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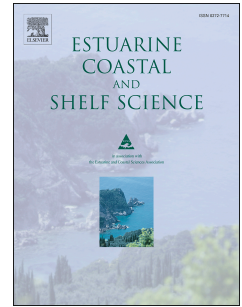
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Alan Cottingham, Norman G. Hall, Ian C. Potter



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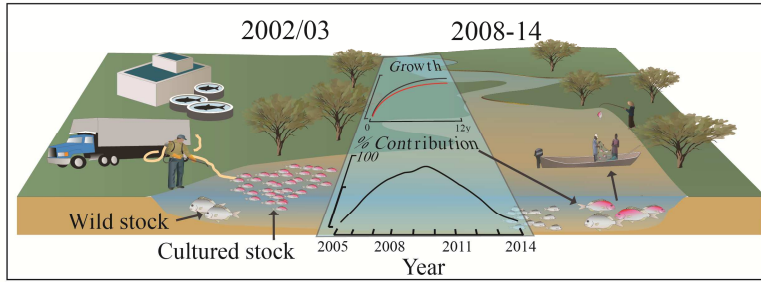
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Performance and contribution to commercial catches and egg production by restocked

Acanthopagrus butcheri (Sparidae) in an estuary.

Alan Cottingham^a, Norman G. Hall^a, Ian C. Potter^a

^a Centre for Fish and Fisheries Research, School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, Western Australia 6150.

*Corresponding author.

E-mail address: a.cottingham@murdoch.edu.au (A. Cottingham)

Centre for Fish and Fisheries Research, School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, Western Australia 6150.

Ph. +61 8 9239 8808

n.hall@murdoch.edu.au

i.potter@murdoch.edu.au

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Abstract

This study has explored whether the restocked fish of a species, which is confined to an estuary, perform as well as its wild stock, and has estimated their contribution to the commercial fishery and egg production. The biological characteristics of the 2001 and 2002 year classes of *Acanthopagrus butcheri*, which had been cultured and introduced into the Blackwood River in south-western Australia at seven and four months old, respectively, were thus determined from samples collected regularly between 2002 and 2014. The restocked fish could always be distinguished from the wild stock because their otoliths retained the pink coloration of the alizarin complexone with which they had been stained prior to release. Detailed analyses demonstrated the growth and maturity schedules of restocked fish were only slightly inferior to those of the wild stock and the mean gonad weights of these two groups did not differ significantly in any month. As increasing numbers of restocked *A. butcheri* attained the MLL of 250 mm for retention, their contribution to the commercial fishery increased from 6% in 2005 to 74% in 2010. That contribution subsequently declined to 39% in 2012 and 10% in 2014, due predominantly to the introduction of the very strong 2008 year class in the commercial catches, the first substantial recruitment into the population since 1999. Restocked fish were estimated as contributing ~55% to the eggs produced in 2008, suggesting that substantial numbers of the 2008 year class were derived from spawning by restocked fish. The results of this and a previous genetic study imply that restocking is an effective and appropriate way for replenishing stocks of an estuarine species such as *A. butcheri*, especially as its recruitment is highly episodic.

1. Introduction

Restocking is increasingly being used to restore fish stocks that have become depleted through over-exploitation and to meet the global demand for seafood (*e.g.* Bell *et al.*, 2006; Gomez & Mingo-Licuanan, 2006; Strøttup *et al.*, 2008; Loneragan *et al.*, 2013). The use of restocking overcomes the need to impose politically and socially less acceptable options for the recovery of a depleted stock, such as reducing size limits and total allowable catch and closing areas to fishing (Travis *et al.*, 1998; Bell *et al.*, 2006). The introduction of the cultured fish of a species can have, however, a negative effect on the wild population of that species through, for example, increasing inbreeding, introducing diseases and leading to a decrease in the abundance of wild fish (Einum & Fleming, 2001; Lorenzen *et al.*, 2012; Camp *et al.*, 2014).

Restocking is a particularly useful method of replenishing a depleted stock of those species that complete their life cycle within estuaries or inland waters and whose stocks cannot thus be augmented naturally by recruitment from outside that system (Lenanton *et al.*, 1999; Taylor *et al.*, 2005; Eby *et al.*, 2006; Ward, 2006). Furthermore, if restocking does have a deleterious impact on the genetic composition of a species in such water bodies, it is restricted to the population within the system into which the individuals were introduced (Potter *et al.*, 2008; Gardner *et al.*, 2013).

The Black Bream *Acanthopagrus butcheri* is an iconic recreational fish species in southern Australian estuaries and is fished commercially in some of these systems (Lenanton & Potter, 1987; Kailola *et al.*, 1993; Prior & Beckley, 2007; Jenkins *et al.*, 2010). This sparid, which can live for ~30 years (Morison *et al.*, 1998; Potter *et al.*, 2008), completes its life cycle within its natal estuary and typically spends most of the year in its upper reaches, where it spawns during spring and early summer (Potter & Hyndes, 1999; Sarre & Potter, 1999; Williams *et al.*, 2013).

Several stocks of *A. butcheri* in south-western Australia were reported by Lenanton *et al.* (1999) to have become depleted. It is not clear, however, whether increased fishing pressure and/or environmental changes were responsible for the decline in the abundance of this species in the Blackwood River Estuary, which supports a recreational fishery and a small commercial gill net fishery (Prior & Beckley, 2007; Gardner *et al.*, 2013). As the recruitment of *A. butcheri* into a population can be highly episodic, the recreational and commercial fisheries in some systems rely almost entirely on a few year classes (Morison *et al.*, 1998). Concern for the status of the stock of *A. butcheri* in the Blackwood River Estuary led to a study aimed at determining the efficacy of using restocking to replenish the population of this species in this system (Potter *et al.*, 2008; Gardner *et al.*, 2013). Brood stock from this estuary were thus used in 2001 and 2002 to culture juveniles, whose otoliths were stained with alizarin complexone to allow these fish to be distinguished from the wild stock in samples collected in years following their release (Potter *et al.*, 2008). This marking method has been successfully employed with other species, such as the Chum Salmon *Oncorhynchus keta* and the Pink Salmon *Oncorhynchus gorbusha* (Sato *et al.*, 2011) and the Black Rockfish *Sebastes schlegeli* (Nakagawa *et al.*, 2007).

Comparisons of data derived from sampling *A. butcheri* in the Blackwood River Estuary, between the introduction of the cultured fish in 2002 and 2003, and the years up to 2010, indicated that, in comparison with wild fish, the cultured fish, on average, had not grown quite as rapidly as the wild stock (Potter *et al.*, 2008; Gardner *et al.*, 2013). While there were indications that the restocked fish reached maturity at a slightly greater length and age, this was not tested statistically. Analyses of commercial gill net catches between 2005 and 2010 demonstrated that many restocked *A. butcheri* exceeded the minimum legal length (MLL) for retention of 250 mm and thus made an important contribution to the commercial fishery for this species in the Blackwood River Estuary and therefore, presumably, also to its recreational fishery (Gardner *et al.*, 2013). However, the age structure of *A. butcheri* in those

catches demonstrated that there had been little or no recruitment of year classes into the fishery since the introduction of the restocked fish.

