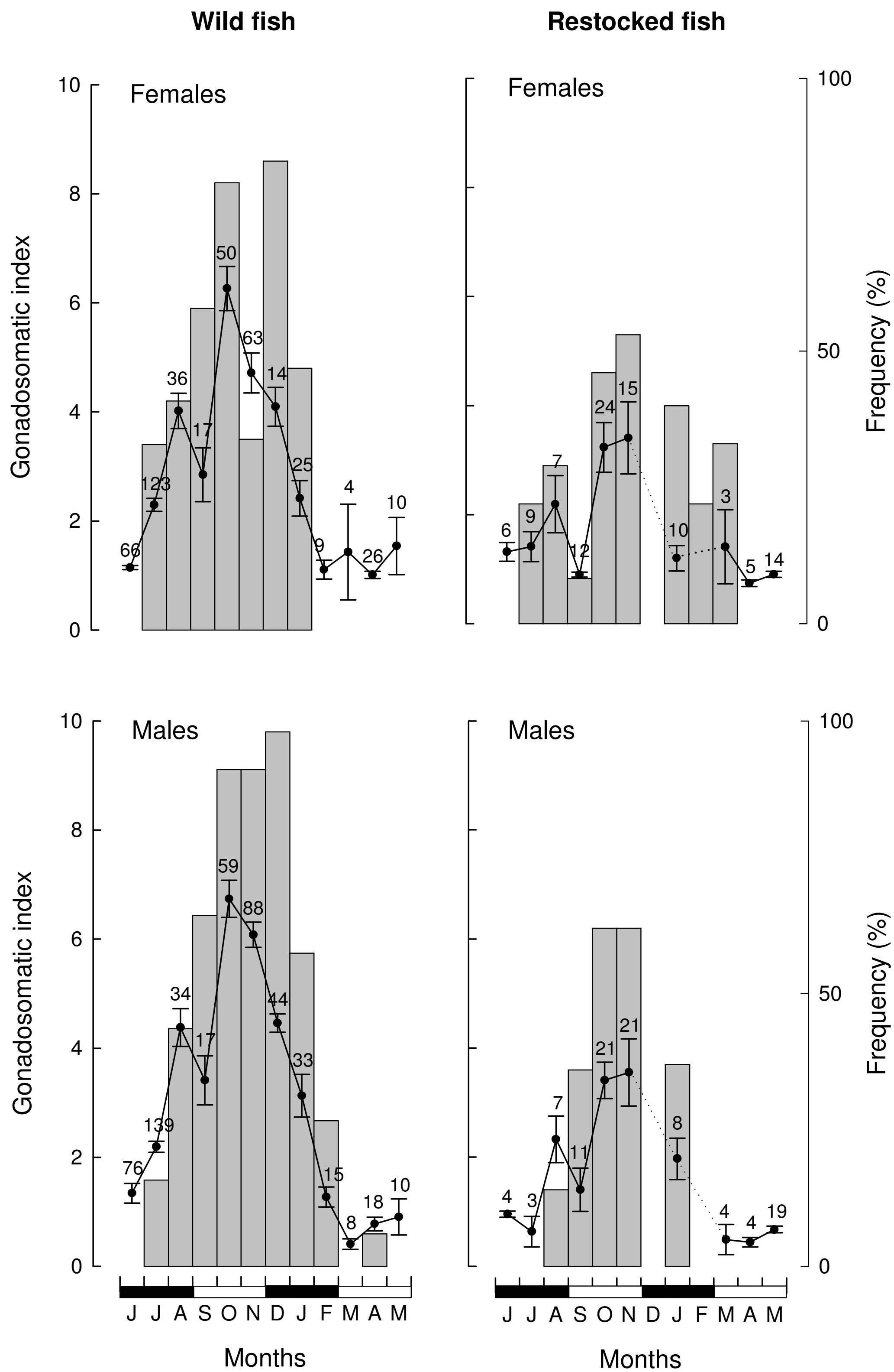

Figure 3.8 Mean monthly gonadosomatic indices ± 1 SE and the mean percentage frequencies of Stage V/VI gonads of wild and restocked females and males of *Acanthopagrus butcheri* ≥ L_{50} in the Blackwood River Estuary. Numbers refer to sample sizes in each month. Open rectangles refer to spring and autumn months and closed rectangles to summer and winter months.

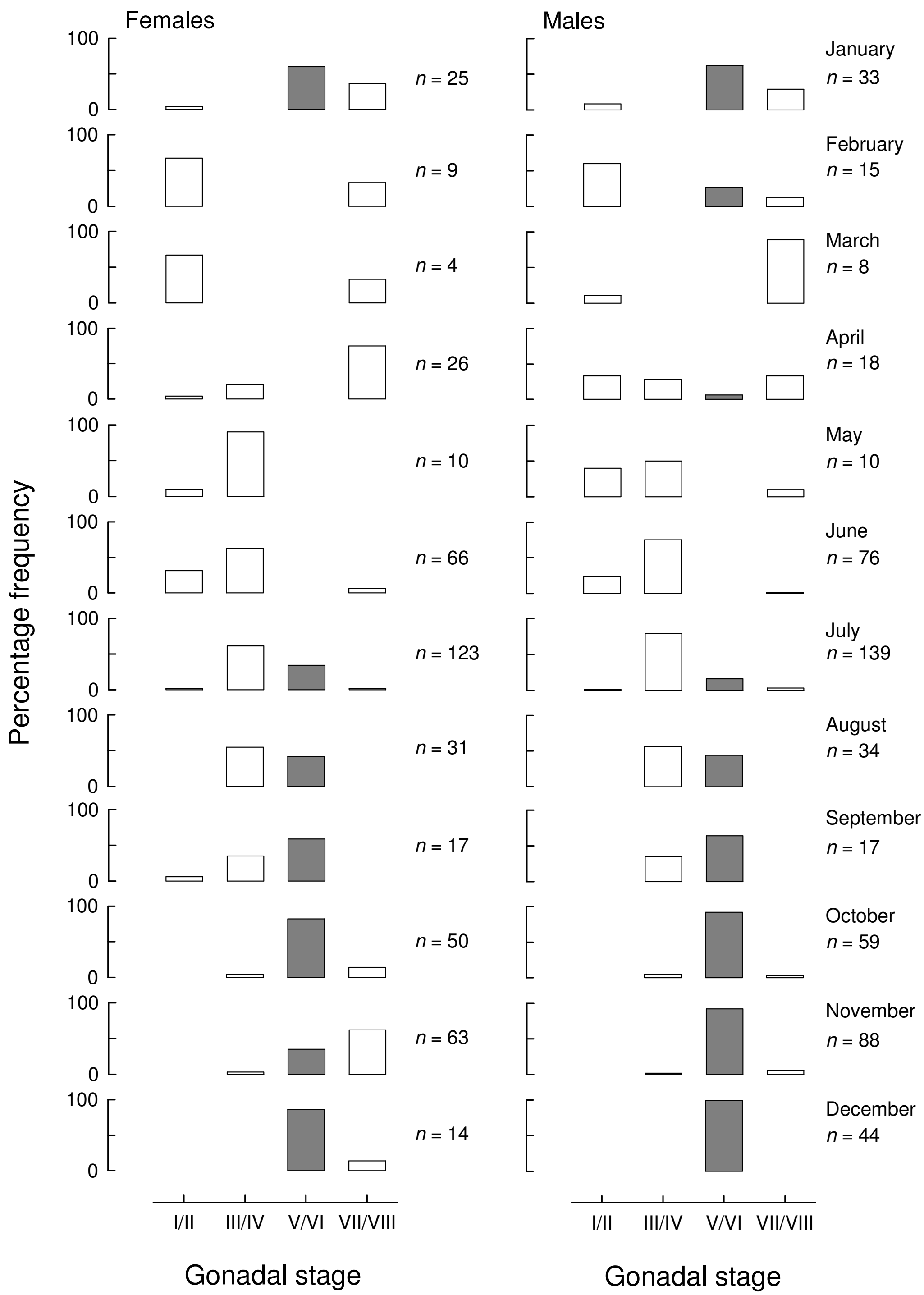

Figure 3.9 Monthly percentage frequencies of occurrence of sequential gonadal stages in wild female and male *Acanthopagrus butcheri* $\geq L_{50}$ at maturity in the Blackwood River Estuary. *n* = sample size in each month.

