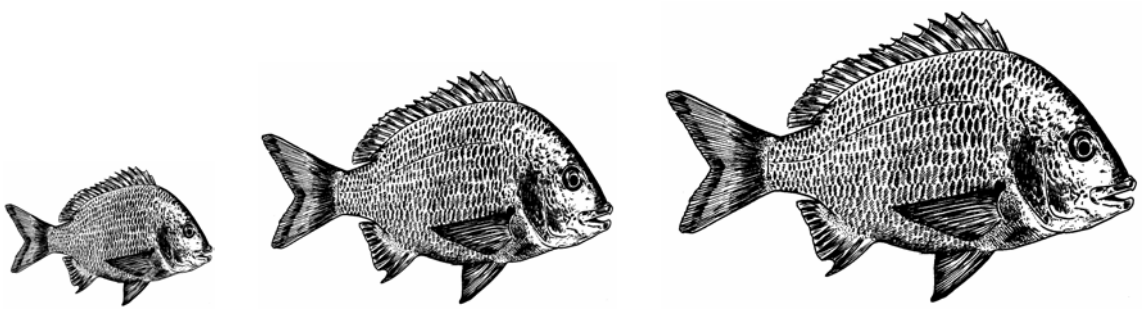
Selection for faster growing black bream *Acanthopagrus butcheri*



By

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at Murdoch University, Western Australia

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Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed…….

Let me be a little braver
When temptation bids me waver,
Let me strive a little harder
To be all that I should be.
Let me be a little meeker
With the brother that is weaker
Let me think more of my neighbour
And a little less of me

Acknowledgements

It is approaching 15 years since I left the Kimberley at the onset of a late monsoon to pursue a higher education in the conservation and management of aquatic resources. I wasn't sure what I was getting myself into and with the benefit of hindsight that was probably a good thing too! It took a lot of beer but I finally got here.

My most sincere thanks go to my good friend and mentor Alan Lymbery. I also wish to recognise Mark Starcevich for his outstanding assistance in all manner of things throughout this project. In addition, I would like to express my gratitude to the staff at the Aquaculture Development Unit in Fremantle, especially Greg Jenkins, Ken Frankish and Gavin Partridge. Others who have helped in various ways include Gavin Sarre, Johan Greeff and Stan Malinowski, and a handful of anonymous reviewers of this work. My final thanks are extended to my family and friends for their encouragement over the years.

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Abstract

In Australia, the widespread clearing of native vegetation has resulted in large areas of once-productive agricultural land being affected by rising saline groundwaters. There is considerable interest among farmers and rural landowners throughout Western Australia, in the possibilities that inland saline aquaculture may offer for a potentially productive use of land and water resources that can no longer support traditional agriculture. Black bream (*Acanthopagrus butcheri*) appear to be an ideal candidate for the developing saline aquaculture industry of inland Western Australia, however their current maximum growth rates are too slow for profitable production. The high productivity of modern breeds of terrestrial livestock species is primarily due to genetic improvement programs utilising selective breeding, and similar gains have also been made where they have been implemented for aquatic species. Before the growth rate of black bream can be genetically improved, however, it is necessary to estimate both the extent of genetic improvement required and the extent of genetic (co)variation in those growth traits which will be subject to, or affected by, selection. The aims of this study were to:

(1) Determine the extent of genetic improvement in growth rate required for black bream to be considered as a profitable aquaculture species.

(2) Estimate the potential for growth rate to be improved through heterosis when different black bream strains are crossbred.

(3) Estimate the additive genetic variation for growth rate, which exists within populations of black bream.

(4) Estimate the genetic (co)variation which exists between growth rate and other production traits.

A partial budget analysis investigated whether enhanced growth rates of black bream would improve profitability and justify a genetic improvement program. It was conducted for two different fish production systems; a commercial operation that incurred more operating expenses due to costs associated with farm initiation (stand-alone farm model) and an existing farm that diversified into aquaculture using the saline water resources of established farm dams (integrated farm model). Sensitivity analyses indicated that a 33% increase in growth rate to at least 200g/annum would allow either production system to return a profit at a farm-gate price of AUS$6/kg whole fish, with fish survival rates of 98% for the stand-alone farm and 65% for the integrated farm model. These results provided a breeding objective, being an improvement in growth rate by at least 33%.

A complete diallel cross of two black bream populations was used to estimate the comparative advantages that might be gained from straight-breeding and crossbreeding. At 90 days of age, the growth traits of standard length, total length and wet weight, varied significantly among all straight-bred and crossbred lines, and among half-sib groups within lines. Differences among half-sib groups explained 6.8% of the total variance in standard length, 8.3% in total length and 7.1% in wet weight, giving estimated heritabilities over all lines of 0.27 ± 0.11 for standard length, 0.33 ± 0.13 for total length and 0.28 ± 0.12 for wet weight. There was no evidence for heterosis in any traits when straight-bred and crossbred lines were compared, and phenotypic ($r_P = 0.95 – 0.98$) and genetic ($r_G = 0.63 – 0.69$) correlations were high among all growth traits.

I used the estimated heritability for wet weight of 0.28 to optimise a factorial mating design from a single population, and to estimate the contribution of additive genetic, non-additive genetic and maternal effects to variation in growth traits of black bream at 75, 130 and

180 days of age in the hatchery. Maternal genetic and environmental effects were greatest at 75 days of age, accounting for 9.1% of total phenotypic variance in wet weight, 11.4% of variance in standard length and 8.8% of variance in total length. At later ages maternal effects were much reduced, explaining 0.8 – 3.7% of phenotypic variance in growth traits. Additive genetic effects were greatest at 130 days of age, when they accounted for 17.4% of total phenotypic variance in wet weight, 21.4% of variance in standard length and 18.7% of variance in total length. Additive genetic effects were negligible (<1%) at 75 days of age and 4.8 – 5.5% of total phenotypic variance in growth traits at 180 days of age. Non-additive genetic effects (which also included common environmental effects due to families being raised in the same tank) explained 5.8 – 7.3% of total phenotypic variance in growth traits at 75 days of age, but were much smaller at later ages. Variable stocking densities among tanks up to 75 days significantly affected all growth trait measurements below 180 days of age.

One of the most important of these traits is feed conversion efficiency. Feed conversion efficiency (FCE) is the effectiveness with which feed is converted to saleable fish product. Feed costs are a major input to aquaculture production systems and genetic changes in FCE may therefore have an important influence on profitability. FCE is usually expressed by a composite measure that combines feed intake and growth rate. The two most common measures are feed conversion ratio (feed intake/weight gain over a specified time interval) and its inverse, feed efficiency. Feed conversion ratio and feed efficiency are measures of gross FCE, because they do not distinguish between the separate energy requirements of growth and maintenance. There is abundant evidence of substantial genetic variation in FCE and its component traits in terrestrial livestock species and, although data are few, the same is likely for cultured fish species. The major problems with selecting from this variation to genetically improve FCE in fish species are:

- It appears impractical to measure feed intake on individual fish, so that family mean data must be used.
- We do not know the optimal time period over which to test fish for FCE.
- We do not know the genetic correlations between FCE under apparent satiation or restricted intake conditions, or between FCE at different times in the production cycle.

I measured the relationships between feed intake to apparent satiety and weight gain in replicate half-sib families of black bream at four times over a 56-day test period. After 42 days, I found significant additive genetic variance in both weight gain and feed intake, and a stabilisation in family group variation in both traits. This indicates that 42 days is the minimum test period over which to measure genetic variation for FCE in black bream. There were high, positive phenotypic (and probably genetic) correlations between weight gain and feed intake after 42 days. There was no detectable genetic variation for either feed efficiency (weight gain/feed intake), or residual feed intake, which is the difference between the actual feed intake of an individual and the intake predicted from its body weight and growth rate. I argue that selection for improved FCE might be better achieved not by using a composite measure, but by using a weighted selection index that accounts for the genetic covariance among weight gain, feed intake and other correlated traits.

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Chapter 1

Introduction

Dryland salinity and inland aquaculture

In Australia, the widespread clearing of native vegetation has resulted in about 2.5 M ha of once-productive agricultural land being affected by rising saline groundwaters and hence, dryland salinity. Over 70% of Australia's salt-affected land occurs in the wheatbelt region of southern Western Australia, a cropping and livestock agricultural zone covering approximately 18 M ha (Doupé *et al*. 2003a). Without appropriate and strategic management, the extent of dryland salinity is expected to significantly worsen before any equilibrium is reached (Robertson 1996).

Strategies to address the salinity problem involve: (1) water management practices, such as introducing deep-rooted perennial plants, protecting and managing remnant native vegetation, collecting and disposing of surface water, and draining and pumping groundwater (Barson & Barrett-Lennard 1995; Hatton & Nulsen 1999); and (2), developing new productive uses for land that has already been affected by salinity (Doupé *et al*. 2003b). There is considerable interest among farmers and rural landowners throughout Western Australia, in the possibility of recreational fishing enhancement and commercial aquaculture production using saline groundwater in inland areas (Trendall & Pitman 1998; Doupé *et al*. 1999). Inland saline aquaculture provides an opportunity for the diversification and expansion of agriculture through a potentially productive use for salt-affected land and water resources that can no longer support standard agricultural enterprises; it may also provide a means of defraying all or part of the cost of surface and subsurface water management systems (Doupé *et al*. 2003b).

The role of black bream

Black bream (*Acanthopagrus butcheri*) is one of the most important recreational and commercial fish species in the estuaries of southern Australia (Kailola *et al*. 1993; Lenanton *et al*. 1999). In the south of Western Australia, black bream populations are mostly reproductively isolated, which suggests that there is limited individual movement among estuaries (Chaplin *et al*. 1998). These estuaries are also highly variable environments (Lenanton & Potter 1987; Loneragan *et al*. 1989) and different black bream populations have different temperature and salinity tolerances, dietary preferences, and reproductive phenology (see Sarre & Potter 1999, 2000; Sarre *et al*. 2000). The extent to which this variation might be due to genetic differences among populations is unknown.