Examination of *A. butcheri* in fishery-independent samples and commercial gill net catches obtained from the Blackwood River Estuary between 2012 and 2014, and thus after the completion of the previous study (Gardner *et al.*, 2013), demonstrated that the alizarin complexone stain was still clearly visible in the central region of the otoliths of restocked fish and had thus persisted for 12 to 13 years. It also demonstrated that, for the first time for many years, a new and strong cohort of *A. butcheri* had recruited into the fishery and which, from its age, could have been derived, in part, from restocked fish.

During the present study, biological and commercial catch data for *A. butcheri* in the Blackwood River Estuary were obtained for 2012 to 2014 and collated with those for 2002 to 2010 (Potter *et al.*, 2008; Gardner *et al.*, 2013). These composite data, covering a period of more than a decade, were used to compare statistically the biological performance of cultured and wild *A. butcheri* and explore the relative contributions of cultured and wild stock *A. butcheri* to commercial catches. Emphasis was placed on the following. (1) Extend comparisons of the growth of restocked and wild stock fish by including data for a further four years and explore more fully the basis for any differences between these two groups. (2) Determine whether the relationship between the lengths and ages at maturity of restocked fish differed significantly from those of the wild stock and, if so, the extent and basis for such differences. (3) Elucidate whether restocked fish are continuing to contribute substantially to the commercial catches of *A. butcheri* and the extent of any such contributions. (4) Ascertain whether restocked fish could have made a major contribution to total egg production by *A. butcheri* and if there was strong circumstantial evidence that the progeny of restocked fish could have contributed to commercial catches.

2. Materials and Methods

2.1. Collection and identification of restocked and wild *Acanthopagrus butcheri*

Full details of culturing *Acanthopagrus butcheri* from brood stock obtained from the Blackwood River Estuary, the staining of the otoliths of the resultant juveniles with alizarin complexone and the timing of the release of the fish cultured in 2001 and 2002 are given in Potter *et al.* (2008). In brief, the 70,000 juveniles cultured in 2001 were released into the Blackwood River Estuary in the winter of 2002 at ~ seven months old, whereas the 150,000 cultured in 2002 were released at ~ four months old in autumn 2003. As mentioned previously, the purple colour in the central region of the otoliths of the cultured *A. butcheri*, which was produced by the alizarin complexone stain, enabled these fish to be clearly identified in samples collected in subsequent years.

Samples of *A. butcheri* were collected from the Blackwood River Estuary in each year between 2000 and 2014, except for 2008 and 2011. They were obtained using fishery-independent methods at six sites in the upper and 'riverine' region of the estuary and/or from the commercial gill net fisher who fishes in this region of the estuary (Fig. 1). This region is stratified and undergoes pronounced seasonal changes in salinity, with annual bottom salinities typically ranging from ~5 to 30 and surface salinities from ~0 to 15 (Brearley, 2013). The fishery-independent methods employed a sunken composite gill net and a seine net. The gill net typically comprised eight panels, each 20 m long and 2 m high and with different mesh sizes, ranging in approximately equal intervals from 35 to 127 mm. The seine net was 41.5 m long and contained a 1.5 m bunt made of 9 mm mesh and two 20 m long wings comprising 25 mm mesh. The sunken gill net of the commercial fisher was 20 m long and 2 m high and made of 105 mm stretched mesh. It was chosen by this fisher so that it essentially caught only *A. butcheri* with total lengths ≥ 250 mm, *i.e.* the minimum legal length (MLL) for retention. The commercial samples of *A. butcheri* were provided as frames, *i.e.* with fillets removed, but with their intact gonads. The reader is referred to Potter *et al.*

(2008) and Gardner *et al.* (2013) for further details of the sampling gear, locations and procedures.

2.2. Ageing and growth

Each *A. butcheri* caught by fishery-independent sampling and by the commercial fisher was measured to the nearest 1 mm (total length) and sexed through macroscopic examination of its gonads. The fish were aged using annually-formed growth zones in whole otoliths when the number of such zones in the otolith was ≤ 6 and in sectioned otoliths when it was > 6 (see Cottingham *et al.* (2014) for full details and rationale for this procedure). The ageing of each fish took into account the date of capture and a birth date of 1 November, the mid-point in the spawning period (see Results). In brief, the otoliths of each fish were removed and those of restocked fish identified through the presence of the purple alizarin complexone stain. von Bertalanffy growth curves were fitted to the lengths at age of the restocked and wild stock fish, which for both groups comprised the 2001 and 2002 year classes collectively, and to the lengths at age of individuals in the 2008 year class. Note, however, that while all fish caught by fishery independent methods were used, the commercially-caught fish were restricted to those > 5 years old, *i.e.* the minimum age by which the total length of all fish would be expected to have reached 250 mm (see Fig. 2 in Results), the MLL for retention of *A. butcheri*.

Although preliminary analyses, using likelihood-ratio tests, demonstrated that the von Bertalanffy growth curves of the females and males of both the restocked and wild stock of *A. butcheri* differed significantly ($P < 0.001$ and 0.01 , respectively), the differences between the lengths at age of the two sexes at all ages, in the cases of both the restocked and wild stock fish, were always less than 5%. As such differences are unlikely to be of major biological significance, the lengths at age for both sexes of the restocked fish and of the wild stock fish have been pooled for comparing the growth of these two groups of fish, an

approach adopted in a previous study of *A. butcheri* in the Blackwood River Estuary (Gardner *et al.*, 2013) and in studies on other species (*e.g.* White *et al.*, 2002; Hesp *et al.*, 2004). Likelihood-ratio tests were then used to determine whether the von Bertalanffy growth curves of the restocked and wild stock fish differed and, if so, the extent of any differences in lengths at age across the ages sampled. If the latter differences exceeded 5%, likelihood-ratio tests were then used to ascertain whether the growth coefficients, k , and/or the asymptotic lengths, L_{∞} , of the restocked and wild stock fish were statistically significant.

2.3. Reproductive biology

The gonads of each restocked and wild *A. butcheri* were examined macroscopically and allocated to one of the following maturity stages described by Sarre & Potter (1999), *i.e.* I/II = virgin/resting adult, III = developing, IV = maturing, V = prespawning, VI = spawning, VII = spent and VIII = recovering spent. Previous histological studies of ovaries had demonstrated that assignment of gonads to these stages on the basis of macroscopic appearance was appropriate (Sarre & Potter, 1999; Chuwen, 2009).