December to March. Mature females (gonad stages V/VI) were caught in each month between July and January, and spent/recovering females (stage VII/VIII) were largely found between October and April. The trends exhibited by the corresponding monthly data for the males paralleled very closely those just described for females (Fig. 3.9). The trends exhibited by the prevalences in each month of the different stages in ovarian and testicular development of stocked fish were similar to that described for wild fish (Fig. 3.10).

The lengths of the smallest mature female and male in the samples of wild Black Bream collected during the spawning season, *i.e.* possessed gonads at stages V/VI or VII, were 181 and 147 mm (Fig. 3.11). Virtually all wild females and males with lengths above 180 mm were mature. The L_{50} for females was greater than that of males, *i.e.* 179 *vs* 156 mm, respectively (Table 3.4). The trends exhibited by the data for female and male wild Black Bream, when the fish were restricted to those < 4 years old, were essentially the same as those described above for all wild fish (Table 4, Fig. 3.11). The smallest female and male stocked fish which became mature were 156 and 153 mm, respectively (Fig. 3.11). In each length class ≥ 180-199 mm the proportions of both mature females and males were less in stocked fish than in wild fish and a substantial number of stocked fish ≥ 180 mm had not become mature, whereas most wild fish above this length had become mature. The L_{50} for females of stocked (187 mm) and wild fish (179 mm) was similar, but was substantially greater for stocked (192 mm) than wild fish (156 mm) in the case of males (Fig. 3.11, Table 3.4).

None of the wild or stocked female fish, nor any of the males of the stocked fish, had become mature at the end of their first year of life (Fig. 3.12). However, by the end of their second year of life, maturity had been attained by 84 and 94% of the females and males of wild fish, respectively, whereas only about one third of the females and males of

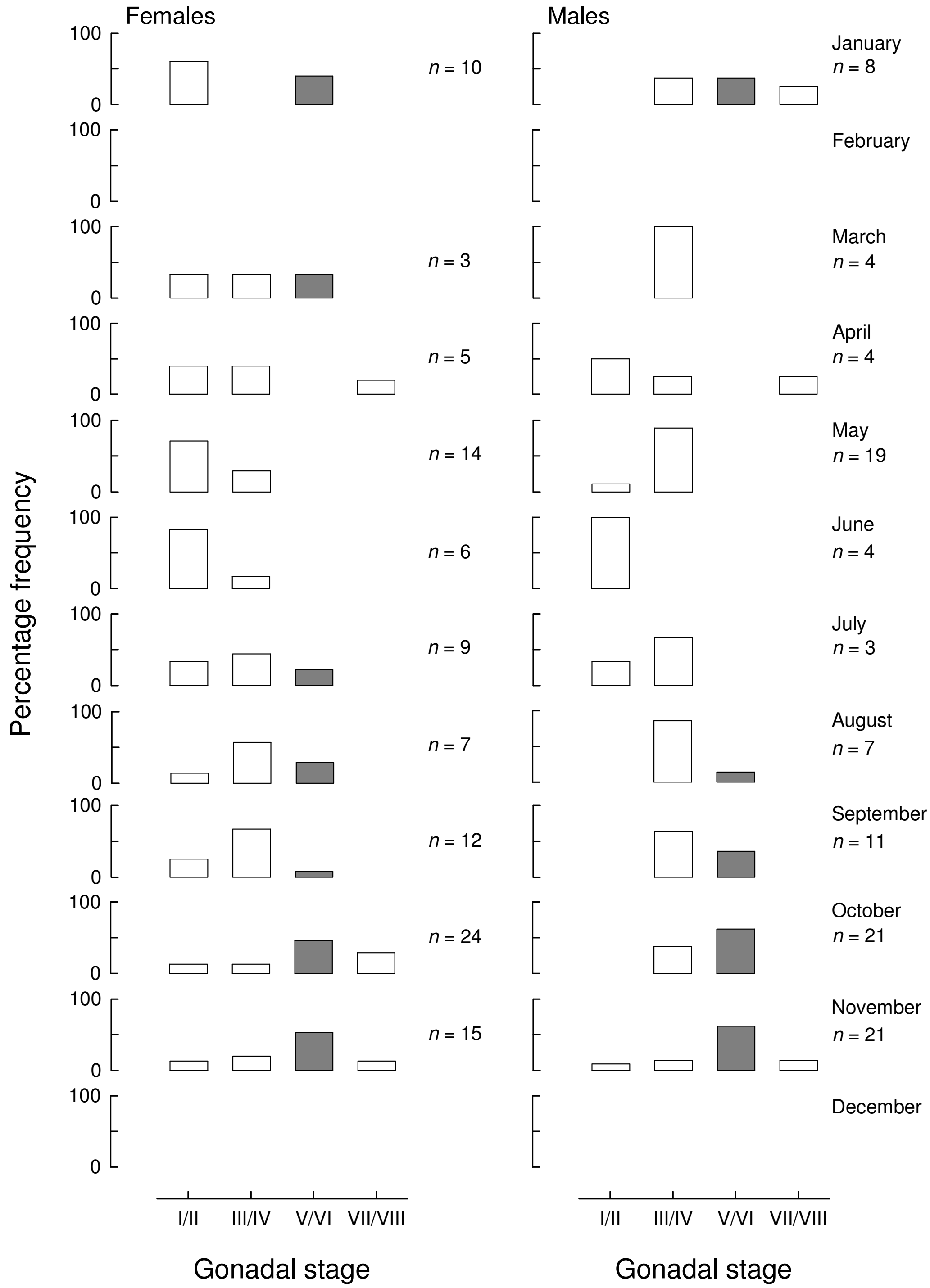

Figure 3.10 Monthly percentage frequencies of occurrence of sequential gonadal stages in restocked female and male *Acanthopagrus butcheri* $\geq L_{50}$ at maturity caught in the Blackwood River Estuary. *n* = sample size in each month.