Black bream are also a highly regarded sport and table fish, and there has been considerable interest in their aquaculture potential for inland waters (Morison *et al.* 1998; Doupé *et al*. 1999; Sarre *et al*. 1999, 2003). In many ways they are an ideal candidate species for inland saline aquaculture. For example, they are endemic to a wide region of southern Australia and they appear to be very hardy; high survival rates (> 95%) have been maintained under a variety of inland farm pond conditions and feeding regimes (Sarre *et al*. 1999, 2003). Importantly, hatchery techniques are now well established for this species (Jenkins *et al*. 1999; Partridge *et al*. 2003), which has led to large numbers of juvenile black bream being stocked in population-depleted estuaries for recreational fishing enhancement, and in farm dams and ponds throughout inland Western Australia for farm-stay tourist development and commercial aquaculture trials.

The major factor limiting the further development of black bream as a commercial aquaculture and impounded fishery species is their slow growth rate; the fastest growing natural populations require 2 years to reach 250g or 250 mm (Sarre & Potter 1999, 2000). It is generally

believed that, to be commercially viable, fish stocked at 5g into farm dams or ponds need to reach a target weight of 250g within 12 months, a growth rate of 0.67g/day. It is presently thought that the maximum achievable on-farm growth rate using unimproved hatchery strains of black bream is 0.3 – 0.4g/day (Sarre et al. 2003). A doubling of growth rate is therefore required if black bream are to fulfill their potential as an important species for commercial inland aquaculture and recreational fishing.

Genetic improvement of growth rate in black bream

The high productivity of modern breeds of terrestrial livestock species is primarily due to genetic improvement. For example, Havenstein *et al.* (1994) compared the performance of broiler chickens maintained with and without genetic selection since 1957, and found that nearly all of the 300 – 400% improvement in growth rate of modern chicken strains was due to selection, with only 14% being attributed to improved diet or other management practices. Most aquaculture species have not yet benefited from genetic improvement programs, but where they have been implemented, genetic gains have typically been of the same order as gains for terrestrial livestock species (e.g. Gjedrem 2000; Knibb 2000). There is a need within Australia for aquaculture industries to implement genetic improvement programs, but a major constraint is the lack of appropriate resources and expertise (Lymbery 2000).

It is possible to increase the growth rate of black bream distributed to farm dams and ponds by instituting a genetic improvement program. The two most commonly used and immediately applicable methods of genetic improvement, are crossbreeding and selection. Before a genetic improvement program can be implemented, however, it is necessary to estimate a number of genetic parameters through directed research. First, the amount of genetic improvement made in a single generation of crossbreeding is a function of the degree of

heterosis in the trait resulting from the dominance effects of alleles. The extent of heterosis may differ depending upon the strains being crossed and the direction of the cross. Theoretically, the two most genetically divergent strains should provide the maximum heterotic response (Charcosset & Essioux 1994), but the optimum direction of the cross needs to be determined empirically (see Gjerde 1988). Second, the rate of genetic improvement in a selection program is a function of the selection intensity, the phenotypic variance of the trait and the heritability of the trait, which is the proportion of phenotypic variance due to additive genetic differences among individuals. Greater selection intensities produce faster genetic responses, but at the risk of inbreeding. Furthermore, it is important to estimate the phenotypic variance and heritability prior to selection, so that the desired rate of genetic improvement can be obtained with minimum selection intensity (Falconer & Mackay 1996). Third, genetic improvement in one trait may also have indirect effects on other traits, and these are a function of the genetic correlations among the traits of interest. It is of paramount importance, therefore, to estimate the genetic correlations between the selection criterion (growth rate) and the other traits of interest, such a survival and feed conversion efficiency.

Thesis objectives and structure

The broad objective of this study was to estimate the extent of genetic (co)variation in selected growth traits of some black bream populations from Western Australia to facilitate a genetic improvement program for the species. More specifically, the aims of this study were to:

(1) Determine the extent of genetic improvement in growth rate required for black bream to be considered as a profitable aquaculture species.

(2) Estimate the potential for growth rate to be improved through heterosis when different black bream strains are crossbred.

(3) Estimate the additive genetic variation for growth rate, which exists within populations of black bream.

(4) Estimate the genetic (co)variation which exists between growth rate and other production traits.

This thesis contains five research chapters. Chapter 2 uses an economic analysis of the biology of production to define a breeding objective for the genetic improvement of black bream. Chapter 2 identifies growth rate as a suitable selection criterion for black bream, and emphasises the importance of investigating the genetic relationships between (a) growth rate at different ages, and (b) growth rate and feed intake. Chapter 3 explores the relative importance of heterosis and selection in improving growth rates in black bream, from straight-breeding and crossbreeding two distinct populations. In chapter 4, a factorial mating design is used in conjunction with repeated measures of growth traits in family groups to identify in more detail, the important components of genetic (co)variance in a single population. Chapter 5 is a review of evidence for the presence of genetic variation for feeding efficiency in fish species and how this might be investigated, and chapter 6 details a test for genetic variation in feed intake and growth rate in black bream.

Chapter 2

Justification for genetic improvement in growth rates of black bream using a partial budgeting analysis

Preamble Given an underlying assumption that a genetic improvement program aims to improve the profitability of a commercial enterprise, then the program firstly needs to know what sort of production and marketing system is available, what costs and returns are in those systems, and which biological traits of the species influence costs and returns. This is essentially an economic analysis of the biology of production and provides a breeding objective for genetic improvement.

Introduction

Aquaculture often competes with other economic activities that either require similar resource inputs like capital, land and water, or use the same product markets, such as with catches from wild fisheries (Shang 1990). An aquaculture industry founded upon the vast areas of salt-affected land and water of inland Western Australia presents opportunities to provide an alternative production system and economic base to dependent rural communities, and contribute to the supply of fish protein that can no longer be achieved by diminishing capture fisheries (Doupé *et al*. 1999). The inland saline aquaculture industry of Western Australia is developing, but remains at hobby status. Presently, about 150 crop/livestock farmers grow-out rainbow trout (*Oncorhynchus mykiss*) yearlings, predominantly in existing farm dams built for other purposes, and only during the cooler winter months (Starcevich *et al*. 2003). A handful of farmers are experimenting with black bream (*Acanthopagrus butcheri*) because this species displays biological attributes that suggest it is an ideal candidate for inland aquaculture. For example, black bream are endemic to a wide region of southern Australia (Kailola *et al*. 1993), optimal

hatchery techniques are established (Jenkins *et al*. 1999; Partridge *et al*. 2003), and they are a highly regarded sport and table fish (Lenanton *et al*. 1999) that survive over a wide range of salinities and temperatures (Sarre & Potter 1999; Sarre *et al*. 2000). The major limiting factor to using black bream for inland aquaculture is their slow growth rate; the fastest growing natural populations require 2 years to reach an acceptable plate size of 250g/250 mm (Sarre & Potter 1999, 2000).

The extent to which inland aquaculture will successfully compete for limited resources with alternative opportunities, such as salt-tolerant crops, depends on the relative efficiency of production which, in turn, can be increased by reducing costs through improved culture practices and technology (Allen *et al*. 1984). Lymbery (2000) argued that despite the high fecundity and significant potential for genetic improvement of most aquaculture species, their relatively poor production efficiency was largely because these stocks were derived from wild populations that had not benefited from modern developments in animal breeding. Selection for improved growth rate, for example, has the potential to increase yield, reduce the production cycle and utilise pond capital more efficiently (Gjedrem 1983; Gjedrem *et al*. 1991; Cann 1997). The capability for *a priori* evaluation of technology, especially for fundamental input and output variables, represents an important application of bioeconomic modelling (Cacho 1997). However, few workers have considered a bioeconomic analysis of fish production in the context of genetic improvement. In this chapter, I investigate whether shorter production periods or increased yield resulting from improved growth rates of black bream, will improve resource efficiency through a positive return on investment. A partial budget analysis is used to determine whether improved growth rate constitutes a suitable breeding objective for the genetic improvement of this species in the developing inland aquaculture industry in Western Australia.

Materials and methods

Two fish farm models each comprising 20, 100 m^2 ponds stocked with 375 fish/pond were designed to represent the current level of investment and activity in inland aquaculture. A stand-alone farm model, in which borrowed capital has been invested in culture facilities using saline land and water resources, was derived in consultation with an existing black bream aquaculture enterprise in inland Western Australia. The alternative, integrated farm model assumes fish farming occurs using existing water resources (i.e. farm dams) in a diversified agricultural system where other livestock and cropping activities remain. This leads to cost differences between the models in establishment, interest and depreciation on the initial investment, water delivery, and also to differences in returns due to expected survival rates of the fish.

Biological assumptions were based on values for survival, growth rate, feed intake and feeding ration from studies of wild and cultured populations of black bream (Jenkins *et al*. 1999; Sarre *et al*. 1999; Sarre & Potter 2000; Partridge *et al*. 2003). Briefly, the survival rate of the species in grow-out trials in constructed aquaculture ponds over a range of water bodies of variable water quality has consistently averaged 98%. The base growth rate of 0.4g/day was taken from available data on the fastest growing wild populations known from Western Australia and from farm dam grow-out trials, and translates to a growth rate from 5g (when juveniles are distributed) to 150g in 12 months. Feed intake was calculated from growth rate by simple multiplication using a constant feed conversion ratio (FCR, the ratio of dry weight of food offered to wet weight gained) of 1.2. There is no evidence of variation in FCR over the size and age ranges of black bream considered in the analysis. Feeding ration is the portion of artificial

feed and natural feed expected to constitute the black bream diet under semi-intensive culture conditions.

A simple, static, partial budget analysis was constructed for each model and based on the best available industry information. The model was designed to account for distinctions between the two culture systems in initiation or start-up costs, and fixed and variable operating costs. An example of the costs involved in each system, that is based on commercial-scale production and maximum growth rates (see later) is detailed in Table 2.1. Interest was calculated as the average of available commercial rates, and is presented as an annualised repayment.

A series of sensitivity analyses were undertaken to compare the performance, measured by break-even price (AUS$/kg) and the profitability or farm net return (AUS$), of the following input parameters:

Growth rate: The range of growth rates considered in each model was from a base rate of 0.4g/day to a maximum growth rate of 0.8g/day, producing a 300g fish in 12 months of grow-out. The increase in growth rate was assumed to be entirely due to the implementation of a genetic improvement program. Standard rates of genetic improvement for growth in most livestock including fishes are at least 10% per generation (Falconer & Mackay 1996).

Price: There is a small, seasonal market for wild-caught black bream in Western Australia, but no established market for cultured fish. I have therefore assigned estimated minimum (AUS$6/kg) and maximum (AUS$10/kg) farm gate prices for fresh, chilled whole fish. These prices are based on interviews with fish producers and retailers, and limited test marketing of cultured black bream. No on-farm processing is expected to occur in the medium term.