For determining the length and age at maturity, *A. butcheri* with gonads at stages I/II during the spawning period were considered immature, whereas those with gonads at stages III to VIII were considered mature, *i.e.* destined to spawn or had spawned during that period. This procedure was adopted after careful recent examination of the pattern of sequential change in gonadal stages in the months immediately prior to and during the spawning period in the Swan River Estuary (see Cottingham *et al.*, 2014). The designation of fish with gonad stages III and IV as mature leads to lower estimates of the L_{50} and A_{50} than when they are classified as immature, as in earlier studies of *A. butcheri* in the Blackwood River Estuary (Potter *et al.*, 2008; Gardner *et al.*, 2013) and other estuaries (Sarre & Potter, 1999). Note also that the reproductive schedules were restricted to data derived from samples collected by fishery independent methods and thus, in contrast to a previous study of *A. butcheri* in the

Blackwood River Estuary (Gardner *et al.*, 2013), did not include commercial samples as these were not always collected during the spawning period.

The gonad weights, in each calendar month, of the restocked and wild stock females \geq the corresponding L_{50} s at maturity (see Results) were standardised for length (231 mm) using ANCOVA. Prior to analyses, the total length and gonad weight for each individual was $\ln(x + 1)$ transformed, where 'ln' is the natural logarithm. To account for bias introduced with the logarithmic transformation, when $\ln(\hat{x})$ is back-transformed, the correction factor $\exp[\text{mean of squared residual}/2]$ was employed (Beauchamp & Olson, 1973).

Logistic regression analysis was used to determine, for both the restocked and wild stock, the probability P that a female or male of a given length and age possessed gonads at stages III-VIII during the spawning season in the Blackwood River Estuary, *i.e.* October, November and December. Likelihood-ratio tests were employed to determine whether the ogive relating maturity to length for both the females and males of restocked fish differed from that for the corresponding sex of wild stock fish and, if so, whether this was accompanied by differences in the values of the L_{50} and/or L_{95} , the lengths at which 50 and 95%, respectively, of fish were expected to be mature. The same approach was used to determine whether the ogives relating maturity to age of the restocked females and males differed from that of the corresponding sex of wild stock fish and, if so, whether this was accompanied by differences in the values of the A_{50} and/or A_{95} , the ages at which 50 and 95%, respectively, of fish were expected to be mature. The reader is referred to Cottingham *et al.* (2014) for details of the maximum likelihood method used to determine the relationship between the length and age at maturity and the Bayesian approach employed to determine the 95% credible limits.

Comparisons of the relationships between the lengths and the ages at maturity of the females and males of restocked fish (2001 and 2002 year classes collectively) with those of the wild stock employed data collected during the spawning period, with the data for wild

stock fish of the 1999 and 2000 year classes being added to those of the wild stock of the 2001 and 2002 year classes. This increased the number of fish in length and age classes of the 2001 and 2002 year classes of the wild stock that were poorly represented in, or absent from, samples collected during the spawning season.

The relative contributions of each year class of the wild stock and restocked *A. butcheri* to egg production in the Blackwood River Estuary in 2007 and 2009 were estimated using the lengths of individual fish in each year class in the commercial catches and the equation of Sarre & Potter (1999), which described the relationship between batch fecundity and the lengths of females in the Swan River Estuary. These two years were chosen for analysis because they straddled 2008, for which no commercial samples were available (see Results), and because the 2008 year class was very well represented in commercial samples from 2012-14, *i.e.* when they exceeded the MLL for *A. butcheri* of 250 mm. The total number of eggs produced (F) in 2007 and 2009 by each year class of the wild stock and restocked fish was thus calculated as $F_{yc,y} = \sum_{j=1}^{n_{yc,y}} \left[10^{-4.65+4.25 \log_{10} L_j} \right]$, where L_j is the total length of the j 'th fish, with the summation covering all $n_{yc,y}$ fish within each year class, yc , separately for both the restocked fish and wild stock in each year, y . This enabled the number of eggs produced by restocked and wild stock *A. butcheri* in commercial samples in 2007 and 2009 to be estimated. These data were then used to estimate the percentage contribution to total egg production by each year class of wild stock and restocked *A. butcheri* in 2007 and 2009 and thus the overall relative contributions by wild stock and restocked fish in those years. As the GSIs of females during the spawning period in the Swan River Estuary were similar to those in the other two estuaries that were sampled during the main part of that period (Sarre & Potter, 1999), the relationship between batch fecundity and length is considered unlikely to differ markedly among systems.

3. Results

During the 12 years of sampling since cultured fish were first introduced into the Blackwood River Estuary, *i.e.* from 2002 to 2014, a total of 1584 restocked fish were obtained from fishery-independent sampling and commercial gill net catches. These restocked fish comprised 193 individuals of the 2001 cohort and 1391 of the 2002 cohort.

3.1 Growth

The von Bertalanffy growth curve provided a good fit to the lengths at age of both the wild stock and restocked fish, derived in both cases from pooled data for the same two year classes collectively, *i.e.* 2001 and 2002 (Fig. 2a-c, Table 1). The growth curve for the restocked fish lay below and differed significantly from that of wild stock fish ($P < 0.001$). The differences between the lengths at age across all ages from 1 to 12 years ranged from 7 to 11%. While the growth coefficient (k) of 0.29 y^{-1} for restocked fish did not significantly differ ($P > 0.05$) from the 0.31 y^{-1} of wild stock fish, the L_{∞} of 323 mm for restocked fish was less than and differed significantly ($P < 0.01$) from the 350 mm of wild stock fish. The growth curve for the 2008 year class lay between those for the restocked and wild stock fish (Fig. 2c).

Based on the von Bertalanffy growth equation, the TLs of female restocked *A. butcheri* at two and six years of age were 179 and 278 mm, respectively, and thus less than the corresponding TLs of 198 and 306 mm for the wild stock.

3.2. Maturation

The mean monthly gonad weights of the females of both the restocked and wild stock fish rose to a sharp peak in October and then declined progressively in the following months (Fig. 3). The mean gonad weights of restocked and wild stock fish did not differ significantly ($P > 0.05$) in any month.

All wild stock females <179 mm and all restocked females <155 mm were immature, *i.e.* possessed gonad stages I/II (Fig. 4a,b). The wild stock females in the 180-199 mm and subsequent length classes were mature (Fig. 4a). The prevalence of mature restocked females increased from 25% in the 140-159 mm length class to 76% in the 180-199 mm length class, and all restocked females ≥ 240 mm were mature (Fig. 4b). The ogive describing the relationship between the proportion of mature restocked females and total length differed significantly from the corresponding ogive for wild stock females ($P < 0.01$, Fig. 4a-c, Table 2). The L_{50} at maturity of 174 mm for restocked females was greater than the 165 mm of wild stock females, but the difference was not significant ($P > 0.05$). The L_{95} at maturity of 224 mm for restocked females was far greater than the 168 mm for wild stock females and the difference was significant ($P < 0.001$, Fig. 4c).