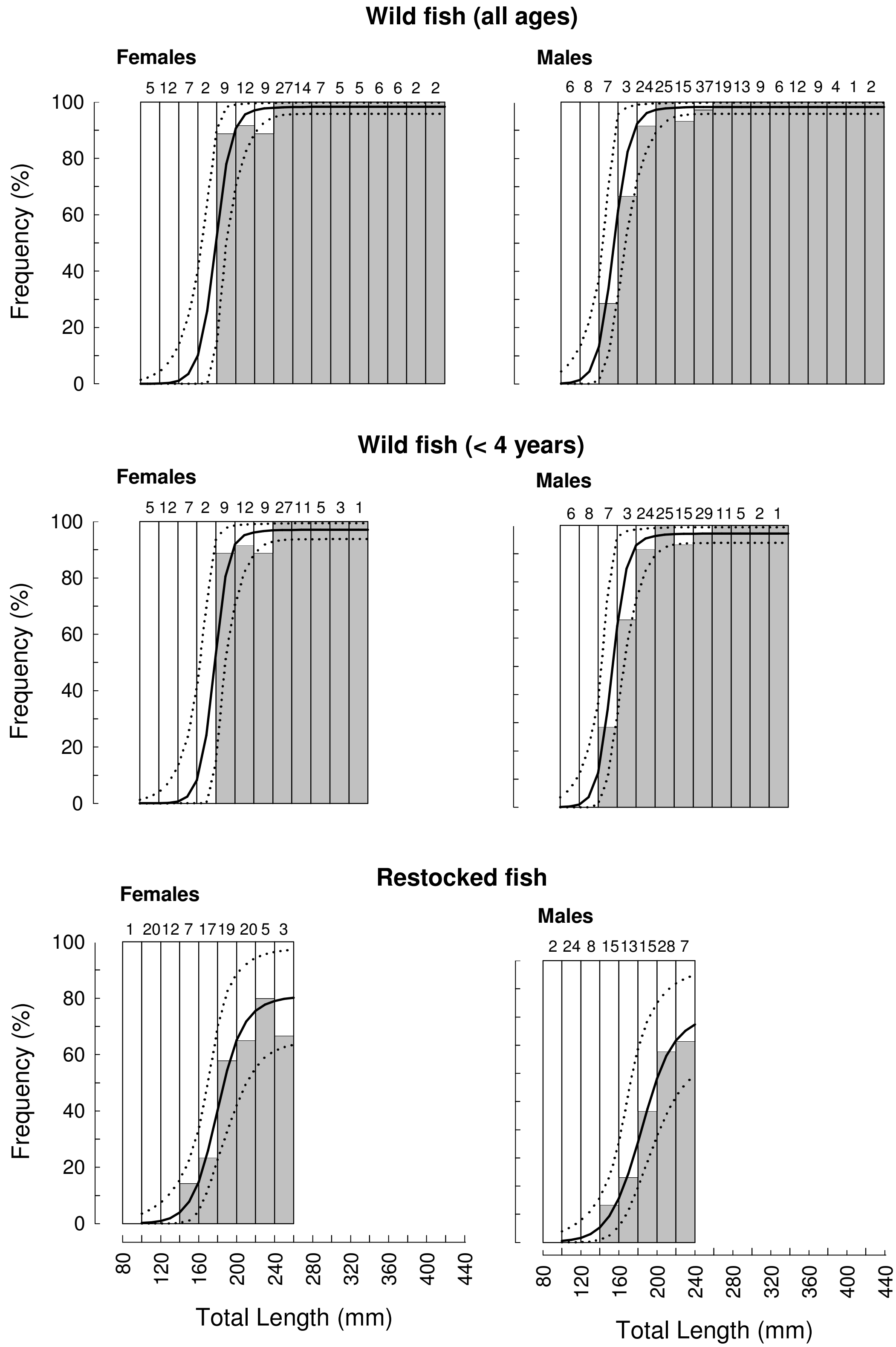

Figure 3.11 Percentage contributions made during the spawning season to each 20 mm length class of both wild and restocked female and male *Acanthopagrus butcheri* by fish with stage V-VIII gonads caught from the Blackwood River Estuary. The predicted percentage ± 95% confidence intervals of mature fish at each length derived using Bayesian analysis are shown (solid and dotted lines). Sample size for each 20 mm length class is shown. Grey bars denote percentage mature in each 20 mm length class.

Table 3.4 Length at maturity (L_{50}) and other parameters (Pmax and $L_{95\%\mathrm{Pmax}}$) of the logistic equation relating, to its length, the probability that a wild and restocked *Acanthopagrus butcheri* is mature, *i.e.* possesses gonads at stages V-VIII, together with 95% confidence limits.

		L_{50} (mm)	$L_{95\%P\mathrm{max}}$ (mm)	P_{max}	*n*
Wild fish (all ages)					
Females	Estimate	**179**	**204**	**0.98**	130
	Upper	190	231	1.00	
	Lower	165	181	0.93	
Males	Estimate	**156**	**181**	**0.98**	200
	Upper	168	210	1.00	
	Lower	145	157	0.95	
Wild fish (≤ 4 yrs)					
Females	Estimate	**179**	**200**	**0.97**	103
	Upper	189	229	1.00	
	Lower	164	179	0.90	
Males	Estimate	**155**	**178**	**0.97**	150
	Upper	167	206	0.99	
	Lower	145	155	0.92	
Restocked fish					
Females	Estimate	**187**	**220**	**0.80**	104
	Upper	202	268	0.98	
	Lower	175	190	0.60	
Males	Estimate	**192**	**233**	**0.82**	112
	Upper	177	278	0.99	
	Lower	207	193	0.58	

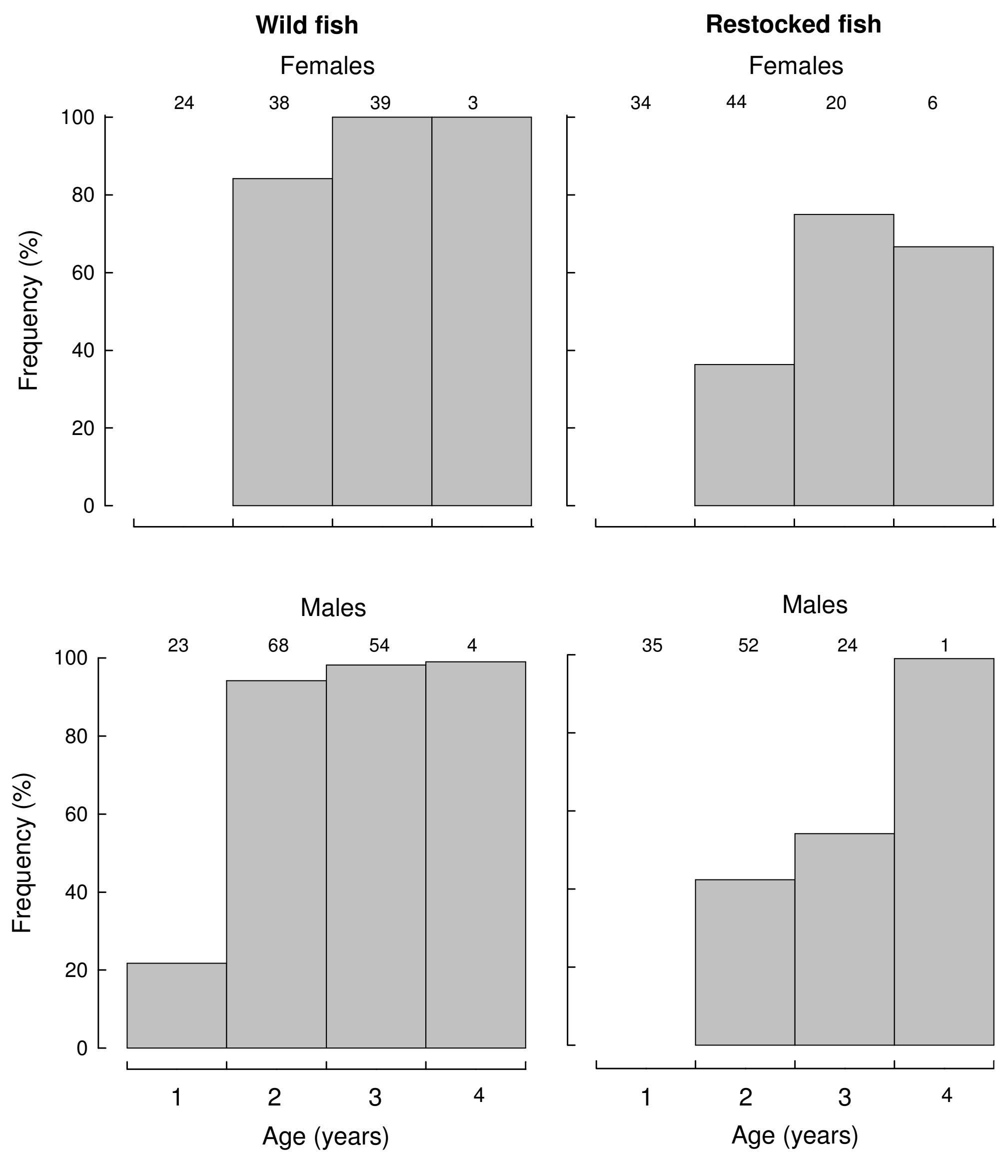

Figure 3.12 Percentage frequency of the occurrence of wild and restocked female and male *Acanthopagrus butcheri* in sequential age classes with gonads at stages V-VIII during the spawning season. Sample sizes for each age class are given.

the stocked fish had become mature by the end of their second year of life. Furthermore, only 75 and 54% of the females and males of stocked fish, respectively, had matured by the end of their third year of life (Fig. 3.12).