Table 2.1 Summary estimates of black bream production costs (AUS$) for stand-alone and integrated farm models comprising 2 ha of ponds at a stocking density of 3750 fish/ha with optimal survival, and a final 12-month growth rate of 300g

Parameter	Stand-alone farm model	Integrated farm model
Biological assumptions		
Survival	98%	65%
Feeding ration (artificial food:natural food)	65:35	65:35
Feed conversion ratio	1.2	1.2
Start-up cost		
Construction and site development (Earthworks, plumbing, fencing)	4200	500
Fixed operating costs		
Labour (Management)	400	400
Feed (1200/t)	2064	1369
Repayment on initial investment	340	___
Variable operating costs		
Stock	4500	4500
Power for water recovery	365	___
Casual labour (Husbandry, harvesting)	1738	1738
Vehicle costs	90	90
Repairs and maintenance	200	200

Survival: The survival rate of 98% for the stand-alone farm model was derived from existing levels for a commercial aquaculture enterprise. I assumed that in an integrated farm model, reduced management inputs may result in higher fish mortality and have therefore considered survival rates of 65%, 50% and 25%. Mortality was assumed to be highest in the early stages of pond establishment. I also assumed, however, that these reductions in survival rate did not affect other biological variables such as feed intake, feed conversion ratio, or feeding ration.

Results

Table 2.1 shows a $3700 difference in start-up costs borne by the stand-alone farm for site development and the construction of fishponds. The difference in establishment costs for the stand-alone farm model is also responsible for the subsequent differences associated with fixed annual operating costs arising from interest and depreciation on initial investment ($340), and variable annual operating costs associated with water pumping and delivery to ponds ($365). Essentially, the cost of infrastructure for fish culture has already been incurred in the integrated farm model. I am assuming that the integrated farm system is operating on freehold land, with unlimited access to water at no additional cost to that already borne by existing agricultural activities. The drawback to this system is that reduced investment in infrastructure for fish culture will impact on the survival of the fish, and in the sensitivity analyses I have considered survival rates of 65%, 50% and 25% for the integrated farm model, compared with 98% for the stand-alone model.

At current growth rates for black bream, which give a final weight of 150g following 12 months of grow-out, farm-gate prices well in excess of $6/kg would be needed for either farm model to break-even (Table 2.2). For the stand-alone model, and for the integrated farm model assuming a survival rate of 65%, final weights of over 200g/annum are required to break-even at

a price of $6/kg. At survival rates of 50% and 25% on the integrated farm, much greater final weights are required (Table 2.2).

Table 2.2 Comparative farm model break-even price (AUS$/kg, rounded) for a sensitivity analysis accounting for variable growth and survival rates

	Survival rate	Weight (g) following a 12-month grow-out						
		150	175	200	225	250	275	300
Stand-alone farm model	98%	7.90	6.90	6.15	5.60	5.10	4.75	4.40
Integrated farm model	65%	8.20	7.15	6.35	5.75	5.30	4.90	4.55
	50%	9.25	8.05	7.15	6.45	5.90	5.45	5.10
	25%	12.95	11.20	9.95	8.95	8.15	7.50	6.95

Table 2.3 shows the effects of farm-gate price on the profitability of the stand-alone and integrated farm models. The table reaffirms that final weights exceeding 200g/annum are required for each production system to yield positive net returns at a base farm-gate price of $6/kg, while both models show positive net returns at current growth rates if a price of $10/kg can be attained.

Table 2.3 Comparative net return (AUS$, rounded) for a sensitivity analysis accounting for variability in growth rates over 12 months and two extreme farm-gate prices, for a stand-alone farm model and an integrated farm model with a 65% survival rate

Model	Price	Weight (g) over a 12-month grow-out						
		150	175	200	225	250	275	300
Stand-alone	$6/kg	-2080.00	-1156.00	-231.00	694.00	1620.00	2545.00	3470.00
	$10/kg	2329.00	3989.00	5649.00	7309.00	8969.00	10630.00	2290.00
Integrated	$6/kg	-1633.00	-1016.00	-399.00	218.00	835.00	1451.00	2068.00
	$10/kg	1307.00	2415.00	3521.00	4628.00	5735.00	6841.00	7948.00

Discussion

I have attempted to understand the relationship between profitability and improved growth rates for two black bream production systems that differ in fixed and variable operating costs, and have concluded that growth improvements of at least 33% (i.e. from 150 to 200g/annum) could sustain a farm-gate price of $6/kg, and yield positive net returns to either farming system.

My spreadsheet models have allowed a partial budget analysis of the black bream production system. In contrast to systems models, component bioeconomic models like the ones

presented here, ignore the complex interactions among production parameters, market prices and resource constraints that may characterise real production systems.

In the analysis, I have assumed that there is no opportunity cost associated with the use of ponds or dams for aquaculture. Typically, these will be situated low in the landscape, on degraded land that has no other productive uses. The returns I have estimated therefore represent a base with which competing land uses could be compared. The relative inexperience of farmers involved in inland aquaculture would be expected to add a high degree of uncertainty surrounding cost structures and the performance of species under various culture conditions. I have therefore chosen to handle the risk or uncertainty in my investment analysis by conducting sensitivity tests for those parameters likely to have a range of values, but have calculated trait economic values based on the presumption that improved growth rates of black bream is entirely due to genetic selection practised in the best available rearing environment (see Barwick 1992; Henryon *et al*. 1999).

Trait economic value of improved growth rates

The aim of this bioeconomic analysis was to determine the suitability of improved growth rate as a breeding objective for black bream aquaculture. The analysis indicates that increasing growth rates of black bream to at least 200g/annum will improve profitability, and provides a breeding objective for a genetic improvement program, but the objective is part of a decision-making process that accounts for predictable genetic changes to the biological traits of economic importance (Goddard 1998). The relative economic importance of traits may change as a consequence of the response to selection on other traits. Reliable estimates of genetic and phenotypic parameters are needed for all traits of economic importance, to predict responses to selection, to choose among various breeding plans, to estimate economic returns, and to predict

breeding values of candidates for selection (Gjerde & Schaeffer 1989). For the genetic improvement of black bream, two of the most important parameters are the genetic correlations between growth rate and feed intake, and between growth rate at different ages.

Growth rate and feed intake

Yield of fish, determined by stocking density, growth rate and mortality, is a major determinant of profitability. An increase in yield, however, entails higher production costs, particularly feed costs, which normally comprise 30 – 60% of the variable production costs of aquaculture enterprises (Goddard 1996). I have assumed that selection for increased growth rate does not change FCR. Despite its economic importance, FCR has remained difficult to accurately measure in fishes (Gjedrem 1983; Helland *et al*. 1996). FCRs vary with size and age, and within and among species (Brett 1979; Ahmad *et al*. 2000). Theoretically, one might expect that a higher relative feed intake that is associated with a higher genetic capacity for growth should lead to improved feed utilization by increasing the amount of metabolizable energy available for gain relative to the maintenance energy cost (McCarthy & Siegel 1983). For example, Thodesen *et al*. (1999) found a better feed conversion ratio in selected versus wild lines of Atlantic salmon (*Salmo salar*), and estimated a compounded selection response of 4.6% in feeding efficiency per generation. Therefore, increasing genetic growth capacity through selective breeding may result in more of the feed consumed per generation being effectively utilized for growth, although the magnitude of any correlated selection response in feeding efficiency to improvements in black bream growth rates awaits measurement.

Growth rate at different ages

It is important to distinguish between the breeding objective, those traits that should be improved to provide most economic benefit, and the selection criteria, the measures of those

traits in the breeding objective. The breeding objective for black bream is improved on-farm growth rate between the time that the fish are stocked and 12 months later, when fish are harvested. The selection criterion, however, is likely to be growth rate from birth to that time in the hatchery when fish are either sold for grow-out, or kept for breeding. The question that needs to be answered is: how accurately does early growth in the hatchery correlate with later growth on-farm? That is, what are the genetic correlations between growth at different ages? There have been a number of studies of the heritability of growth rate in fish (e.g. Aulstad *et al*. 1972; Hershberger *et al*. 1990; Knibb *et al*. 1997), and of genetic correlations among growth rate and other traits (e.g. Gunnes & Gjedrem 1978; Robinson & Luempert 1984; Gjerde & Schaeffer 1989; Jonasson 1993). Only a very few have looked at correlations among growth at certain ages (e.g. Rye *et al*. 1990; Winkelman & Peterson 1994a), but there are none of which I am aware that have followed the genetic correlations among growth and other production traits from early life to harvest.

Implementation of a genetic improvement program for black bream

The analysis was primarily concerned with determining the economic rationale for a particular breeding objective, not with testing the economic justification for investment in a genetic improvement program. Nevertheless, the financial models indicate that an improvement in growth rates of black bream could be economically viable for a farm enterprise at two levels of production intensity. Whether this warrants investment in a genetic improvement program remains to be proven, because the analysis has not accounted for the costs of the research associated with implementing a genetic improvement program for a black bream grow-out industry. I estimate research and implementation costs of AUS$150 000 – $200 000 over 5 years. With an improvement in net returns of AUS$5 000 – $10 000/farm/year (Table 2.3), the justification for a genetic improvement program for black bream clearly depends on the

estimated scale of investment in black bream aquaculture. This, in turn, depends on how inland saline aquaculture progresses from being a small-scale remedial use of salinised farmland to an agricultural industry in its own right. The successful development of a new industry is governed by a number of factors: production technology, the identification and establishment of sustainable markets, the establishment of environmentally sustainable production systems and industry management. For a new industry to succeed, concurrent progress is needed across all these fields (Doupé *et al*. 2003b).

Chapter 3

Genetic variation in growth traits of straight-bred and crossbred black bream at 90 days of age

Preamble My breeding objective is to increase black bream growth rate measured as wet weight, by at least 33% following 12 months of grow-out. Before pursuing this goal, however, I need to identify which mating system is most appropriate, and which traits can be improved and how they should be measured. These are my selection criteria. In this chapter, I use reciprocal crossbreeding as an investigative tool to provide genetic information on those traits being investigated as possible selection criteria, by estimating the proportion of phenotypic variance that is due to additive genetic differences among individuals (the heritability of the traits), the degree of allelic dominance at loci affecting the trait(s) of interest (the heterosis effect on the traits), and their genetic covariance (the genetic correlations among the traits).