All wild stock males <147 mm and all restocked males <146 mm were immature (Fig. 4d,e). While all wild stock males in the 160-179 mm and subsequent length classes were mature, about 25% of the restocked males in the 160-179 mm length class were immature (Fig. 4d,e).

The ogive describing the relationship between the proportion of mature restocked males and total length did not differ significantly from the corresponding ogive for wild stock males ($P > 0.05$, Fig. 4d-f, Table 2). Although the L_{50} of 154 mm and L_{95} of 177 mm at maturity for restocked males were both greater than the corresponding values of 149 and 159 mm, respectively, for wild stock males, the differences were likewise not significant ($P > 0.05$, Fig. 4f).

No wild stock or restocked females reached maturity by the end of its first year of life (Fig. 5a,b). While virtually all wild stock females had become mature by two years of age, only 54% of restocked females had become mature by that age and some restocked females had not become mature by 3 and 4 years of age.

The ogive describing the relationship between the proportions of mature restocked females and age was significantly different ($P < 0.001$) from the corresponding ogive for wild stock females (Fig. 5a-c, Table 2). The A_{50} for maturity of 2.1 years of restocked females was greater than the corresponding value of 1.7 years for wild stock females, but this difference was not significant ($P > 0.05$). The A_{95} for restocked females of 3.2 years was appreciably greater ($P < 0.001$) than the 2.0 years for wild stock females (Fig. 5c).

Some wild stock males attained maturity at the end of their first year of life and all became mature at the end of their second year of life, whereas no restocked males became mature at the end of their first year of life and nearly 20% were still immature at the end of their second year of life (Fig. 5d,e).

The ogive describing the relationship between the proportions of mature restocked males and age differed significantly ($P < 0.001$) from the ogive for wild stock males (Fig. 5d-f, Table 2). The A_{50} of 1.8 years and A_{95} of 2.3 years for restocked males were both greater than and differed significantly ($P < 0.001$) from the corresponding values of 1.2 and 1.6 years, respectively, for wild stock males (Fig. 5f).

3.3. Contribution of restocked fish to commercial catches

In any description of the length compositions of *A. butcheri* in commercial catches from the Blackwood River Estuary, it must be borne in mind that the stretched mesh size of the commercial gill net (105 mm) was chosen by the commercial fisher so that it essentially caught only *A. butcheri* with lengths ≥ 250 mm, the MLL for retention of this species. The commercial catches of *A. butcheri* in 2005 comprised almost exclusively wild stock fish, with their lengths ranging from 257 to 412 mm and producing a modal length class of 280-299 mm (Fig. 6a). The samples in 2005 did contain, however, two restocked fish of the 2002 year class and 14 restocked fish of the 2001 year class, whose lengths collectively ranged from 255 to 295 mm. In 2006, the overall modal length class decreased to 260-279 mm, which was

due to the recruitment into the fishery of appreciable numbers of restocked fish of the 2002 year class, whose lengths ranged from 253 to 325 mm. The modal length class of restocked fish, produced predominantly by the 2002 year class, increased from 260-279 mm in both 2006 and 2007 to 280-299 mm in 2009 and 2010. During this period, the lower end of the overall distribution of lengths was dominated by cultured fish, whose lengths were almost invariably <340 mm, whereas the upper end contained several wild fish with lengths extending up to 426 mm. In contrast to 2010, when virtually all fish with lengths <320 mm were restocked fish, the majority of *A. butcheri* <320 mm caught in 2012, and even more particularly in 2013 and 2014, were fish whose otoliths were not stained with alizarin complexone, and were thus spawned naturally in the wild (Fig. 6a).

The ages of *A. butcheri* in commercial catches in 2005, which comprised almost exclusively wild stock fish, ranged from 2 to 10 years old, with one fish 15 years old and another 18 years old (Fig. 6b). The samples in that year were dominated by the 5+ and to a lesser extent the 4+ age class, *i.e.* fish spawned in 1999 and 2000, respectively. The 1999 year class remained the most abundant year class of wild stock fish in the years up to 2010, being represented, for example, by substantial numbers of the 7+ age class in 2007 and of the 10+ age class in 2010. However, restocked fish, mainly of the 2002 year class, increasingly dominated the catches in this period, with their contributions to commercial catches increasing progressively from 32% in 2006 to 74% in 2010 (Fig. 6b).

Although there had been little or no recruitment of year classes of wild stock fish into the fishery in 2006 to 2010, this situation changed dramatically in 2012, when a strong new cohort, representing the 2008 year class, appeared in samples, at which time they were thus 3 years old (Fig. 6b). This strong recruitment was reflected in the marked increase in the prevalence of smaller fish in 2012 to 2014 (Fig. 6a). The 2008 year class became so strong in 2013 and 2014, when its individuals were 4 and 5 years old, respectively, that it dominated the age-frequency distributions for those years (Fig. 6b). The dominance of this year class in

2013 and 2014 accounts, in part, for the decline in the relative contribution of restocked fish from 74% in 2010 to 19% in 2013 and 10% in 2014 (Fig. 6b).

3.4. Egg production

On the basis of the relationship between fecundity and total length (Sarre & Potter, 1999) and the lengths and numbers of fish in each year class, the contribution to egg production by the 1999 year class of wild stock females was 25% in 2007 and 18% in 2009, in which years these fish were 8 and 10 years old, respectively (Fig. 7). These were the greatest contributions by any year class of wild stock females in both years and particularly in 2007. The contribution to total egg production by females of restocked fish was 54% in 2007 and 55% in 2009, due predominantly to that made by the 2002 year class. Thus, the restocked fish made a greater contribution than wild stock fish to total egg production in 2007 and 2009 (Fig. 7).

4. Discussion

Although the number of *A. butcheri* cultured in 2001 and released into the estuary in 2002 was nearly half that cultured in 2002 and released in 2003, *i.e.* 70,000 vs 150,000, only 6% of the restocked *A. butcheri* taken by commercial gill netting between 2005 and 2014 belonged to the 2001 cohort. The lower survival of the 2001 than 2002 cohort of cultured fish may have been due, in particular, to the former cohort having been released in winter, when freshwater discharge was high, whereas the latter cohort was released in autumn, when freshwater discharge was negligible (see Gardner *et al.*, 2013 for discussion of other possibilities).

4.1. Comparisons of the performance of restocked and wild fish

This study demonstrated that, in terms of growth and maturity schedules, restocked *A. butcheri* did not perform as well as its wild stock in the Blackwood River Estuary.