3.3.6 *Catch rates*

The mean catch rate of stocked fish was significantly greater ($P < 0.05$) than that of wild fish in each season between summer 2004 and spring 2005, except during the autumn of 2004 and summer of 2005 (Fig. 3.13). In the 6 out of 8 seasons when those catch rates were significantly different, the mean seasonal catch rates, expressed as numbers of fish caught per 12 hours, exceeded 17 fish in the case of stocked fish, whereas they were always less than 8 for wild fish. Stocked fish comprised 75 and 92% of the 215 and 475 individuals of the 2001 and 2002 year classes of *Acanthopagrus butcheri* that were caught during this study, respectively (Fig. 3.14). The contributions of stocked fish of the 2001 and 2002 year classes to the total catches in those year classes in 2003, 2004 and 2005 were 87, 83 and 90%, respectively.

3.3.7 *Mortality*

The point estimate of the instantaneous coefficient for natural mortality, *M*, for wild *A. butcheri*, derived by refitting Hoenig's (1983) equation for fish and inserting the maximum age of 31 years for wild fish in this estuary, was 0.16 y^{-1}. The 95% confidence interval for this estimate was broad, *i.e.* 0.06 to 0.44 y^{-1}. The point estimate for *M* for stocked *A. butcheri* in the 12 month period after their release into the estuary at *c*. 6 months of age, derived using a modification of the Lincoln-Petersen method and data for both otolith and t-bar tagged fish, was 1.05 y^{-1}, *i.e.* 35% survival over the 12 month period.

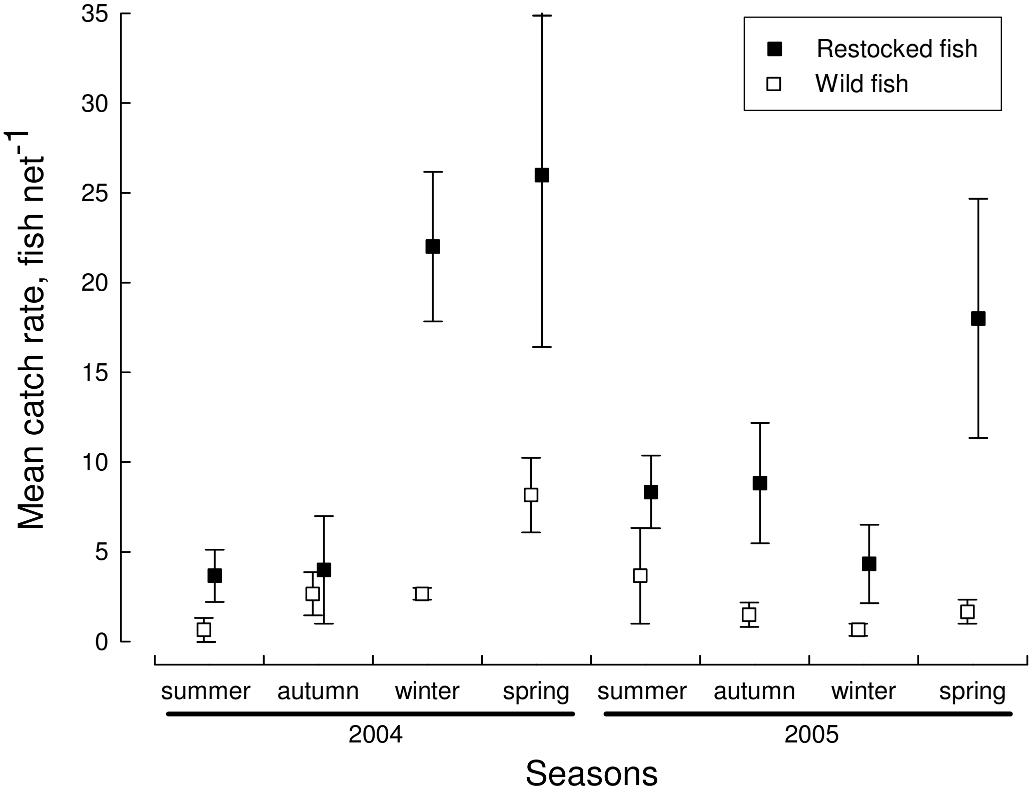

Figure 3.13 Mean catch rate $\pm$ 1 SE of the total catch of wild and restocked *Acanthopagrus butcheri* in 20m long gill nets set for 12h in the Blackwood River Estuary in each consecutive season between 2004 and 2005.

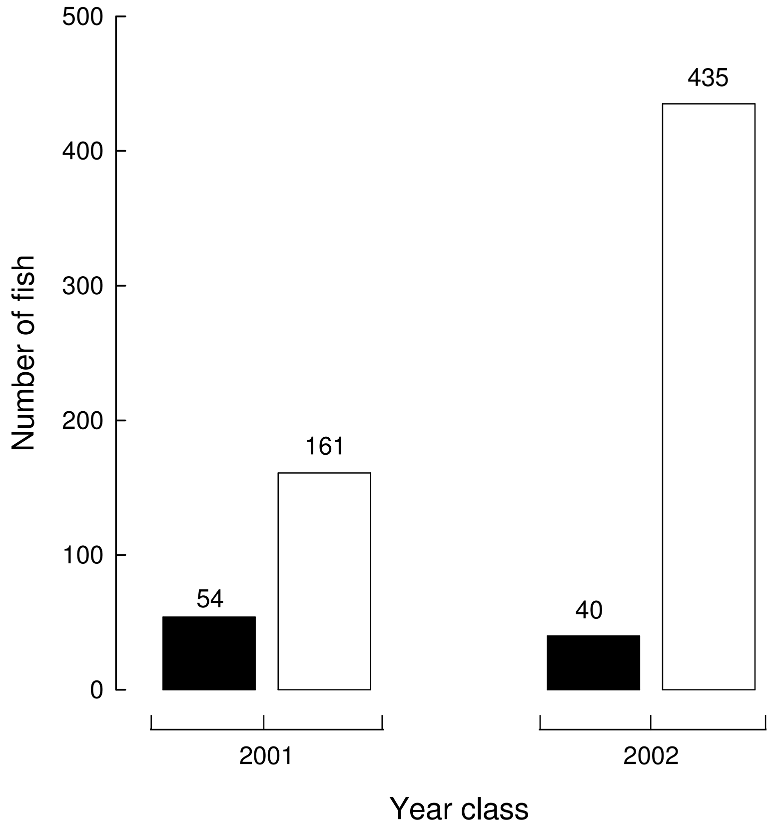

Figure 3.14 Number of wild (black histograms) and restocked (open histograms) *Acanthopagrus butcheri* of the 2001 and 2002 year classes caught between 2000 and 2005. Numbers refer to sample size.

3.3.8 *Yield, egg and spawning potential ratio per recruit*

Yield per recruit (YPR) analysis for wild *A. butcheri*, which assumed that fish were fully recruited to the fishery at *c*. 3 years of age, *i.e.* the age at which they are estimated to reach minimum legal length (MLL), indicate that YPR will continue to increase as F increases from 0 year^{-1}, to reach a maximum, F_{max}, when F equals 0.72 y^{-1} (Fig. 3.15a). The level of F which results in the slope of the yield-per-recruit curve equating to one-tenth of the slope of the curve at its origin, $F_{0.1}$, was 0.19 y^{-1}. As fishing mortality increases, the egg per recruit (EPR) for wild *A. butcheri* is predicted to undergo a decline exponentially (Fig. 3.15b,c). The biological reference point, $F_{40\%SPR}$, which is the level of fishing mortality at which SPR is predicted to be reduced to 40% of that of the unfished stock, was estimated to be 0.16 y^{-1}.

3.3.9 *Costs*

The total cash and in-kind cost of this project (as itemised in the FRDC 2000/180 Project Agreement with Challenger TAFE), involving the culturing and restocking of 220,000 Black Bream in the Blackwood River Estuary, together with the substantial amount associated with preliminary sampling, a range of research activities and monitoring, was $633,810. However, the cost solely of culturing and releasing Black Bream into this estuary was only $82,000, *i.e.* $0.37 per released fish (Table 3.5). Furthermore, the cost of each stocked fish that was estimated to survive to reach the MLL of 250 mm amounted to only $1.60 (Table 3.5). The cost that would be incurred if the otoliths of the stocked fish were to be stained with ALC to facilitate monitoring (two annual sampling trips to a range of carefully selected sites) of the progress of the stocked fish is also provided in Table 3.5.