Introduction

The slow growth rate of hatchery-derived fish is a major factor limiting the widespread commercial aquaculture of black bream in southern Australia's inland saline waters. In chapter 2, I found that an increase in growth rate of at least 33% was necessary to yield positive net returns to grow-out producers at realistic market prices. Genetic improvement programs can provide permanent improvement in growth rate.

Before implementing a genetic improvement program, it is important to estimate the extent of genetic variance in the trait on which selection will be based, and the genetic covariance with other traits of interest. These estimates are needed to predict the response to selection, choose among different breeding plans, determine the cost effectiveness of the program, and estimate breeding values of the candidates for selection (Gjerde & Schaeffer 1989).

Gjedrem (2000) has estimated the response to selection for increased growth rate over a range of fish species to be 10 – 15% per generation, so we might expect a 33% improvement in growth rate of black bream to require at least 3 generations. In chapter 2, I suggested that the rate of genetic improvement could be increased by crossing genetically distinct populations of black bream prior to implementing a selection program; this is expected to provide an initial boost to growth rate through heterosis and a greater response to selection because of increased phenotypic variance (Falconer & Mackay 1996). Black bream populations found in the estuaries of southern Western Australia display significant, but moderate population genetic subdivision (mean F_{ST} = 0.091), attributed to limited movement by individuals among estuaries, and differences in regional and local environmental conditions (Chaplin *et al*. 1998).

My broad aim is to measure the genetic (co)variation in growth traits, such as wet weight and length, at different ages in black bream from different populations in Western Australia, and to estimate how much growth rate can be improved by selection and/or crossbreeding. In this chapter, I present the results of a preliminary experiment, aimed at providing initial estimates of additive genetic variance and heterosis in growth traits at 90 days of age.

Materials and methods

Mating design and rearing conditions

Broodstock were collected from the Swan and Blackwood Rivers in southwestern Australia. Swan River black bream are thought to be the fastest growing population in the region (Sarre & Potter 2000), whereas fish of the Blackwood River, about 300 km south of the Swan, appear to be among the slowest growers (G. Sarre pers. comm.). The mating design was a complete diallel cross, with two straight-bred lines (Swan x Swan, Blackwood x Blackwood) and two crossbred lines (Swan x Blackwood, Blackwood x Swan). For each line, eggs from four

randomly selected females were combined, mixed, divided and fertilized with sperm taken from eight individual, randomly selected males. This design provides half-sib groups for the estimation of sire components of variance, with an adequate sampling of genetic diversity within each population, given time and cost constraints on the number of tanks required to rear separate family groups.

Trait measurements

Half-sib groups were randomly allocated among 300 litre aquaria connected to a common recirculating seawater system for hatching to occur. The fish were raised using established, optimal rearing techniques (Jenkins *et al*. 1999; Partridge *et al*. 2003), and from age 50 days, were fed to satiation on standard, pelleted food. At age 90 days, all fish were removed and counted, and a random sample of 20 – 50 individuals from each group ($\bar{x}$ = 30) was measured for wet weight, standard length (the straight line distance from the anterior most point of the head to the base of the caudal fin, after Howe 2002) and total length (as for standard length but including the caudal fin).

Data analyses

The effect of different crosses on growth traits at 90 days was analysed using the mixed model:

$$Y_{ijkl} = \mu + L_i + S_{j(i)} + D_k + e_{ijkl}$$

where μ is the mean growth trait, L_i is the fixed effect of the *i*th line, $S_{j(i)}$ is the random effect of the *j*th half-sib group nested within the *i*th line, D_k is a covariate accounting for the effect of the *k*th stocking density, and e_{ijkl} is the residual error.

Heterosis (H) was calculated as the difference between mean trait values of straight-bred and crossbred half-sib groups. Variance and covariance components were estimated using restricted maximum likelihood. Heritabilities and genetic correlations for growth traits were determined from the half-sib components of (co)variance, and approximate standard errors of these estimates were calculated according to Becker (1984). Genetic parameters estimated from the nested model (above) are referred to here as across-line parameters for each trait, because they are calculated from the (co)variance components over all lines. I also estimated parameters separately for each trait for the half-sib groups from each straight-bred and crossbred line, but these estimates were similar to the across-line estimates and are not reported here.

Results

A summary of trait measurements for the family groups is provided in Appendix 1. Poor hatching success in a large number of crosses meant that the fish from only 18 half-sib groups were available for measurement. These groups were evenly distributed between straight-bred and crossbred lines, and between Swan and Blackwood sires. Values for standard and total length were normally distributed, however wet weight was highly left-skewed and I normalised this variable with logarithm and square root transformations. Stocking densities ranged between 0.04 and 7.14 fish.L^{-1} for the 18 half-sib groups, and the environmental effect of variable stocking density on weight was highly significant ($r^2 = 0.28$, $P < 0.0001$).

There were significant differences in standard length ($F = 6.34$, $P < 0.007$), total length ($F = 4.27$, $P < 0.02$) and wet weight ($F = 5.00$, $P = 0.002$) among different lines, and also among half-sib groups within lines (standard length $F = 4.29$, $P < 0.0001$; total length $F = 4.54$, $P < 0.0002$; wet weight $F = 3.76$, $P < 0.001$). Table 3.1 shows the least square mean, coefficient of variation and range over family group means for wet weight, standard length and total length

at 90 days in the two straight-bred and two crossbred lines. There is clearly no heterotic effect (standard length H = – 1.8 mm; total length H = – 2.1 mm; wet weight H = – 0.54 g; see Table 3.1). It appears that crosses involving Blackwood River sires produced faster growing fish than crosses using Swan River sires, although the only statistically significant difference in mean weight was between the Blackwood x Blackwood and Swan x Blackwood lines. There were significant differences in phenotypic variances among lines for all traits, as shown by Levene's test (standard length $F = 8.81$, $P < 0.001$; total length $F = 12.58$, $P < 0.001$; wet weight $F = 14.25$, $P < 0.001$), although this was not due to consistently greater variances in the crossbred lines (Table 3.1).

Differences among half-sib groups explained 6.8% of the total variance in standard length, 8.3% in total length and 7.1% in wet weight, giving an estimated across-line trait heritability of 0.27 ± 0.11 for standard length, 0.33 ± 0.13 for total length, and 0.28 ± 0.12 for wet weight respectively. Similar estimates were obtained when each line was analysed separately. There were high phenotypic and genetic correlations between wet weight, standard length and total length (Table 3.2).

Discussion

The potential advantages of crossing genetically different lines come from heterosis and increases in phenotypic variance for the traits of interest. There was no evidence for heterosis or increased phenotypic variance for 90-day growth traits when black bream from the Swan and Blackwood Rivers were crossed. Theoretically, the magnitude of the heterotic response should be a function of genetic divergence (Charcosset & Essioux 1994), so this may indicate that the Swan and Blackwood River populations are not genetically different for the loci that control growth rate. Although differences have been found among populations in reproductive biology

Table 3.1 Least square mean (LSM ± SE), coefficient of variation (CV) and range over family means for standard length, total length and wet weight at 90 days of age in straight-bred and crossbred lines of black bream. Means with the same superscript were significantly different using Tukey's HSD test

Line (Sire x Dam)	Standard length			Total length			Wet weight		
	LSM (mm)	CV (%)	Range (mm)	LSM (mm)	CV (%)	Range (mm)	LSM (g)	CV (%)	Range (g)
Swan R x Swan R	39.0[1] ± 0.6	16	37.5-41.5	45.0 ± 0.8	15	43.1-47.7	2.30 ± 0.14	47	2.04-2.86
Blackwood R x Blackwood R	44.8[1,2] ± 1.4	11	43.5-46.1	50.3 ± 1.6	10	48.9-51.8	3.35[1] ± 0.30	30	2.97-3.74
Swan R x Blackwood R	38.9[2] ± 1.5	14	38.3-39.5	44.0 ± 1.7	14	43.1-44.8	1.86[1] ± 0.31	46	1.69-2.03
Blackwood R x Swan R	41.3 ± 0.6	19	39.3-42.8	47.0 ± 0.8	19	45.2-48.9	2.71 ± 0.14	54	2.22-2.99

(Sarre & Potter 1999) and age and growth parameters (Sarre & Potter 2000), the proportion of this variation that is genetic in origin is not known. Paradoxically, there was some evidence that the progeny of sires from the Blackwood River grew faster than the progeny of sires from the Swan River, which is opposite to the phenotypic differences seen in natural populations (Sarre & Potter 2000). An alternative explanation for the lack of heterosis may be that it is an artefact of

limited sampling of male and/or female breeders in each population. Further studies are needed to resolve this question.

Table 3.2 Phenotypic (above diagonal) and genetic (below diagonal) correlations (± SE) for standard length, total length and wet weight

	Standard length	Total length	Wet weight
Standard length	–	0.99	0.96
Total length	0.63 ± 0.08	–	0.95
Wet weight	0.69 ± 0.16	0.64 ± 0.14	–

The substantial variation in growth traits among half-sib families within each line suggests there is encouraging room for improving growth rate of black bream through traditional selection. The estimated heritability of 0.27 – 0.33 is in line with those found for growth traits in many other fish species (Tave 1986; Gjedrem 2000). However, I urge caution for this estimate because the experimental design only allowed sire components of variance to be measured, and the loss of a number of half-sib groups reduced the extent to which the genetic variance in each population was sampled. In addition, I did not standardise stocking densities prior to measurement. Large differences in stocking density were found among groups, resulting from variation in fertility and survival, although this variation was randomly distributed among lines. Elvingson & Johansson (1993) suggest that standardisation of stocking density should be done as early as practicable to minimise tank effects. The trade-off to earlier measurement is, however,

the introduction of further unknown environmental effects related to handling, anaesthesia and possibly increased mortality. For this preliminary experiment I preferred a statistical correction to potential tank effects, by using stocking density as a covariate in the analysis of variance model. Nevertheless, the differences in stocking density introduced a potentially complicating environmental effect among half-sib groups, which may have confounded these estimates of genetic effects on growth traits.