However, any consideration of the efficacy of using restocking as a tool for replenishing the depleted stock of this species in this system should take into account the extent of the differences and how the performance of cultured fish compares with that of this species in other estuaries. Thus, while the L_{∞} s of restocked and wild stock fish differed significantly, the difference was only 8% and the rates, k , at which the total lengths at age of restocked and wild stock fish approached their respective asymptotic lengths, were not significantly different. The fact that the growth curve, fitted to the lengths at age of the 2008 year class lay between that for the restocked and wild stock, suggests that the growth of this year class is intermediate, recognising, however, that the difference between the growth of the restocked and wild fish was relatively small.

On the basis of the von Bertalanffy growth equation, restocked fish took 4.3 years to reach the MLL of 250 mm for *A. butcheri* and thus longer than the 3.4 years taken by the wild stock. However, the MLL was reached at an earlier age by restocked fish in the Blackwood River Estuary than recorded by Sarre & Potter (2000) for the wild stock of this species in 1993-95 in two other south-western Australian estuaries (~6.7 and 7 years) and at a similar age in another estuary during that period (4.4 years). Restocked *A. butcheri* took longer to reach the MLL in the Blackwood River Estuary than in a fourth system, the Swan River Estuary, in that earlier period, *i.e.* ~2.8 years (Cottingham *et al.*, 2014).

Some restocked fish, and particularly their females, were still immature in the length and age classes at which all wild stock fish had become mature. This accounts for the L_{50} and A_{50} at maturity, and, to an even greater extent, the L_{95} and A_{95} , all being greater for restocked females than wild stock females, recognising that the differences were statistically significant only in the case of the L_{95} s and A_{95} s. Although restocked males matured at a greater length and age than wild stock males, the differences were statistically significant only for the A_{50} s and A_{95} s at maturity. Furthermore, restocked females attained maturity at an age very similar to the 1.9 years for *A. butcheri* in 1993-95 in the Swan River Estuary, ~300 km further north,

and at a slightly younger age than the 2.5 years estimated for that system in 2007-11 (Cottingham *et al.*, 2014).

Although the differences in growth and maturity schedules of restocked and wild *A. butcheri* in the Blackwood River Estuary were relatively small, they parallel the trend in other studies for the long-term performance of the cultured fish of a species to be inferior to that of its wild stock (Jonsson & Jonsson, 2006; Lorenzen *et al.*, 2012).

4.2. Contributions of restocked and wild fish to commercial catches and egg production

The progressive rise, in the relative contributions of restocked fish to the annual commercial catches of *A. butcheri*, from negligible levels in 2005 to about three-quarters in 2010 is attributable to two factors. First, through growth, the number of restocked fish that attained the MLL of 250 mm increased progressively each year. Second, the recruitment into the commercial fishery of all year classes of the wild stock of *A. butcheri*, except for the 1999 year class, was relatively low between 2005 and 2010, probably representing poor spawning success in earlier years. It is thus evident that restocked fish made a major contribution to the commercial catches for a number of years and thus, implicitly, also to those of recreational fishers.

The decline in the contribution of restocked fish to commercial catches in 2012 was largely due to an increase in the contribution of the 2008 year class. The subsequent increase in the proportion of the 2008 year class in 2013 and 2014 reflected an increase in the numbers of that year class that would have reached the MLL and a decline in the abundances of both the 2002 cohort of restocked fish and the 1999 cohort of wild stock fish as a result of natural and fishing mortality.

The question now arises as to whether the progeny of cultured fish could potentially have contributed to the substantial number of individuals that comprised the 2008 year class. On the basis of the A_{50} at maturity of ~2 years, the restocked *A. butcheri* of the 2002 year

class would, on average, have matured in 2004 and thus could potentially have spawned in that and subsequent years. However, as mentioned above, the age compositions in commercial catches imply that spawning success was very limited between 2000 and 2007. Thus, as the vast majority of wild fish in 2007 and 2009, and thus implicitly in 2008, were ≥ 8 years in age, the relatively strong spawning success in 2008 was presumably due to spawning by cultured fish (particularly of the 2002 year class) and/or wild stock fish or to interbreeding between these two groups.

Although commercial catch samples were not available in 2008, it is relevant that restocked fish were estimated to contribute 54 to 55% to the total egg production by legal-sized *A. butcheri* in the Blackwood River Estuary in 2007 and 2009. Restocked fish would thus presumably have made a similar contribution in 2008, the year in which recruitment into the population, and thus implicitly spawning success, was particularly strong.

The spawning success of the restocked individuals of some species has been shown to be inferior in certain respects from that of the wild stock of that species, due, for example, to differences in the timing of breeding and/or selection of suitable nest sites (Fleming & Petersson, 2001, McGinnity *et al.*, 2003; Weir *et al.*, 2004). However, as the monthly trends in ovarian weights and locations of capture were the same in restocked fish as wild stock fish, the two groups spawn at the same time and in the same region of the estuary. Furthermore, as discussed earlier, the differences in the reproductive schedules of the restocked and wild stock fish were relatively small. It is also relevant that the results of a thorough genetic study strongly indicated that the prevalence of inbreeding was not demonstrably greater among the restocked than wild stock *A. butcheri* (Gardner *et al.*, 2013).

In summary, this study has shown that the growth of restocked *A. butcheri* in the Blackwood River Estuary was only slightly less than that of wild fish likewise hatched in 2001 and 2002 and thus exposed to the same environmental conditions for the twelve to thirteen years since those cultured fish were introduced into this system. It also demonstrated

that an appreciable number of restocked fish were still present in the estuary, eleven years after their introduction to this system, and that all restocked fish that survived to five years of age reached maturity and therefore potentially could spawn and contribute to future generations of *A. butcheri* in this estuary. In addition, the restocked fish were shown to make a very substantial contribution to the commercial gill net fishery for this sparid in the Blackwood River Estuary in 2007-10. There is also strong circumstantial evidence that the cultured fish had contributed progeny that were caught by the commercial fisher for *A. butcheri* in the Blackwood River Estuary. The results of this study provide a rare example of a continuing investigation into the consequences of a fish restocking programme in an estuary and demonstrate that it is a worthwhile proposition to consider the use of restocking with appropriately cultured fish to replenish a stock of a species such as *A. butcheri*, whose individuals are confined to their natal estuary. *Acanthopagrus butcheri* is also a very good candidate for restocking in the Blackwood and some other estuaries as natural recruitment is so episodic in these systems (Morison *et al.*, 1998) and density dependent interactions between wild and restocked fish would be likely to be low.

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LIST OF FIGURES

Figure 1. Map showing the six sites at which *Acanthopagrus butcheri* was sampled in the Blackwood River Estuary by fishery-independent methods (open circles) and the stretch of the estuary in which the commercial gill net fisher typically operated. Arrow in insert map shows location of the Blackwood River Estuary in Western Australia.

Figure 2. von Bertalanffy growth curves fitted to the lengths at age of (a) wild stock and (b) restocked *Acanthopagrus butcheri* of the same year classes, *i.e.* 2001 and 2002, collectively, and (c) comparison of the growth curves shown in a and b for wild stock fish (long dashed line), restocked fish (continuous line) and the 2008 year class (short dashed line).