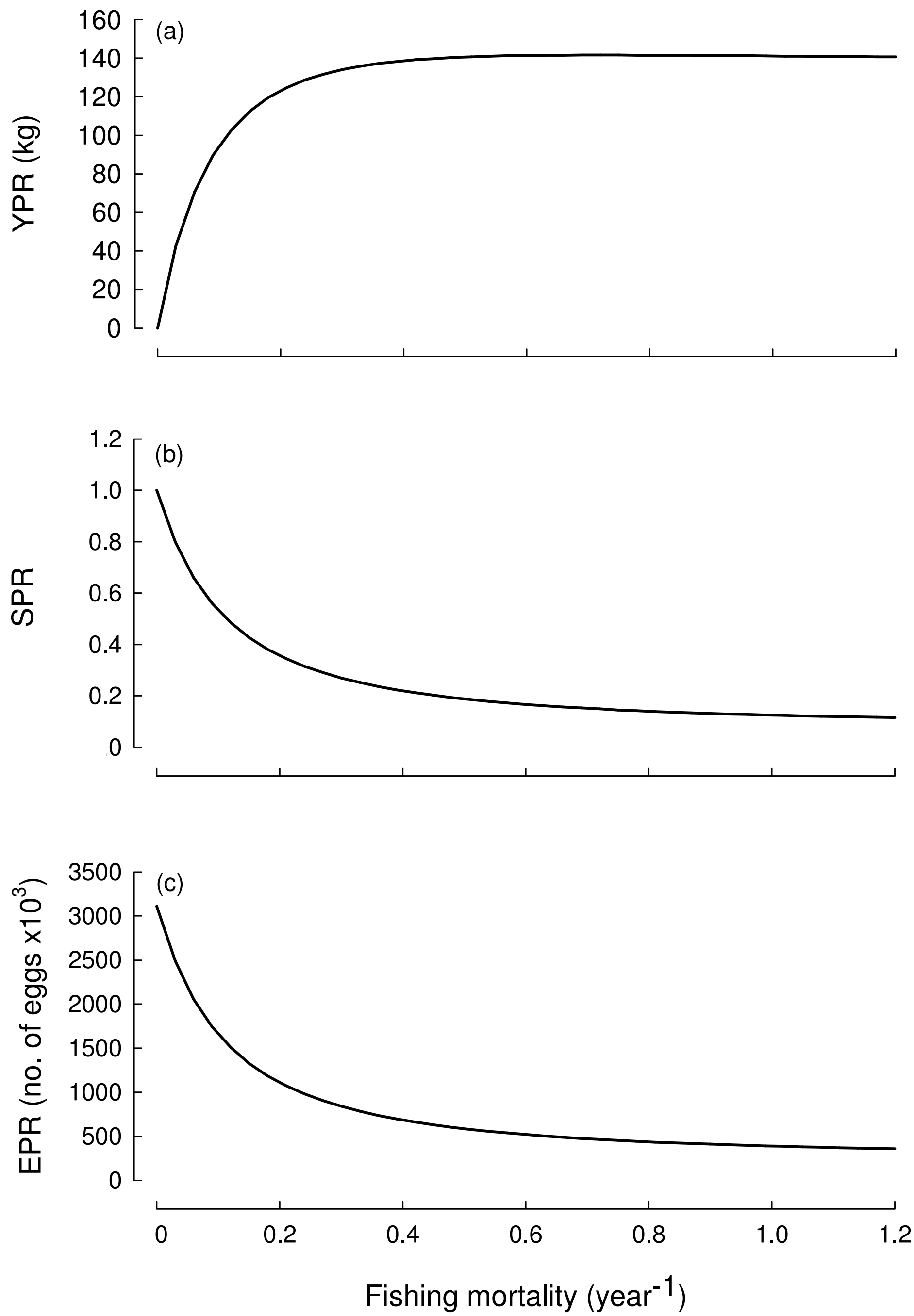

Figure 3.15 Effect of different levels of fishing mortality on (a) yield per recruit, (b) yield per recruit and (c) spawning potential ratio for wild *Acanthopagrus butcheri* from the Blackwood River Estuary.

Table 3.5 Costs involved in culturing the 220,000 Black Bream that were restocked in the Blackwood River Estuary, and the cost per recruit for those that were estimated to survive to reach the MLL. The additional costs of staining and monitoring that would be required, if the restocking was to be accompanied by a monitoring program, are included at the bottom of the table.

	Total cost ($)	Cost per released fish ($)	Cost per recruit ($)
Capture and maintenance of brood stock	5,000	0.02	0.13
Production and release of juveniles	77,000	0.35	1.99
Sub-total (excluding staining and monitoring)	**82,000**	**0.37**	**1.60**
Staining of otoliths with ALC	17,000	0.08	0.44
2 monitoring trips year^{-1}	6,000	0.03	0.15
TOTAL	**105,000**	**0.48**	**2.05**

3.3.10 *Tag returns and recreational survey*

A total of 57 fish with t-bar tags have been returned by recreational fishers, of which just over half were caught in the uppermost region of the upper Blackwood River Estuary. The majority of recaptures, *i.e.* 52%, occurred during autumn.

Unfortunately, there were only eleven responses to the questionnaires that were sent to the 57 fishers who had reported having captured external t-bar tagged fish between mid-2003 and 2005 (Appendix 2). Thus, extreme caution must be exercised in drawing conclusions from these limited numbers of responses.

3.4 DISCUSSION

3.4.1 *Use of alizarin complexone for staining otoliths*

This study showed that the purple-pink colour produced in the otoliths of Black Bream by ALC was still visible *c.* 3.5 years after those otoliths had been stained and these otolith-tagged fish had been released into the estuary. The ability to detect this stain in the otoliths for such a long period demonstrates that otolith staining by this chemical provides an excellent method for enabling stocked Black Bream to be identified and thus facilitate the tracking of their biological performance. The possibility that the stain will persist for a further substantial period is supported by the fact that the stain in the otoliths of the ocellate puffer *Takifuga rubripes* was still detectable more than five years after the otoliths had been stained with ALC (Matsumura, 2005). In studies conducted in eastern Australia, the stains were still visible in Rainbow Trout and Mulloway *Agyrosomus japonicus*, that were harvested 150 and 120 days, respectively, after treatment with ALC (van Der Walt and Faragher, 2003; Taylor *et al.*, 2005a). However, the mark produced by ALC in the otoliths of the turbot *Scophthalmus maximus* had became so faint seven months after staining that it was necessary to cut or polish the otoliths to detect that mark (Iglesias and Rodríguez-Ojea, 1997). This fading of the mark was due to the otoliths having been stained during the larval stage, rather than in juveniles as in our study, and thus, by seven months, for the otoliths to have increased greatly in thickness.

3.4.2 *Growth of restocked* vs *wild fish*

Comparisons between the mean lengths of sequential age classes of otolith-tagged Black Bream in the Blackwood River Estuary with those of the corresponding age classes of wild fish demonstrate that stocked fish did not grow quite as rapidly as wild fish in this estuary. On the basis of von Bertalanffy growth curves, stocked Black Bream in the Blackwood River Estuary had reached a length of *c*. 220 mm by the end of their third year of life, whereas by that age, wild fish had attained a slightly greater length of *c*. 245 mm. The stocked fish in the Blackwood River estuary grew faster, however, than the wild stocks of Black Bream in the Wellstead, Nornalup-Walpole and Moore River estuaries of Western Australia. This point is illustrated by the fact that, by the end of the third year of life, they had attained lengths of only *c*. 205, 160 and 145 mm, respectively, in these estuaries, compared with *c*. 220 in the Blackwood River Estuary (Sarre and

Potter, 2000). Thus, cultured Black Bream grow relatively well in the Blackwood River Estuary.