The high trait economic value of growth rate in aquaculture species makes it a desirable character to improve (Lymbery 2000). Although the breeding objective in a genetic improvement program for growth rate is usually size at harvest, this is impractical to use as a selection criterion. When choosing fish as candidates for future broodstock or for grow-out, selection decisions are made on the basis of size at earlier stages of development (Fishback *et al*. 2002). The high phenotypic and genetic correlations between length and wet weight at 90 days of age, suggest that length could serve as an indirect selection criterion for young fish. The practical application in measuring this trait over weight could be especially useful if genetic improvement was undertaken on farms.

The assumption that is made when choosing broodstock based on their early growth in a hatchery is that this selection criterion is genetically correlated with the breeding objective. There are surprisingly few data relating the genetic variation of growth rate at different developmental ages, even for long-cultured groups like the salmonids (Su *et al*. 1996; Vandeputte *et al*. 2002). It is important therefore, to precisely estimate both the heritability of growth traits for black bream in the hatchery, and the genetic correlations between these selection criteria and growth traits when fish are transferred to grow-out conditions. Accurate and repeatable estimates of genetic variance and covariance for growth traits at different ages

would allow me to predict the suitability and reliability of using traits associated with juvenile growth rate as indirect selection criteria for choosing future broodstock.

Chapter 4

Additive genetic and other sources of variation in growth traits of juvenile black bream

Preamble In chapter 2, I suggested that a potentially important influence on the profitability (and success) of a genetic improvement program for black bream concerned the genetic associations between growth rate at different ages, because the same or different genes may variably affect a trait at different stages of development. Although the breeding objective is final weight after 12 months of grow-out, knowing the genetic relationships among growth traits at different ages during the hatchery stage of development could determine whether early growth in the hatchery is a suitable selection criterion to satisfy the breeding objective. In chapter 3, I found substantial additive genetic (co)variation for length and weight growth traits, however these estimates were derived only once from paternal half-sib groups that were clearly affected by variable mortalities and tank stocking densities. Consequently, rearing space was under-utilised, nothing could be said of maternal genetic and environmental effects, and these estimates were not repeated. It is for these reasons that the 90-day data should be regarded as preliminary because they do not provide a complete indication of the additive and non-additive components of phenotypic variance, and therefore, of the likely responses to selection for faster growth rate.

Introduction

Chapter 3 described a diallel cross using two populations of black bream. At age 90 days there was no evidence for heterosis, but there was substantial genetic variance in growth-related characters among paternal half-sib families (estimated across-line heritability for standard length, total length and wet weight was 0.33, 0.27 and 0.28 respectively), and moderate to high genetic (r_G = 0.63 – 0.69) and phenotypic (r_P = 0.95 – 0.98) correlations among all traits. I also identified that the sires of one black bream population (the Blackwood River) appeared to produce faster growing progeny, and that any future work should concentrate on it. The accuracy of my findings at 90 days of age was compromised by several factors. First, variable stocking densities up until the point of measurement means that there were common environmental effects among

half-sibs; although I attempted to account for these effects with a statistical correction, heritability estimates may still have been inflated. Second, paternal half-sib analyses ignore potentially important maternal environmental and genetic effects (e.g. Hill & Nicholas 1974), and any non-additive genetic effects on the traits being measured.

In this chapter, I attempt to measure the importance of stocking density, maternal effects and non-additive genetic effects on heritability estimates of growth traits in juvenile black bream by utilising a factorial mating design. For most fish species, their high fecundity and relative ease of applying *in vitro* fertilisation techniques means that complex mating designs utilising full- and half-sib families are possible (e.g. Berg & Henryon 1998; Dupont-Nivet *et al*. 2002; Blanc 2003). Inclusion of both full- and half-sib groups in a mating design provides the necessary information for estimating maternal effects, and for separating additive genetic variance from non-additive (dominance and epistasis) effects, thereby improving the accuracy of conclusions (Lynch & Walsh 1998; Gall & Bakar 2002).

Materials and methods

Factorial mating design

In a factorial, or cross-classified, mating design, each sire is mated with each dam to produce a number of progeny, which are then reared until the trait of interest can be measured. The sire and dam variance components from such a design are equivalent to the covariance of paternal half-sibs and maternal half-sibs, respectively. The paternal half-sib covariance therefore provides an estimate of the additive genetic variance for the trait, while the maternal half-sib covariance contains the additive genetic variance, plus any maternal genetic or environmental effects. The interaction variance component from the factorial design is equivalent to the

covariance of full-sibs less the covariance of paternal half-sibs, less the covariance of maternal half-sibs, and provides an estimate of non-additive genetic variance.

I had 30 rearing tanks and 50 (Blackwood River) black bream broodstock comprising about equal numbers of sires and dams from which to select parents. Given these limited facilities, I used simulation studies to determine the combination of number of sires, number of dams and number of progeny per dam that minimised the variances of the intraclass correlations (t) of paternal half-sibs (t_{PHS}) and maternal half-sibs (t_{MHS}). The large-sample variances for t_{PHS} and t_{MHS} were derived from formulae in Lynch & Walsh (1998). These formulae require an estimate of t_{PHS} and t_{MHS}, which I obtained from my previous estimates of heritabilities for growth traits in black bream (chapter 3). In my simulations, I varied sire number from 2 – 8, dam number from 4 – 16, and progeny number from 10 – 50. In general, both Vart_{PHS} and Vart_{MHS} could be minimised, within the constraints of my rearing facilities, by using 4 – 6 dams and 5 – 7 sires. Both Vart_{PHS} and Vart_{MHS} were relatively unaffected by progeny number, providing that it was greater than 20 individuals.

Mating and rearing conditions

The eggs from five randomly selected females were separately fertilized with sperm from each of six randomly chosen sires to establish 30 full-sib families. Family groups were randomly allocated among 300 L tanks, on a common recirculation system, for hatching to occur. Poor hatching success occurred in two of the 30 family groups, and these were removed from the trial. Following hatching, the common recirculation among tanks was replaced with flow-through seawater (35 ppt at 4 L.min^{-1}). Fish were raised using established, optimal rearing techniques (Jenkins *et al*. 1999; Partridge *et al*. 2003) and from age 35 days, were fed to satiation on standard, pelleted food. Limited tank numbers meant that I could not replicate family groups, so

that any common environmental effects among full-sib families raised in the same tank were associated with the interaction variance component. Between age 35 and 55 days, progeny numbers were progressively thinned where necessary to achieve approximate uniformity and reduce common environmental effects associated with uneven stocking densities (chapter 3).

Trait measurements

Tanks were first harvested at 75 days of age, and tank stocking densities were standardized by returning only one hundred randomly selected individuals to each, with the first 30 of these being measured for wet weight, standard length (the straight line distance from the anterior most point of the head to the base of the caudal fin, after Howe 2002), and total length (as for standard length but including the caudal fin). An unidentified toxin caused significant losses in three families at about 90 days and they were removed from the trial. Growth traits and tank densities (being analogous to family survival) were again measured at 130 and 180 days of age for the remaining 25 families.

Data analysis

For each trait measured at each time, I calculated the mean for each family and over all families, the within-family coefficient of variation (mean of the coefficient of variation for each family) and the among-family coefficient of variation (coefficient of variation of family means).

The effects of sire, dam and interaction components of variance on growth traits were analysed using the mixed model:

$$Y_{ijklm} = \mu + S_i + D_j + I_{ij} + T_k + e_{ijkl}$$

where μ is the mean growth trait, S_i is the random effect of the *i*th sire, D_j is the random effect of the *j*th dam, I_{ij} is the interaction between the *i*th sire and the *j*th dam, T_k is a covariate accounting for the effect of the *k*th stocking density (i.e. the number of progeny per family group up until 75 days, when stocking densities were equalised), and e_{ijkl} is the residual error. Variance and covariance components were estimated using restricted maximum likelihood. Convergence for variance component estimation was considered satisfactory when two successive rounds of iteration changed by less than 0.1%. Likelihood ratio tests were used to evaluate the significance of variance components and the difference between variance components. For each trait, I estimated additive genetic effects (heritabilities) as four times the sire component of variance divided by the total phenotypic variance, maternal effects as the difference between the dam and sire components of variance divided by the total phenotypic variance, and non-additive genetic effects as four times the interaction component of variance divided by the total phenotypic variance (Lynch & Walsh 1998).

Results

Mean survival across the 25 families was 96% at 130 days and 93% at 180 days. There were no significant differences in survival among families at either 130 ($\chi^2 = 26.44$, $P = 0.33$) or 180 ($\chi^2 = 6.03$, $P = 0.99$) days. Initial fish density in the rearing tanks (i.e. prior to 75 days, when stock numbers were equalised) ranged from 0.29 – 4.63 fish.L^{-1}, and analysis of variance indicated that initial stocking density significantly affected all growth traits at 75 days (for wet weight $F = 19.7$, $P < 0.05$; for standard length $F = 20.9$, $P < 0.0001$; for total length $F = 25.6$, $P < 0.0001$) and 130 days (for wet weight $F = 4.6$, $P < 0.05$; for standard length $F = 4.7$, $P < 0.05$; for total length $F = 4.5$, $P < 0.05$), but not at 180 days (for wet weight $F = 1.1$, $P = 0.29$; for standard length $F = 0.4$, $P = 0.54$; for total length $F = 0.3$, $P = 0.56$).

Table 4.1 shows the mean over all families, and the within-family and among-family coefficients of variation for each growth trait at each age of measurement. Although the within-family variance was very similar at each age for all growth traits, the among-family variance decreased sharply at 180 days of age (Table 4.1). A summary of trait measurements for these groups is provided in Appendices 2 – 4.

Table 4.1 Overall mean and coefficients of variation within (CV_W) and among (CV_A) families of black bream for growth traits at 75, 130 and 180 days of age

Age (days)	Wet weight (g)			Standard length (mm)			Total length (mm)		
	Mean (± SE)	CV_W	CV_A	Mean (± SE)	CV_W	CV_A	Mean (± SE)	CV_W	CV_A
75	0.6 (± 0.0)	37.2	18.5	25.4 (± 0.1)	14.7	7.0	29.7 (± 0.2)	13.9	6.7
130	5.0 (± 0.1)	42.8	15.9	51.4 (± 0.3)	16.8	6.2	58.7 (± 0.3)	15.9	5.8
180	17.2 (± 0.2)	34.8	8.4	76.9 (± 0.4)	12.1	3.1	86.8 (± 0.4)	11.5	2.8

Table 4.2 shows the variance component estimates and Table 4.3 the estimates of additive genetic, maternal and non-additive genetic effects for wet weight, standard length and total length at 75, 130 and 180 days of age. At 75 days of age, dam components of variance, but not sire or interaction components of variance, were significant for all growth traits, and dam

components of variance were also significantly greater than sire components of variance for all traits (for wet weight, $P < 0.001$; for standard length, $P < 0.0001$; for total length, $P < 0.0001$).