Figure 3. Mean gonad weights and 95% confidence intervals in sequential months for wild stock (closed circles) and restocked (open circles) females of *Acanthopagrus butcheri* with lengths $\geq L_{50}$ at maturity and which were caught by fishery-independent sampling in the Blackwood River Estuary between 2000 and 2014. Gonad weights have been standardised for fish of a common length using ANCOVA. Black bars, summer and winter; white bars, autumn and spring. Sample size for each month is shown.

Figure 4. Percentage frequency of occurrence, in sequential 20 mm length classes, of females and males of wild stock and restocked *Acanthopagrus butcheri* with immature (white bars) and mature gonads (grey bars), derived from samples collected during the spawning season of *A. butcheri*. Logistic curves (solid lines) and their 95% credible intervals (dotted lines) for the probability that a fish at a given length is mature are superimposed on Fig. 3a, b, d and e. Sample size for each length class is provided. Comparisons of logistic curves for wild stock (dashed line) and restocked (continuous line) females and males are shown in c and f.

Figure 5. Percentage frequency of occurrence, in sequential ages, of females and males of wild stock and restocked *Acanthopagrus butcheri* with immature (white bars) and mature gonads (grey bars), derived from samples collected during the spawning season of *A. butcheri*. Logistic curves (solid lines) and their 95% credible intervals (dotted lines) for the probability that a fish at a given age is mature are superimposed on 4a, b, d, e. Sample size for each length class is provided. Comparisons of logistic curves for wild stock (dashed line) and restocked (continuous line) females and males are shown in c and f.

Figure 6. a) Length and b) age-frequency distributions for samples of commercial gill net catches of *Acanthopagrus butcheri* in the Blackwood River Estuary between 2005 and 2014. White and black bars represent the 2001 and 2002 year classes of restocked fish, respectively, and the grey bars the wild fish, *i.e.* those whose otoliths did not contain the purple colouration of the alizarin stain that characterises those of cultured fish. Arrows track the 1999 year class of the wild stock and the 2002 year class of restocked fish, which were the dominant year classes between 2006 and 2010.

Figure 7. Percentage contributions made to egg production by the different year classes of wild stock and restocked *Acanthopagrus butcheri* in 2007 and 2009 and by the wild stock and restocked fish overall in those two years. Data were derived from the lengths of individuals of each age class in commercial gill net samples and employed the fecundity-length relationship for *A. butcheri* in Sarre & Potter (1999).

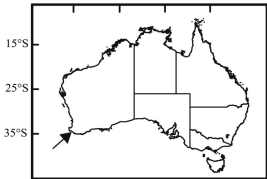
Table 1. von Bertalanffy growth parameters and their 95% confidence intervals for wild stock and restocked *Acanthopagrus butcheri* in the Blackwood River Estuary. L_{∞} , asymptotic length (mm); k , growth coefficient (year^{-1}); t_0 , hypothetical age at fish would have zero length (years). r^2 , coefficient of determination; n , number of fish in sample.

	L_{∞} (mm)	k (year^{-1})	t_0 (years)	r^2	n
Wild stock fish					
Estimate	350	0.31	-0.70	0.88	163
Lower	336	0.26	-1.01		
Upper	364	0.36	-0.40		
Restocked fish					
Estimate	323	0.29	-0.79	0.90	1321
Lower	318	0.26	-0.93		
Upper	328	0.30	-0.64		

Table 2. Estimates of total lengths (mm) and ages (years) and associated 95% credible limits at which 50% (L_{50} and A_{50} , respectively) and 95% (L_{95} and A_{95} , respectively) of the females and males of the wild stock and restocked *Acanthopagrus butcheri* were mature. ML = Maximum Likelihood. n is the number of fish.

	L_{50} (mm)	L_{95} (mm)	A_{50} (years)	A_{95} (years)	n
Wild stock fish					
Females					
ML Estimate	165	168	1.7	2.0	87
Lower	150	153	1.4	1.8	
Upper	179	196	1.9	2.2	
Males					
ML Estimate	149	159	1.2	1.6	133
Lower	141	138	1.1	1.4	
Upper	153	203	1.3	2.0	
Restocked fish					
Females					
ML Estimate	174	224	2.1	3.2	139
Lower	166	210	1.8	2.8	
Upper	184	245	2.2	3.9	
Males					
ML Estimate	154	177	1.8	2.3	176
Lower	146	168	1.6	2.2	
Upper	160	194	1.9	2.8	