A number of factors could have contributed to the slower growth of stocked than wild fish, most of which are probably associated with hatchery rearing (*e.g.* Kellison *et al.*, 2000; Svåsand, 2004) and are not necessarily mutually exclusive. It would appear particularly relevant, for example, that, at 20-35 days, the juveniles were weaned from a diet of live, hatchery-produced zooplankton to one of formulated pellets *ad libitum* on which they remained for about five months prior to their release into the estuary. These juveniles were thus fed a diet of low diversity and did not have to forage or compete for food, as do individuals of the wild stock. Furthermore, the juveniles of fish species cultured under hatchery conditions using artificial diets often accumulate large amounts of fat in their livers, which reduces the ability of these fish to cope with stress and probably lowers their vigour (Coz-Rakovac *et al.*, 2002; Smith *et al.*, 2003). It is also relevant that, as the survival rate of the larvae and young juveniles grown under culture conditions was high, it would presumably have exceeded that which occurs under natural conditions in the estuary. Furthermore, while in the hatchery, the juveniles would not have been exposed to predators and thus to the type of selection pressures which, in the estuary, would have favoured the survival of individuals best adapted to coping with the far more rigorous conditions of the natural environment. It is also possible that the number of individuals used as brood stock in the hatchery was insufficient to produce progeny with a level of genetic diversity that approached that of the wild population. Consequently, it is suggested that, for one or more of the above reasons, the stocked fish would have been less adapted to compete for food when released into the Blackwood River Estuary.

The possibility that the process of staining their otoliths by placing cultured juveniles in a strong concentration of ALC influences the subsequent growth of these fish when released into an estuary cannot be excluded. It is relevant, however, that reared, alizarin-marked turbot *Psetta maxima* displayed similar growth to that of wild fish (Støttrup *et al.*, 2002) and that, on the basis of a five year study, the tagging of the otoliths of *T. rubripes* with ALC did not have a detrimental influence on the growth of this species (Matsumara, 2005). Such tagging was also found to have no detectable effect on either growth or mortality of other species (see Taylor *et al.*, 2005b). Although the stocked fish did not grow as rapidly as wild fish in the Blackwood River Estuary, their rate of growth exceeds that of wild Black Bream in some other estuaries. For example, on the basis of von Bertalanffy growth curves, the otolith-tagged Black Bream in the Blackwood River Estuary reached a length of *c*. 220 mm at the end of their third year of life, whereas those of natural populations in the Wellstead, Nornalup-Walpole and Moore River estuaries had attained lengths of only 207, 161 and 146 mm at the that age (Sarre and Potter, 2000). Thus, from a growth perspective, cultured Black Bream still grow relatively well in the Blackwood River Estuary.

3.4.3 *Gonadal development of restocked* vs *wild fish*

The trends exhibited by the mean monthly GSIs and monthly prevalences of the different gonadal stages demonstrate that wild *A. butcheri* start becoming mature in mid-winter, when water temperatures are at their minima, and that they spawn mainly between late winter (August) and mid-summer (January), when water temperatures are rising from *c.* 12 to 24^{o} C. The above timing of the onset of maturation and the duration of spawning parallels closely that recorded for *A. butcheri* in the Swan River Estuary, 300 km to the north (Sarre and Potter, 1999). The success of Black Bream in the Blackwood River Estuary in the past and in many other south-western Australian estuaries can be attributed to its ability to spawn over both a wide range of salinities as well as temperatures. During the spawning season in the Blackwood River Estuary, the salinities ranged from a minimum of just over 1‰ throughout the water column to a maximum of 31 and 25‰ at the bottom and top of the water column, respectively. Indeed, in the normally-closed Wellstead Estuary, to the east of the Blackwood River Estuary, *A. butcheri* spawns in salinities in excess of 40‰ (Sarre and Potter, 1999).

Our data showed that, while maturity was attained by most 2 year old and all older females of the wild stock, this was not the case with most of the 2 year old and several of the 3 and 4 year old stocked females. Furthermore, in the case of males, the stocked fish attained maturity at a greater length than wild fish. Thus, the above difference in age at maturity is partly, but not entirely explained by the slower growth of stocked than wild fish. Other studies have indicated that hatchery-reared fish may have lower reproductive fitness than their wild counterparts (Fleming and Petersson, 2001). The genetic implications of the potential risk of unintentional selection of maladaptive traits during hatchery rearing and subsequent release of relatively large numbers of reared individuals to supplement a depleted stock need to be considered (Waples and Drake, 2004).

We have obtained support from the Western Australian Fish Foundation (WAFF) to sample the Blackwood River Estuary during the spring and early summer of 2006, when Black Bream will next spawn in this system, in order to determine whether some or all of the remaining stocked Black Bream progress through to maturity in that spawning season.

3.4.4 *Habitat and contribution of restocked Black Bream*

The very low numbers of Black Bream caught by seine netting nine sites in nearshore, shallow waters of the Blackwood River Estuary at regular intervals during 2000, 2001 and 2002 contrasts with the substantial catches obtained using this method in the Blackwood River Estuary during 1974 and 1975 (Lenanton, 1977). It also contrasts with the substantial catches obtained for this species in comparable habitats in other estuaries in south-western Australia, *e.g.* Moore River Estuary and Swan River Estuary (Young *et al*., 1997; Sarre and Potter, 2000). This paucity of Black Bream in the nearshore shallows of the Blackwood River Estuary is consistent with the indications that the abundance of the stock of this species in this estuary has declined. However, as the juveniles of Black Bream occupy nearshore waters, it also clearly reflects the fact that the recruitment of the juveniles of the 2000, 2001 and 2002 year classes was very poor. Certainly, the data shown in Figure 3.3 provide strong circumstantial evidence that the recruitment of some earlier year classes was stronger than those of 2000, 2001 and 2002.

The large contributions made by stocked Black Bream to the 2001 and 2002 year classes of all individuals of this species in the Blackwood River Estuary provide strong support for the view that the introduction of cultured individuals of this species can enhance the abundance of the stock of this sparid in this system. However, it should be recognised that this high contribution also reflects the very poor recruitment of the 2001 and 2002 year classes compared with some earlier years.

The data derived as a result of the release and recapture of the t-bar tagged Black Bream, when considered in the context of concomitant captures of fish that were otolith-tagged and released 12 months earlier, indicated that *c*. 35% of the stocked fish survived the first 12 months following their release. This relatively high survival of fish from *c*. 6 to 18 months in age is almost certainly related, in part, to the fact that the stocked juveniles had already reached *c*. 50 mm by the time they were released and consequently would have been less prone to predation than if they had been released at an earlier stage.

3.4.5 *Cost and benefit analysis*

The restocking of cultured fish incurs a substantial cost. Our study was designed and conducted in a manner that has allowed a realistic estimate to be made of the cost that was incurred in restocking and monitoring the progress of cultured Black Bream in the Blackwood River Estuary during this study. The total cost per legal-sized recruit to the fishery in this project was approximately $16 (based on the cash and in-kind components of this FRDC project agreement). However, this included the cash and in-kind costs for the following additional research components that would not constitute part of a routine restocking programme in the future. (1) Preliminary sampling of the wild stock, (2) the extensive trials aimed at selecting and trialling a stain for otoliths, and (3) the time-consuming collection and analysis of data required to compare the performance of wild and stocked fish over a period of four years. When these expenses are excluded, the cost per legal-sized recruit declines to only $1.60 (Table 3.5), *i.e.* the base cost per individual for hatching, rearing and releasing 220,000 Black Bream into the estuary.

Taylor et al. (2005b) have recognised that *A. butcheri* is an ideal candidate for stock enhancement because it is a common resident of estuaries and heavily targeted and is thus likely to return a high yield on investment. At the same time, these workers state that this species suffers limitations through not demonstrably being recruitment limited and by taking > 5 years to reach legal size. However, year class strength does vary in some south-western Australian estuaries (Sarre and Potter, 2000) and thus the stocks in such systems would benefit from restocking. Furthermore, in both the Blackwood River Estuary and Swan River Estuary to the north (Sarre and Potter, 2000) the wild stocks, on average, reach the minimum legal length for retention (MLL) at 3 years of age, and a substantial number of the stocked fish in the former estuary had attained the MLL at 3.5 to 4 years of age. Thus, although as pointed out above, the growth of stocked *A. butcheri* was not as rapid as that of wild Black Bream, it was still sufficiently substantial to exceed that of wild *A. butcheri* in other estuaries.