Table 4.2 Sire, dam and interaction components of variance (± standard error) for wet weight, standard length and total length for juvenile black bream measured at 75, 130 and 180 days. * indicates that the effect is significant at $P < 0.05$

Source of variation at Measurement age (days)	Wet weight	Standard length	Total Length
$Sire_{75}$	0.0001 ± 0.0009	0.0002 ± 0.66	0.000
Dam_{75}	0.0058* ± 0.0007	1.776* ± 1.89	1.625* ± 1.498
Sire x dam_{75}	0.001 ± 0.001	0.224 ± 0.250	0.324 ± 0.366
$Residual_{75}$	0.053	13.530	16.414
$Sire_{130}$	0.224* ± 0.215	4.399* ± 4.084	4.444* ± 4.314
Dam_{130}	0.280* ± 0.257	5.060* ± 4.557	5.318* ± 5.05
Sire x dam_{130}	0.005 ± 0.076	0.000	0.001 ± 1.603
$Residual_{130}$	4.587	72.861	85.147
$Sire_{180}$	0.500 ± 0.716	1.149 ± 1.662	1.247 ± 1.880
Dam_{180}	1.170 ± 1.255	4.456* ± 4.212	4.720 ± 4.598
Sire x dam_{180}	0.208 ± 0.671	0.469 ± 1.587	0.631 ± 1.898
$Residual_{180}$	35.004	83.973	98.279

Maternal effects explained 8.8 – 11.4% of total phenotypic variance of growth traits at 75 days, while non-additive genetic effects were slightly smaller and additive genetic effects were negligible. At 130 days of age, both sire and dam components of variance were significant for all growth traits. Although dam components of variance were slightly greater than sire components

of variance for all traits, these differences were not significant ($P > 0.05$). Additive genetic effects were large for all traits, explaining 17.4 – 21.4% of the total phenotypic variance, while maternal and non-additive effects explained 1% or less of the variance. At 180 days of age, the only significant variance component was for dam effects on standard length. In general, dam components were greater than sire components of variance, but these differences were not significant ($P > 0.05$). Additive genetic effects were greater than maternal or non-additive genetic effects for all traits, explaining 4.8 – 5.4% of total phenotypic variance.

Table 4.3 Estimates of additive genetic, maternal and non-additive genetic effects for wet weight, standard length and total length for juvenile black bream measured at 75, 130 and 180 days

Age of measurement	Trait	Proportion of total phenotypic variance due to:		
		Additive genetic effects	Maternal effects	Non-additive genetic effects
75 days	Wet weight	0.004	0.091	0.073
	Standard length	0.001	0.114	0.058
	Total length	0.000	0.088	0.071
130 days	Wet weight	0.174	0.011	0.004
	Standard length	0.214	0.008	0.000
	Total length	0.187	0.009	0.000
180 days	Wet weight	0.054	0.018	0.023
	Standard length	0.051	0.037	0.021
	Total length	0.048	0.033	0.024

Discussion

In chapter 3, I found moderately high heritabilities (0.27 – 0.33) for wet weight, standard length and total length of black bream at 90 days of age, from a paternal half-sib analysis of variance, utilising 16 males and 8 females. Slightly lower estimated heritabilities (0.17 – 0.21) were found in this study from a factorial analysis at 130 days of age, although I was able to utilise only 6 sires and 5 dams. The very small number of parents used in this study means that there is a large degree of uncertainty in the genetic parameter estimates, but this was not the main purpose of the study. Instead, the aim was to measure the importance of variable stocking density, maternal effects and non-additive genetic effects on growth traits of black bream at different ages.

Stocking density

When implementing genetic improvement programs for fish species, a lack of appropriate and cost-effective identification systems for larval and juvenile animals often means that families are hatched and reared separately for considerable periods of time (Rye & Mao 1998; although see Fishback et al. 2002). This creates variable stocking densities and these may confound the estimation of breeding values in potential broodstock. In this study, the gradual thinning of tank numbers prior to first measurement did not achieve my aim of reducing the predictable environmental effect of variable progeny numbers on growth traits, because at 75 days stocking densities significantly affected wet weight, standard length and total length. Moreover, despite stocking densities being equalised after 75 days, this effect on growth traits appeared to linger until some time past 130 days but ceased before 180 days.

Maternal effects

In a factorial mating design, the difference between the dam and sire components of variance provides an estimate of the variance due to maternal genetic and environmental effects. In most fish, these effects are usually considered to be due to egg size and quality, which may have a large influence on growth during the early stages of development (Gjedrem 1983; Kinghorn 1983a; Gjerde 1986). Maternal effects on growth traits should therefore decrease as fish age (Henryon et al. 2002). I found that the dam variance component was significantly greater than the sire variance component for all growth traits in black bream at 75 days of age, but not at 130 days or 180 days. This suggests that maternal genetic and environmental effects dissipate some time after 75 days of age in the hatchery. This is of practical significance, as would like to institute a simple, low cost selection program to genetically improve growth rate in black bream, and it is important to know the minimum age at which potential broodstock will clearly demonstrate additive genetic differences.

Non-additive genetic effects

In addition to providing an estimate of maternal genetic and environmental effects, the factorial mating design adopted in this study also allows the estimation of non-additive genetic effects, which are contained in the interaction variance. I found no significant interaction variance components for any growth trait at any age, which suggests that dominance and epistatic effects on growth rate are relatively minor in this population of black bream. This is consistent with my failure to find heterosis for growth in this species in chapter 3, although substantial non-additive effects on growth have been found in chinook salmon *Oncorhynchus tshawytscha* and Atlantic salmon *Salmo salar* (Winkelman & Peterson 1994b; Rye & Mao 1998). One limitation of my study was that since I had no replication of family groups, so non-additive genetic effects were confounded with common environmental (tank) effects in the

interaction variance component; however, this would be expected to inflate, not decrease, my estimate of non-additive genetic effects.

One surprising feature of my data was the absence of significant additive genetic variance for any growth trait at 180 days of age. This was not due to any increase in within-family variance for growth at later ages, for example due to the establishment of dominance hierarchies causing competition for resources within tanks, but instead appeared to result from a marked decline in among-family variance for growth. I am unsure as to the nature of this effect at 180 days, but it seems relatively unimportant in a practical sense because I have a very strong indication that selection decisions regarding which black bream should be kept as broodstock and which should leave the hatchery for grow-out, could be made at about 130 days when components of additive genetic variation are detectable for all growth traits. Nevertheless, to validate this selection decision and the extent to which any or all of these growth traits could be used as selection criteria, I need to know what happens to them between when they leave the hatchery and when they are ready for harvest after 12 months in grow-out ponds. That information will allow me to understand by how much the same trait at different ages is influenced by the same or different genes, and so predict the response of genotypes in different environments (Falconer 1990; Hershberger *et al*. 1990; Pérez-Rostro & Ibarra, 2003).

Chapter 5

Toward the genetic improvement of feed conversion efficiency in fish

Preamble In my bioeconomic model of chapter 2, I assumed a constant relationship between feed intake and weight gain despite selective improvement of the latter trait. I also proposed that an increased genetic capacity for growth rate through selective breeding, might result in more of the feed consumed being effectively utilized for growth. This implies that these two traits are genetically correlated, and if that were the case, it would positively influence production system profitability. I have found genetic variation for weight gain in black bream, and theoretically, feed intake should also be amenable to selective improvement, but what is the evidence for their genetic (co)variation in cultured fish species?

Introduction

The power of selective breeding in increasing productivity and efficiency has been amply demonstrated in traditional livestock species. Aquaculture species, however, have hardly benefited from modern developments in animal breeding and selection, despite their typically high reproductive capacity, and therefore, high potential for genetic improvement. Where selection has been applied to fish species, the breeding objective is mostly enhanced growth rate, leading to a greater output of fish product (Gjedrem 1997), but the overall aim of fish breeding programs is improved profitability. As profitability is a function of both outputs and inputs, there is also a need to consider how inputs can be reduced through genetic improvement.

Feed costs are a major input, comprising 30 – 70% of the variable costs in almost any animal production system (Dickerson 1978; Bickel 1988; Campo & Turrado 1998), including aquaculture (Shang 1990; Tacon *et al*. 1995; Goddard 1996). Feed conversion efficiency (FCE) is an indicator of biological function that combines feed intake (the input variable) with growth

or weight (the output variable). Significant improvements in FCE using genetic and non-genetic methods have been made in other animal production systems, especially the pig and poultry industries (e.g. Luiting *et al*. 1994). There is no reason to think the same would not occur in fish production.

Aquaculture research effort has primarily focussed on non-genetic means for improving FCE (Kinghorn 1983a; Thodesen *et al*. 2001). FCE has been shown to vary with temperature (Brown 1951), size and age (Brett 1979), feeding level (Fontaine *et al*. 1997), nutritional content of feeds (Shyong *et al*. 1998; Cook & Scurlock 1998; Rudacille & Kohler 2000), body weight and composition (Springborn *et al*. 1992), physical exercise (Petrell & Jones 2000), and density-dependent interactions including competition, antagonism and stress (Jobling *et al*. 1995; Alanärä 1996). Important as non-genetic means are, only genetic changes can permanently improve the FCE of an aquaculture species across variable management regimes. Despite general agreement over the importance of FCE, there has been some confusion in the literature over the prospects for the genetic improvement of FCE in fish species. Kinghorn (1983a,b) calculated that the heritability of FCE in young rainbow trout (*Oncorhynchus mykiss*) was so low that there was little value in selecting for the trait. Conversely, Gjedrem (2000) and Thodesen *et al*. (2001) suggested that considerable additive genetic variation for FCE did exist in salmonid species, although Gjedrem (2000) believed that it was more efficient to improve the trait indirectly through selection for increased growth rate.

The aim of this chapter is to review the potential for genetic improvement of FCE in cultured fish species. Alternative avenues for the genetic improvement of FCE in fish include choice of breed, crossbreeding, and selection within breeds. This review focuses on the potential for within-breed selection in fish, however such is the dearth of information that it is often

necessary to refer to data on other vertebrates. I believe that confusion over the prospects for genetic improvement of FCE in fish species arises from a number of sources: problems with terminology; problems with how to measure feed intake; problems with experimental design for estimating genetic parameters; and problems with the appropriate selection criteria. I review each of these areas in turn, and suggest ways in which the problems might be addressed.