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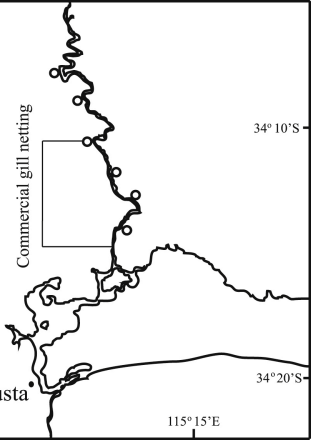
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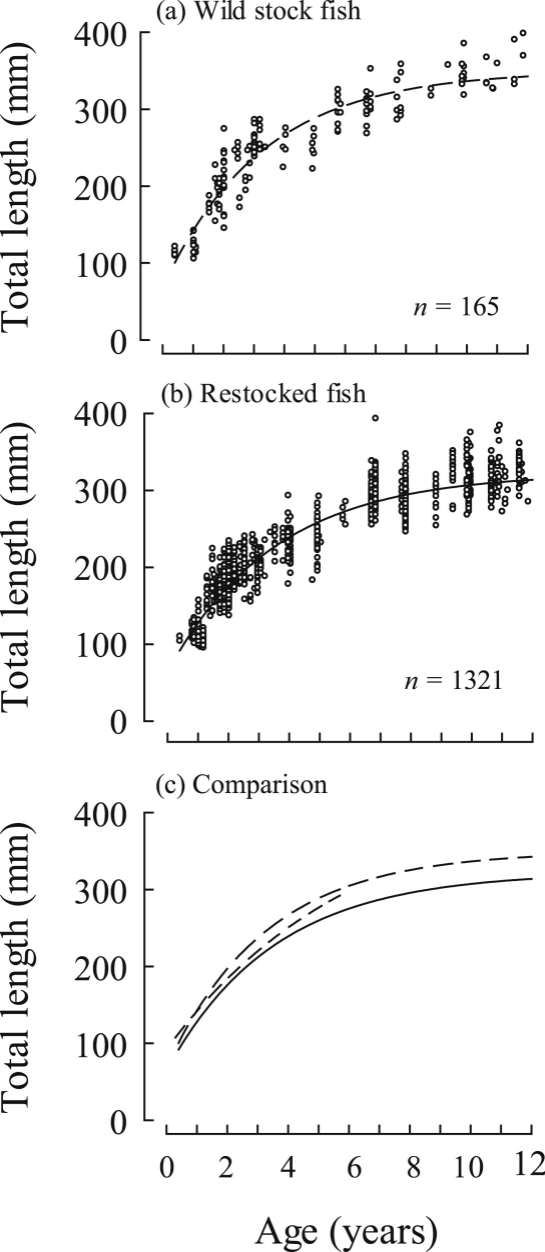
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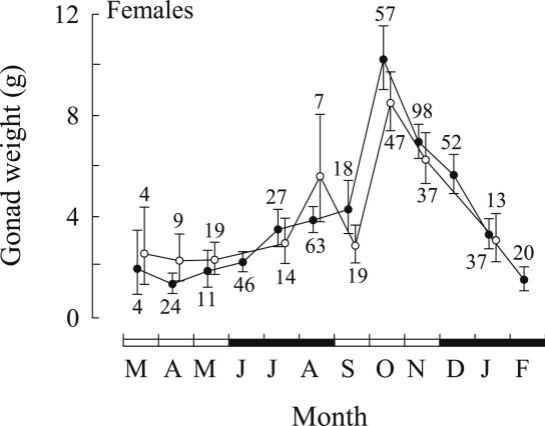
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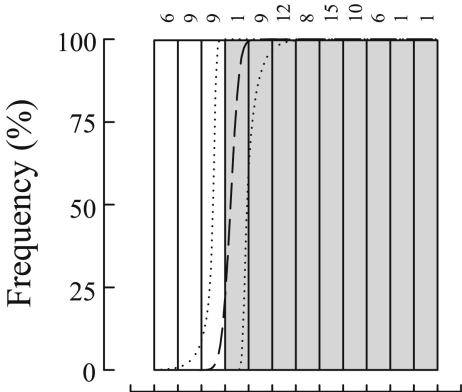
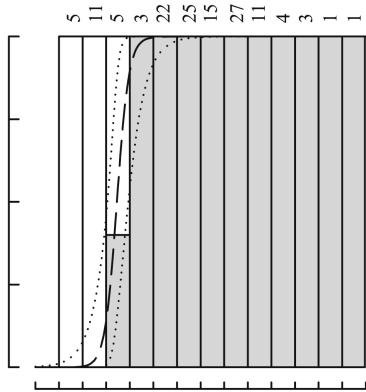
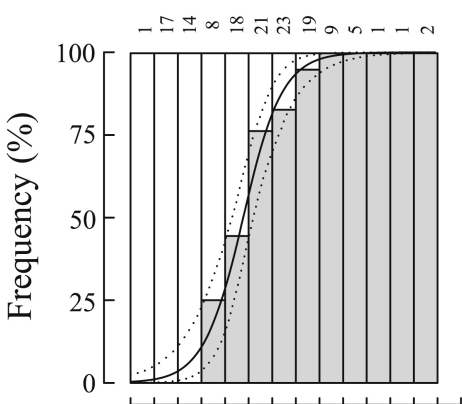
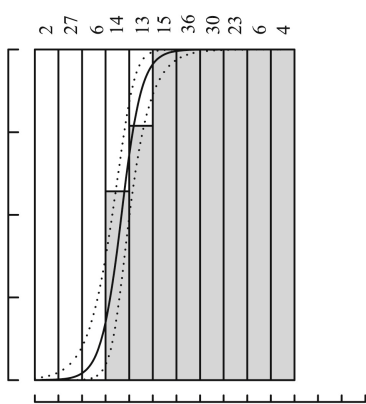
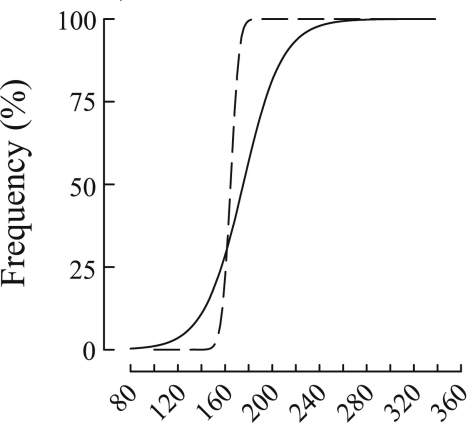
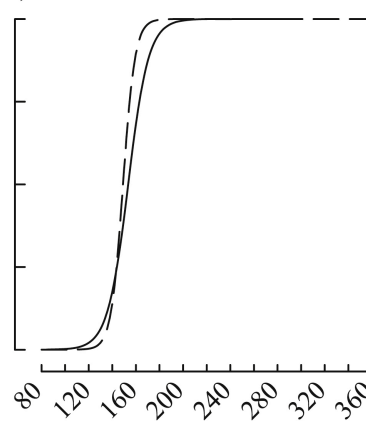
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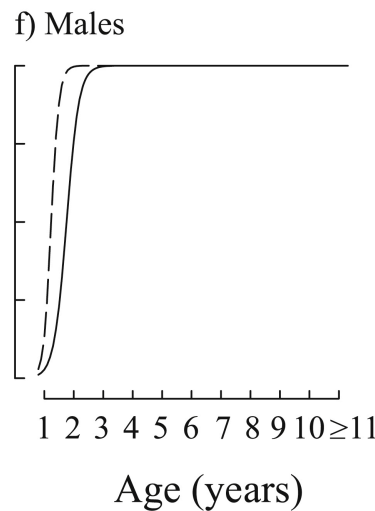
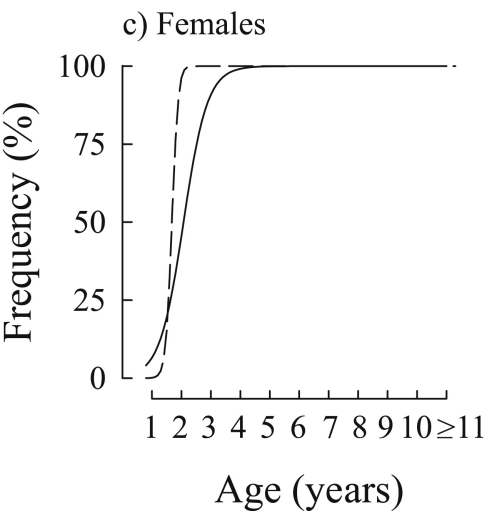
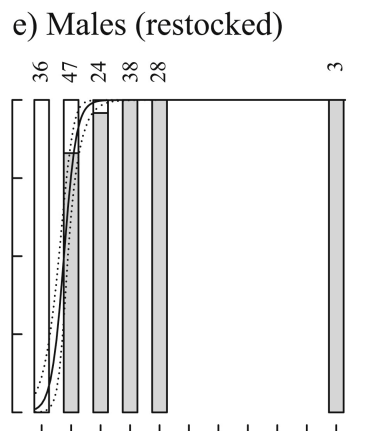
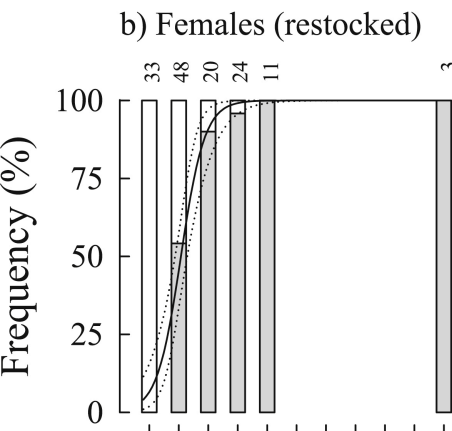
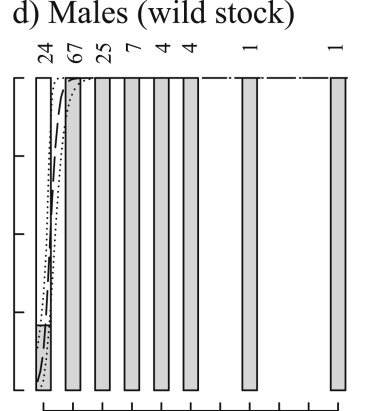
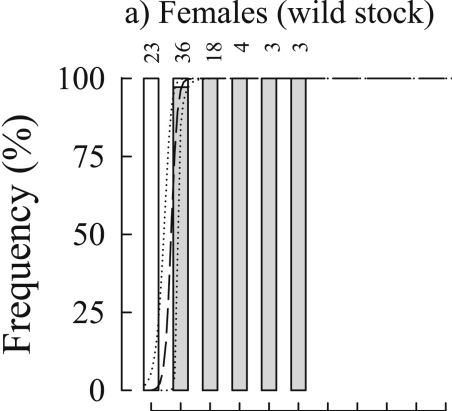
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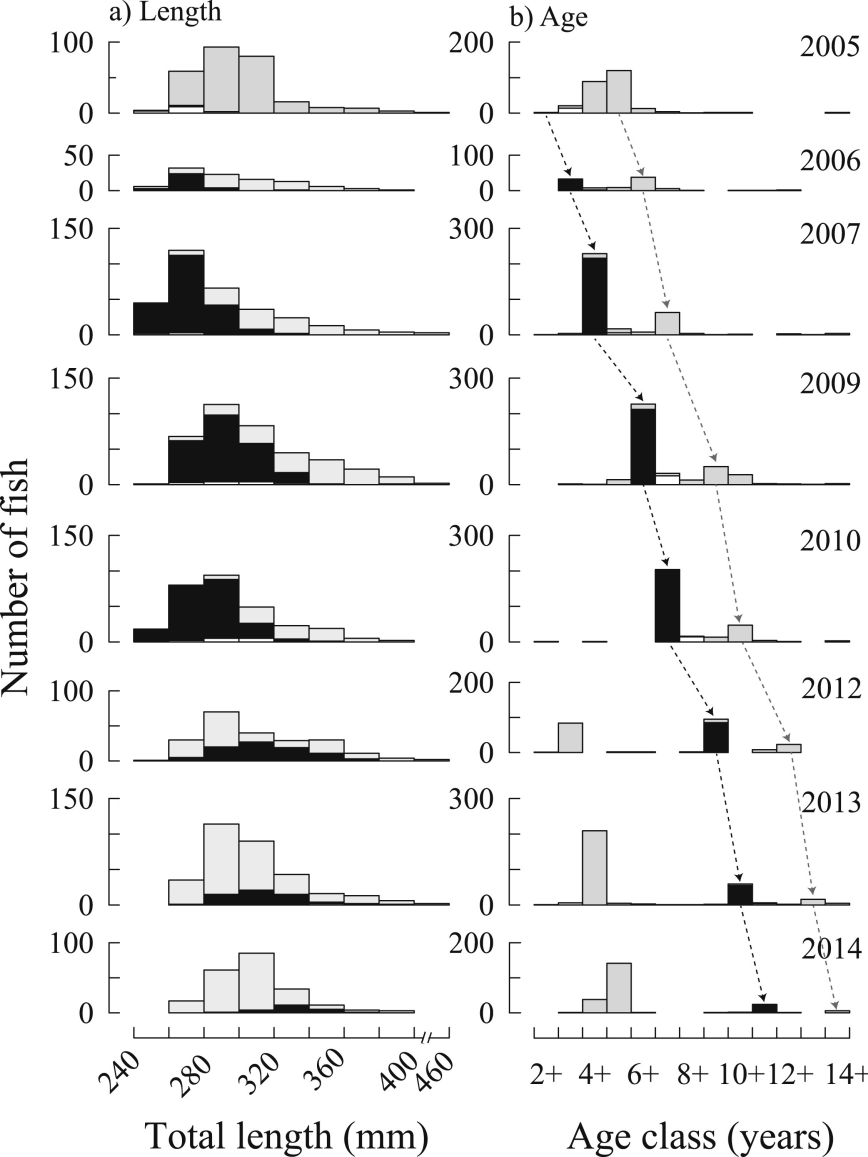