Our calculations indicated that *c*. 16% of the stocked fish that ultimately reach the MLL and thus become vulnerable to capture by the fishery are expected to die each year from natural causes. Thus, those that don't die from such causes will either be caught by fishers or remain available to contribute to spawning at the end of the year and have the potential to remain available to fishers in subsequent years. There is thus a cumulative impact derived from restocking. In years in which recruitment, derived from the wild stock, is naturally low, the supply of fish available to fishers would be evened out through the release of cultured fish, thereby adding to the benefit of all fishers of the Blackwood River Estuary. The addition of stocked fish would help maintain adequate numbers of spawning fish, thereby improving the sustainability of the stock. The benefits to the local community will be substantial due to the flow-on effects of attracting greater numbers of the populace to the Blackwood River Estuary. There will be strong political support for restocking by recreational fishers as they will see it as a positive move to counter the perceived decline in the abundance of Black Bream in this estuary. Furthermore, the cost in the future of enhancing, through culture, the stock of Black Bream the Blackwood River Estuary, *i.e.* \$1.60, is very reasonable, especially as some stocked fish will have the potential of reproducing and contributing to future generations.

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5 BENEFITS AND ADOPTION

This study has demonstrated the following.

(1) Alizarin complexone (ALC) can be used to stain the otoliths of juveniles of Black Bream.

(2) This readily-visible stain persists in the otoliths of Black Bream for at least 3.5 years, thus enabling otolith-tagged, stocked fish to be identified in an estuary for a very substantial period of time.

(3) The retention of the stain ALC by the otoliths of Black Bream enables the performance, *i.e.* growth and gonadal development, of the cultured individuals of this species in the estuary to be tracked and compared quantitatively with those of the wild stock. Such comparisons demonstrated differences between the performance of stocked and wild fish.

(4) A very encouraging proportion of Black Bream, stocked in an estuary as small juveniles, can survive to reach the minimum legal length for retention of 250 mm for this species.

(5) Further studies are required to elucidate the basis for stocked fish not performing at quite the same level as individuals of the wild population, even though the brood stock used to produce the juveniles for restocking the Blackwood River Estuary were collected from that estuary.

(6) The restocking of juveniles of Black Bream and other species in an estuary has considerable potential for counteracting the effects of the depletion of the stocks of such species in that estuary.

(7) Our study provides a basis for refining the approaches for culturing, marking, releasing and monitoring the individuals of an estuarine fish species *sensu stricto* in an estuary.

(8) The ease and relatively low cost estimated for culturing Black Bream, the hardiness of this species and its restriction to its natal estuary make the restocking of Black Bream an economically viable and valid proposition. Restocking provides a further tool for fisheries managers to use to sustain the stocks of Black Bream in Australian estuaries.

6 FURTHER DEVELOPMENT

The results of this study were very encouraging as they demonstrated that the juveniles of Black Bream cultured from brood stock obtained from an estuary could be introduced into an estuary and survive, grow and often reach maturity in the ensuing 3.5 years. However, studies are clearly required to elucidate why, on average, these stocked fish did not grow as rapidly or mature so consistently as wild fish. Such studies should focus, in particular, on elucidating the ways in which hatchery techniques influence the performance of cultured fish after their release into the wild. Hatchery issues that need to be addressed include elucidating the optimal feeding, lighting and temperature regimes required for culturing juveniles that will perform as well as wild fish when stocked in an estuary. Work should also be undertaken to determine, from a genetic point of view, the best approaches to utilising brood stock. For example, in an ongoing, annual restocking programme, would it be better to use 100 brood stock over several years or to use smaller numbers of recently-caught brood stock each year?

Our data will be able to be used as the basis for more informed discussions regarding the practicality and efficacy of restocking estuaries with cultured fish and thus providing the potential for complementing traditional methods for managing exploited fish stocks. Our results have enabled us to apply successfully for local funds to undertake the following. (1) Sample Black Bream in the Blackwood River Estuary in the next spawning season (spring 2006) to determine whether a substantial proportion of stocked fish reach maturity in this period. (2) Apply our restocking approaches in the Swan River Estuary where Black Bream is also heavily targeted.

The data acquired during this study was presented at a stock enhancement workshop in Brisbane, funded by the FRDC, and will be presented at the Third

International Symposium on Sea-ranching and Stock-enhancement to be held in Seattle in September 2006. A workshop will also be held at Challenger TAFE in Fremantle during 2007 to inform Western Australian stakeholders of the results of our study and of those presented at the above international symposium. We are also in the process of preparing a paper for an international symposium on stock assessment and for publication in the international journal *Fisheries Science*.

7 PLANNED OUTCOMES

This study has achieved the following planned outcomes as given in B6 of the original project application.

(1) An increase in the abundance of Black Bream in the Blackwood River Estuary.

(2) An ability to assess the economical viability of the type of restocking programme undertaken during the current study.

(3) Develop sound quantitative data that will inform managers and the public of the practicality, issues and problems associated with the type of restocking programme undertaken during the current study. These issues are considered in detail in this final report.

8 CONCLUSIONS

- Extensive sampling of the Blackwood River Estuary during the first year of this study yielded far lower numbers of Black Bream than had been caught during a detailed study of the fish fauna of this estuary in the 1970s (Lenanton, 1977). This indicated that the abundance of Black Bream in the Blackwood River Estuary had declined during recent years, a view consistent with the anecdotal reports of a commercial fisher who has fished Black Bream in this estuary for more than three decades. Because of the apparent decline of the Black Bream stock in the Blackwood River Estuary, we were encouraged to initiate a trial restocking of this species in this estuary.
- Work carried out on the wild population prior to introducing cultured fish demonstrated that Black Bream largely occupy the riverine component of the Blackwood River Estuary and particularly its upper reaches. The abundance in those upper reaches is greatest in spring. As spawning occurs in spring, and the samples from these upper reaches contained many maturing, mature and spent Black Bream, it is concluded that as individuals of *A. butcheri* migrate some distance upstream as they mature and spawn mainly in those more distal waters of the estuary.
- Alizarin complexone was the most effective of a number of chemicals for staining the otoliths of Black Bream. Optimal staining is achieved by immersion of fish for 24 h in a concentration of alizarin complexone of 30 g L^{-1} and at density of fish equivalent to 5 g L^{-1}.
- As the mark, produced on the otoliths by alizarin complexone, was still visible to the naked eye 3.5 years later, fish that have been tagged using this stain can be used to trace the progress of hatchery-reared fish for at least this period following their release into the wild.

- The wild population of Black Bream in the Blackwood River Estuary grow faster than hatchery-reared Black Bream stocked in this estuary, even though those Black Bream were cultured from broodstock collected from that same estuary. The growth of the stocked Black Bream was, however, still substantial.
- Stocked Black Bream reach maturity later than wild Black Bream.
- The stocked Black Bream, that were cultured and released in 2001 and 2002, survived well and contributed 75 and 92% to the total number of individuals of the 2001 and 2002 year classes, respectively, that we caught.
- The total cost of this project, involving the restocking of Black Bream in the Blackwood River Estuary and the substantial amount associated with preliminary sampling, a range of research activities and monitoring was $633,810. However, the cost solely of culturing and releasing Black Bream into this estuary was only $82,000, *i.e.* $0.37 per released fish.
- In summary, this study has demonstrated that the abundance of Black Bream in the Blackwood River Estuary was enhanced through the introduction of cultured fish. The Black Bream is a particularly suitable candidate for restocking estuaries in south-western Australia as this species is confined to these systems and thus the likelihood of any such stocked fish colonizing other estuaries is remote. The ease and relatively low cost of culturing Black Bream, the hardiness of this species and its restriction to its natal estuary make the restocking of Black Bream an economically viable and valid proposition, when a stock of this species has become highly depleted. Thus, such restocking provides, in addition to traditional approaches, a further tool for fisheries managers to use to sustain the stocks of Black Bream in Australian estuaries.