Problems in terminology

What is FCE?

FCE is the efficiency with which ingested feed is converted to saleable fish product. Although this appears conceptually simple, many different measures have evolved because of the complex nature of feed use within an animal and the different target endpoints of animals in a production system (Arthur 2001). Hence, FCE has been poorly defined because it has been confused with its component traits like feed intake and weight gain.

Comparative FCE measurements between individuals or groups of animals are usually made over a limited part of the production cycle, typically during the growth phase. A measure of efficiency based on a restricted phase of the production cycle, however, is not necessarily representative of a genotype's efficiency across the entire production system (Archer *et al*. 1999; Arthur 2001; Thodesen *et al*. 2001). Consideration of efficiency over the whole production system may depend on the summation of many different traits expressed in several classes of the total stock, each varying in feed inputs and production outputs (Herd & Bishop 2000; Johnston 2001). What I would like to identify is measures of FCE that are correlated with production system efficiency, and in which genetic improvement will provide correlated improvements in the profitability of the production system (Archer *et al*. 1999).

Gross and net FCE

The energy intake of growing animals is partitioned into that used to maintain body functions and that used to increase body mass. A distinction is therefore often made between measures of gross FCE, which do not attempt to separate the partitioning of feed intake between maintenance and growth, and measures of net FCE, which do attempt to partition the amount of feed intake used for maintenance, and the amount used for growth.

The most widely used measure of gross FCE is feed conversion ratio. Feed conversion ratio is the ratio between feed intake and weight gain measured on a time-constant basis (i.e. between 2 set points in time, usually referred to as the test period). The inverse of feed conversion ratio (i.e. the ratio between weight gain and feed intake) is usually called feed efficiency. Net FCE is sometimes measured as the partial efficiency of growth, which is the ratio of weight gain to feed intake, less the expected feed requirement for maintenance (Veerkamp *et al*. 1995). The expected maintenance requirement is estimated from standard feeding tables, which have the disadvantage of assuming that there is no variation among animals in the efficiency of feed used for maintenance (Archer *et al*. 1999). A more biologically realistic measure of net FCE is residual feed intake (Koch *et al*. 1963). Residual feed intake is the difference between actual feed intake and that predicted from observed requirements for production and body weight maintenance; the residual feed is the feed that cannot be accounted for, and the variation is due to both measurement and prediction error, and the ability of more efficient animals to consume less feed for the same output. Predicted feed intake is usually established from a multiple regression of feed intake on weight gain and mean metabolic weight over the test period.

All the estimates of FCE in fish, of which I am aware, are measures of gross FCE rather than net FCE. Unnecessary confusion occurs, however, because a number of different terms have been used to describe the trait. Many studies, for example, measure the ratio between weight gain and feed intake, and this is variously referred to as gross feed efficiency (Kinghorn 1983a,b), feed efficiency ratio (Thodesen *et al*. 1999; Thodesen *et al*. 2001), feed efficiency (Sanchez *et al*. 2001) and feed conversion efficiency (Imsland *et al*. 2000; Henryon *et al*. 2002). I propose that the terminology in Table 5.1 be used to avoid confusion when referring to FCE and its various measures and component traits.

Table 5.1 Terminology for measures of feed conversion efficiency and their component traits

Trait	Definition
Live weight	Weight at a specified age
Weight gain	Increase in weight (per day) over a specified time period
Feed intake	Amount of feed eaten (per day) over a specified time period
Feed conversion ratio	Feed intake per unit weight gain
Feed efficiency	Weight gain per unit feed intake
Partial efficiency of growth	Weight gain per unit feed less expected maintenance requirements
Residual feed intake	Observed feed intake less the expected feed requirements for maintenance and growth

In most livestock species, residual feed intake is usually preferred to feed conversion ratio as a measure of FCE, because it is thought to provide a better indication of production system efficiency. Feed conversion ratio is both phenotypically and genetically highly correlated with growth rate and other production traits (Archer *et al*. 1999), and there seems to be an

antagonistic relationship between increased production (expressed in juveniles) and maintenance costs of the population (expressed in mature adults) in most animals (Luiting *et al*. 1994; Veerkamp *et al*. 1995). Therefore, improved gross efficiency during the production phase will not necessarily improve the efficiency of the whole production system, because the measurement includes both production (growth) and maintenance energy requirements, and they will differ during the life cycle.

For aquaculture species, the situation may be different for two reasons. First, Gjedrem (1983) argued that fish probably have a lower maintenance energy requirement than other domesticated animals because they are poikilothermic, although domestic conditions may increase stress levels, which would raise the maintenance requirement. Second, and more importantly, the high fecundity of most aquaculture species means that particular aquaculture enterprises usually specialise in specific parts of the production cycle. This is especially true for finfish species, where the industry typically consists of comparatively few major hatcheries, where broodstock are held, and a large number of grow-out enterprises, which sell their stock prior to sexual maturation. The proportion of growing animals to breeding animals is therefore much greater in aquaculture than in most livestock industries, and FCE in the growing phase of the production cycle is more likely to be closely correlated with efficiency over the whole production system (Kinghorn 1983a; Henryon *et al*. 1999).

Problems with estimating feed intake

Regardless of which measure of FCE is used, the operational requirements for the fish breeder are similar; an estimate of feed intake and an estimate of live weight change over a defined period of time. A number of problems arise with the accurate estimate of feed intake.

Estimating feed intake in individuals or groups

Maximum information is gained when feed intake is recorded for individual fish (e.g. Cook & Scurlock 1998), but this creates a high cost of measurement and is not practical for schooling species (Jobling *et al*. 1995). Feed intake is therefore mostly measured on groups of fish (e.g. Fontaine *et al*. 1997; Thodesen *et al*. 1999, 2001; Imsland *et al*. 2000; Jonassen *et al*. 2000; Rasmussen & Ostenfeld 2000; Valente *et al*. 2001). This often results in wasted feed, especially if the fish are fed to apparent satiation, which may provide a significant source of measurement error. Alanärä (1996) reviewed the use of automatic feeders that allow fish to self-regulate intake, but these do not eliminate waste and may have the additional complication of increasing within-group variance of intake because of the establishment of dominance hierarchies (see also Gélineau *et al*. 1998).

Helland *et al*. (1996) describe a method for the measurement of daily feed intake of groups of fish that involves the collection of wasted feed from effluent, and calculating the difference between the amounts fed and the waste collected (corrected for leaching loss). This technique shows most promise as a proven, practical, and cost-effective method for measuring feed intake under production conditions. An alternative approach may be to search for an indirect measure; Kinghorn (1983b), for example, used oxygen consumption as an indirect measure of feed intake by considering it as an analogue of energy metabolised, but the accuracy of this approach has been questioned (Gjoen *et al*. 1991; Thodesen *et al*. 1999). A number of physiological and behavioural markers of feed intake and FCE have been investigated in livestock species, including body composition (Di Costanzo *et al*. 1990), physical activity (Luiting *et al*. 1994), and total plasma protein (Richardson *et al*. 1996), but to date no effective marker has been identified. In recent years there has been much interest in the possibility of

genetic markers, but even for species with well developed gene maps, such as cattle and pigs, no putative QTLs or linked markers for feed intake or FCE have been identified.

Apparent satiation versus restricted feeding levels

Little is known of fish satiety mechanisms and debate continues over the relative merits of restricted versus apparent satiation feeding levels when estimating FCE (Gropp & Tacon 1994; Sanchez *et al*. 2001). Feeding to apparent satiation obviously allows more variation in voluntary feed intake to be expressed (Henryon *et al*. 2002), and FCE under apparent satiation and restricted feeding conditions may be regarded as different traits to some extent. Feeding conditions on test should therefore be the same as feeding conditions in the production environment, if the trait to be improved is to have commercial relevance. Problems arise when both apparent satiation and restricted feeding are used in production, or when the restricted feeding levels vary under production conditions. As it would be impractical to routinely test potential broodstock for their FCE under a range of feeding conditions, we must know the genetic correlations between FCE (or feed intake) under the standard testing regime, and the range of production regimes likely to be experienced.

Time of test

Feed intake and weight gain of fishes are often measured over short durations (e.g. one month, Thodesen *et al*. 2001; but see Sanchez *et al*. 2001), although there appears to be little theoretical or empirical justification for these time periods. Studies in cattle, using automatic feeders, have suggested that, following a 21-day adjustment period a 35-day test provides an accurate estimate of feed intake, although estimating weight gain may require a significantly longer test period (Archer *et al*. 1997). Physiological differences among individuals can cause diurnal fluctuations in live weight, implying that multiple, daily measurements might be required

(Knee 2001). Such a test regime would be impractical for most aquaculture species, because of the trauma associated with anaesthesia, removal from water, and post-measurement recovery. For fish, the optimal test period will probably be species-specific, depending on factors including acclimation to the feeding regime(s), and growth rate. This means that for each species, we need an empirical determination of the optimal (i.e. most cost effective) test period to accurately estimate both feed intake and weight gain.

Problems with genetic parameter estimates of FCE

Heritability of FCE measures

Estimated heritabilities of both feed conversion ratio and residual feed intake for cattle, pigs and poultry are typically in the range 0.3 – 0.5 (Luiting 1987; Korver 1988; Katle 1991; Mrode & Kennedy 1993; Veerkamp *et al*. 1995; Archer *et al*. 1999). There have been very few estimates of heritability for any FCE measures in fish. Kinghorn (1983b) reported a heritability of 0.03 (± 0.10, i.e. not significantly different from 0) for feed efficiency and 0.31 (± 0.11) for the partial efficiency of growth in rainbow trout, although he regarded the estimate for partial efficiency of growth to be misleading because it assumed constant maintenance requirements. Thodesen *et al*. (2001) found a significant difference in feed efficiency among full-sib families of Atlantic salmon (*Salmo salar*), and Henryon *et al*. (2002) reported significant additive genetic variance for feed efficiency in a farmed population of rainbow trout, with an upper-bound estimate for heritability of 0.7 (Henryon *et al*. reported only additive genetic variance and did not calculate heritability because within-group variances of feed intake could not be estimated). Gjoen *et al*. (1991) reported a heritability of 0.15 (± 0.30) for feed intake in rainbow trout, however FCE was not calculated.