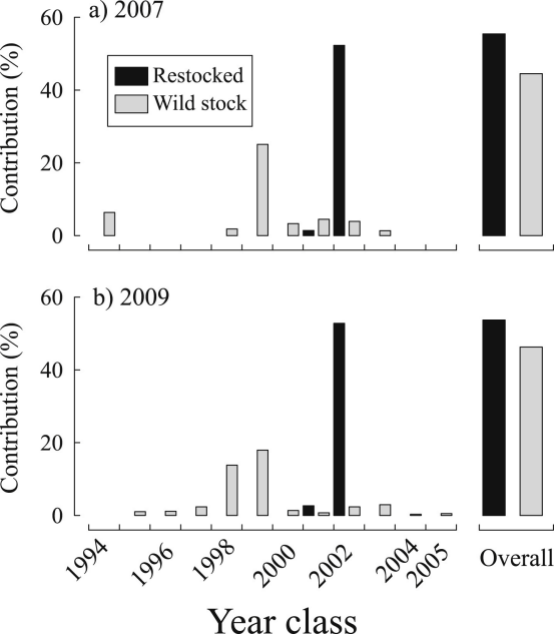




a) Females (wild stock)**d) Males (wild stock)****b) Females (restocked)****e) Males (restocked)****c) Females****f) Males**







Restocking with cultured fish replenished a depleted stock of an estuarine species.

Growth and maturity schedules of restocked fish differed only slightly from the wild stock.

Restocked fish contributed substantially to the fishery.

There is evidence that the progeny of restocked fish now contribute to the fishery.

Recruitment variability provides excellent opportunity for use of restocking.

ACCEPTED MANUSCRIPT