9 APPENDICES

APPENDIX 1 – WinBUGS code

```
# Model parameters are Pmax, TruL50, d, Pmaxm, TruL50m, dm,
# Pmaxf, TruL50f, df, and derived parameters are L50, L95, L50m, L95m, L50f and L95f.
# TruL50, TruL50m and TruL50f represent the lengths at which 50% of the
# population has attained maturity. L50 and L95 represent the lengths at which
# 50 and 95% of Pmax of the fish have attained maturity, where L50<TruL50
# if Pmax<1. Similarly, L50m and L95m represent the lengths at which 50 and 95%
# of Pmaxm of the males have attained maturity, while L50f and L95f represent
# the lengths at which 50 and 95% of Pmaxf of the females have attained maturity.
# The parameters d, dm and df are the differences d=L95-L50, dm=L95m-L50m and
# df=L95f-L50f.
model logistic
{
  for (i in 1:N)
  {
    # Calculate the expected probability associated with the value L[i]
    # logit(p[i]) <- alpha + beta*L[i]
    p[i] <- Pmax/(1+exp(-log(19.)*(L[i]-L50)/d))

    # Ensure that this probability lies between 0 and 1, thereby
    # ensuring that WinBugs does not crash.
    newp[i] <- max(0, min(1, p[i]))

    # Specify the distribution associated with the maturity classification mat[i]
    mat[i] ~ dbern(newp[i])
  }

  for (j in 1:N)
  {
    # Create a copy of the maturity status
    mmat[j]  <- mat[j]

    # Set the parameters for this fish to those for either males or females
    # depending on the sex of the individual. The formulae below
    # set LL50[] and dd[] to L50f and L95f-L50f if sex = 0, and to
    # L50m and L95m-L50m if sex = 1.
    LL50[j] <- step(sex[j]-1) * L50m + (1 - step(sex[j]-1)) * L50f
    dd[j] <- step(sex[j]-1) * dm + (1 - step(sex[j]-1)) * df
    LLPmax[j] <- step(sex[j]-1) * Pmaxm + (1 - step(sex[j]-1)) * Pmaxf

    # Calculate the expected probability of maturity for this fish,
    # based on its length and sex.
    ppp[j] <- LLPmax[j] / (1 + exp(-log(19) * (L[j] - LL50[j]) / dd[j]))

    # Ensure that this probability lies between 0 and 1, thereby
    # ensuring that WinBugs does not crash.
    newppp[j] <- max(0, min(1, ppp[j]))

    # Specify the distribution associated with the maturity classification mmat[j]
    mmat[j] ~ dbern(newppp[j])
  }

  # Calculate the probability of maturity at specified, equally-spaced lengths,
  # for use when plotting
  for (j in 1:45)
  {
     LL[j] <- 100 + (j - 1) * 10
     # Calculate probability if a common logistic equation describes the relationship
     # between the probability that a fish is mature and the length of that fish
     pp[j] <- Pmax/(1+exp(-log(19.)*(LL[j]-L50)/d))
     # Calculate probability if the relationship
     # between the probability that a male is mature and the length of that male
     # is described by a different curve than that of the females
     ppm[j] <- Pmax/(1+exp(-log(19.)*(LL[j]-L50m)/(L95m - L50m)))
```

```
 # Calculate probability if the relationship
      # between the probability that a female is mature and the length of that female
      # is described by a different curve than that of the males
      ppf[j] <- Pmax/(1+exp(-log(19.)*(LL[j]-L50f)/(L95f - L50f)))
  }

  # Prior probabilities of the parameters of the logistic model
  TruL50 ~ dnorm(150., 0.00001)
  d ~ dnorm(100.,0.0000001)I(0.001,)
  Pmax ~ dnorm(1., 0.00001)I(0.5,1.)

  TruL50m ~ dnorm(150., 0.00001)
  dm ~ dnorm(100.,0.0000001)I(0.001,)
  Pmaxm ~ dnorm(1., 0.0001)I(0.5,1.)

  TruL50f ~ dnorm(150., 0.00001)
  df ~ dnorm(100.,0.0000001)I(0.001,)
  Pmaxf ~ dnorm(1., 0.0001)I(0.5,1.)

  # Derived parameters
  temp<-log(2.*Pmax-1)/log(19.)
  tempm<-log(2.*Pmaxm-1)/log(19.)
  tempf<-log(2.*Pmaxf-1)/log(19.)

  L50 <- TruL50+d*temp
  L50m <- TruL50m+dm*tempm
  L50f <- TruL50f+df*tempf

  L95 <- L50 + d
  L95m <- L50m + dm
  L95f <- L50f + df

  alpha <- -log(19.) * (L50 / d)
  beta <- log(19.) / d

  alpham <- -log(19.) * (L50m / dm)
  betam <- log(19.) / dm

  alphaf <- -log(19.) * (L50f / df)
  betaf <- log(19.) / df
}

# The maturity status mat[] assigned to each of 100 fish,  of lengths L[],
# where mat[i] = 0 if the fish is immature and mat[i] = 1 if mature.
# fem=0 and male=1
list(N=330)
L[] mat[] sex[]

END
```

APPENDIX 2 – Responses of the eleven of the fifty seven fishers who replied to the questionnaire that they were sent when they had informed us that they had caught a t-bar tagged fish.

1. How often would you go on a fishing trip to the Blackwood River?
    - ≤ once per year - 27.3 %
    - 2-5 per year - 27.3 %
    - > 5 per year - 45.4 %

2. On how many days would you fish on each trip to the Blackwood River?
    - Once - 9.1 %
    - 2-5 days - 45.4 %
    - everyday - 45.4 %

3. How many fish would you normally catch per day on each trip to the Blackwood River?
    - 1-2 - 9.1 %
    - 3-10 - 63.6 %
    - > 10 - 27.3 %

4. How much money would you spend per day on fishing tackle (including bait) for Black Bream fishing in the Shire of Augusta?
    - None - 27.3 %
    - ≤ $10 - 36.3 %
    - ≤ $20 - 27.3 %
    - ≤ $50 - 9.1 % -

5. How much money would you spend on accommodation per day in the Shire of Augusta?
    - None - 36.4 %
    - ≤ $20 - 27.3 %
    - ≤ $50 - 9.1 %
    - > $50 - 18.2 %

6. How much money would you spend on consumables (*i.e.* food & drink) per day in the Shire of Augusta?
    - None - 27.3 %
    - ≤ $20 - 27.3 %
    - ≤ $50 - 9.2 %
    - > $50 - 18.2 %

7. What dollar value would you place on catching a Black Bream from the Blackwood River Estuary?
    - Impossible to value - 72.8 %
    - ≤ $10 - 18.2 %

8. If you go fishing in the Blackwood River and do not catch a Black Bream are you less likely to return to the river to go fishing next time?

- Yes - 27.3 %
- No - 63.6 %
- Unsure - 9.1 %

9. What percentage of time of your trip would be spent fishing?
   - 0-20 % - 9.1 %
   - 20-50 % - 45.4 %
   - 100 % - 45.4 %

10. How long have you been fishing for Black Bream in the Blackwood River?
    - ≤ 1 year - 9.1 %
    - 1-4 years - 18.1 %
    - 5-10 years - 45.4 %
    - > 10 years - 45.4 %

11. Have you noticed any change in your catches over this period? For example do you now find it harder to catch Black Bream?
    - More fish / easier - 27.3 %
    - Less fish / harder - 9.1 %
    - No difference - 63.6 %

12. Would you continue to go to the Blackwood region if you knew you were unlikely to catch Black Bream?
    - Yes - 54.5 %
    - No - 36.4 %
    - Unsure - 9.1 %

13. Would you support the concept of regularly restocking the Blackwood River if it proved to be successful?
    - Yes - 100 %

APPENDIX 3 – Intellectual property

The FRDC's share of the intellectual property will be 48.7% based on Part C and A7 of the original project and project extension proposals, respectively.

APPENDIX 4 - Staff

Mr Greg Jenkins

Mr Daniel French

Professor Ian Potter

Dr Simon de Lestang

Professor Norm Hall

Dr Gavin Partridge

Dr Alex Hesp

Dr Gavin Sarre

Ms Carly Bruce