Given this diversity of estimated heritabilities, it is clear that more studies are needed to determine the rate of response of FCE to selection in genetic improvement programs. The major problem with heritability estimates for FCE traits in fish, is that feed intake is usually measured on family groups rather than on individuals. Within-family variance is therefore not estimated and genetic parameters relate only to family means. This will overestimate individual heritability, unless a correction is made (e.g. Kinghorn 1983b).

Genetic correlations

Genetic correlations between FCE and other traits have been estimated in many livestock species, both through breeding experiments and by measuring correlated responses to selection. Of major interest are the correlations between FCE measures taken at different points in the life cycle and under different feeding conditions, and the correlations between FCE measures and their component traits, particularly growth rate.

There is some evidence from terrestrial livestock species that, while the genetic correlation between feed conversion ratios measured in growing and mature animals is low, a moderate, positive genetic correlation (0.60 – 0.70) exists between residual feed intake at different ages (Nieuwhof *et al*. 1992; Archer *et al*. 1999; Pitchford 2001). Henryon *et al*. (2002) estimated genetic correlations between feed efficiency in rainbow trout at different times over a 215-day period. Most of the correlations were positive and high, suggesting that feed efficiency was controlled by the same set of genes over the grow-out period. Of more interest are the genetic correlations between FCE at markedly different points on the growth curve, for example in the initial post-hatching phase and at the end of the commercial grow-out phase. There are no data on these correlations in fish species, although physiological considerations indicate that the relationship between age and FCE is likely to be complex and non-linear (Paloheimo & Dickie

1966; Brett 1979), and FCE may therefore be controlled by different sets of genes along the growth curve.

In terrestrial livestock species, there has been concern about the genetic correlations between FCE measures when animals are fed on different types of feed (e.g. Fan *et al*. 1995). In fish, the concern is not so much about FCE on different food types as about FCE under different feeding conditions, particularly apparent satiation or restricted feed intake conditions. There are no data on genetic correlations between FCE measures under these different feeding conditions. Sanchez *et al*. (2001) found that feed efficiency did not differ between strains of brown trout (*Salmo trutta*) selected or not selected for increased growth rate when the fish were fed to apparent satiation, but when the fish were on a restricted diet, feed efficiency was greater in the non-selected line. The implication is that selection for increased growth also increased feed intake, suggesting that there may not be a high genetic correlation between FCE on apparent satiation and restricted diets; more studies are needed to test this hypothesis.

Many studies have reported strong genetic correlations between feed conversion ratio and growth rate in cattle, pigs and poultry, with more efficient animals being faster growing (Pym & Nicholls 1979; Korver 1988; Archer *et al*. 1999; Pitchford 2001). Residual feed intake is, by definition, phenotypically independent of growth rate, but Kennedy *et al.* (1993) showed that it is not necessarily genetically independent. The few studies that have estimated genetic correlations between residual feed intake and growth rate in livestock species show low to moderate correlations, again with more efficient animals being faster growing (Mrode & Kennedy 1993; Pitchford 2001). Thodesen *et al*. (1999) found that Atlantic salmon selected for increased growth rate had greater feed efficiency than wild salmon, and Ogata *et al*. (2002) found that Japanese flounder (*Paralichthys olivaceus*) selected for increased growth rate also had significantly higher

final body weight, weight gain and feed intake (using size as a covariate in the analysis to correct for expected maintenance requirements) than wild flounder. Both Thodesen *et al*. (1999) and Thodesen *et al*. (2001) also reported positive phenotypic correlations between growth rate and feed efficiency (0.79 – 0.90) in Atlantic salmon. Phenotypic correlations, however, are not necessarily good predictors of genetic correlations among traits (Willis *et al*. 1991). The only estimated genetic correlations between feed efficiency and growth rate of which I am aware, are for rainbow trout: 0.80 (Kinghorn 1983b) and 0.99 (Henryon *et al*. 2002).

Problems with selection to improve FCE in fish

Gjedrem (2000) argued that, despite its economic importance, FCE should not be included in the breeding objective for cultured fish species because it was difficult and expensive to measure. Although it may be impractical and uneconomic to measure FCE in an individual aquaculture enterprise, this does not mean it should not be considered on an industry-wide basis. The specialised nature of aquaculture enterprises, with a division into breeding and grow-out sectors, lends itself to a centralised nucleus-breeding scheme. Any decision on the economic advantages of genetically improving FCE requires a formal economic analysis of investment and returns from genetic improvement in an industry-wide context. That is, the costs of implementing selection for improved FCE in the breeding sector of the industry need to be compared with the discounted returns obtained from disseminating the superior genetics into the grow-out sector (Archer & Barwick 1999). Such an analysis has not yet been attempted for any fish species.

There are inherent problems with using any of the common measures of FCE, such as feed conversion ratio or residual feed intake, as selection criteria. Genetic variation in feed conversion ratio or residual feed intake includes genetic variation in their component traits (feed

intake and growth rate/weight gain) and in relationships between them (Campo & Turrado 1998; Kennedy *et al*. 1993). In addition, ratios have poor statistical properties because the ratio of two dependent normal variables is not normally distributed (Iwaisaki & Wilton 1993), and may produce fallacious indications of economic efficiency (Melton & Colette 1993). As a result, the use of a ratio as a selection criterion does not allow an accurate prediction of the expected response to selection, and may result in different responses in the component traits (Gunsett 1984).

These methodological complications mean that it may be more appropriate to use the component traits (feed intake and growth rate/weight gain) as separate criteria in a selection index, rather than combining them in a single measure. Feed conversion ratio and residual feed intake provide no additional genetic information over and above that provided by their component traits, and genetic improvement in FCE will be best achieved through multiple trait selection on growth rate, feed intake and other traits of importance, using an appropriate profit function to determine their relative economic weights (Kennedy *et al*. 1993; Davis & Hetzel 2000).

Chapter 6

Indicators of genetic variation for feed conversion efficiency in black bream

Preamble Genetic variation for feed conversion efficiency, which is an indicator of biological function that combines feed intake and growth rate (or weight gain) traits, exists in most production animals. Before this variation can be fully exploited for economic gain in cultured fish species, however, technical and genetic obstacles relating its accurate measurement and appropriate interpretation need to be better understood. These include the appropriate method and length of time with which to assess components of genetic (co)variation for feed intake and weight gain, and how this knowledge might be incorporated in a genetic improvement program.

Introduction

In chapter 5, I found evidence for substantial genetic variation in FCE and its component traits in terrestrial livestock species and although data are few, the same is likely for cultured fishes. There are, however, a number of problems with selecting from this variation to genetically improve FCE in fish species. First, it is usually impractical (not cost effective) to measure feed intake on individuals (Gjedrem 1983; Helland *et al*. 1996), so that family mean data taken from growing animals needs to be used (Kinghorn 1983b; Helland *et al*. 1996; Henryon *et al*. 1999). Second, the optimal time period over which to test fish for FCE is unknown. Feed intake and weight gain of fishes are measured over a range of time periods from one to many months (e.g. Sanchez *et al*. 2001; Thodesen *et al*. 2001; Henryon *et al*. 2002), although there appears to be little theoretical or empirical justification for these durations. The optimal test period will probably be species-specific, and depend on factors including acclimation to the feeding regime and growth rate. This means that for each species, we need an empirical determination of the optimal (i.e. most cost effective) test period to

accurately estimate feed intake and weight gain (Chapter 5). Third, genetic improvement in FCE will probably best be achieved by incorporating feed intake into a selection index, along with growth rate. However, although moderate additive genetic variation has been found for weight gain in black bream (Chapters 3 & 4), there are no data on the extent of genetic variation in feed intake, or the covariation between feed intake and weight gain.

In this chapter, I make preliminary estimates of the optimal time period over which to test fish for FCE, and of the extent of genetic (co)variation in feed intake and weight gain in families of black bream, based on serial tests in which the fish are fed to apparent satiation.

Materials and methods

Fish and rearing conditions

The trial utilised 300, 260-day old black bream (average initial wet weight = 39.42 ± 10.06 g) arising from an *in vitro* mating design (see Jenkins *et al*. 1999; Partridge *et al*. 2003) where each of five sires were mated to a single dam, providing half-sib groups for the estimation of sire components of variance. Offspring from each sire (n = 60) were randomly divided three ways (i.e. three replicate groups per sire) and arbitrarily allocated among 15, 300 litre aquaria (i.e. 0.066 fish.L^{-1}) connected to flow-through seawater (35 ppt at 4 $L.min^{-1}$) heated to 22 ± 1ºC (Partridge & Jenkins 2002). Fish had been gradually accustomed to these conditions, and to a feeding regime where they were fed to apparent satiety twice daily with a composite diet of 2 and 3 mm extruded pellets (Skretting feeds; 92.5% dry matter, 58% protein, 11% fat). Mortalities were replaced with a clearly marked fish of similar size from a reciprocal 'reserve' full-sib tank maintained under the same stocking densities and feeding conditions.

Feeding and growth trials

Feed intake and weight gain were estimated over a 56-day test period. At the start of the experiment (day 0) all fish were removed, anaesthetised (40 $mg.L^{-1}$ AQUI-S in the presence of pure oxygen), and measured for wet weight. Fish were then returned to their respective tanks to recover under 'black-out' conditions and no feed was offered.

Beginning on the following morning (day one) and 30 minutes before feeding, each tank was vacuumed and a 10% water exchange was undertaken to prevent nitrate accumulation (Partridge & Jenkins 2002). The central standpipe containing 3 mm mesh was exchanged for one containing 500 μm mesh to prevent feed being filtered from the tank. Each tank was administered 30 grams of feed comprising 8 g of 2 mm feed and 22 g of 3 mm feed, for one hour. After one hour, all uneaten feed was siphoned onto a 350 μm screen, the 3 mm mesh standpipe was replaced in the tank, and flow-through filtration was resumed. Collected feed was dried at 95ºC for 24 h, and feed intake was recorded as the total dry weight (g) corrected for the average moisture content and the average water stability (following 1 h of immersion) of 30 g pellets. The procedure was repeated each morning and afternoon.

Weight gain and total feed intake were calculated cumulatively for each replicate full-sib family group at the end of each of four 14-day test phases (i.e. days 1 – 14, 1 – 28, 1 – 42, & 1 – 56). To maintain a consistent effect on fish due to feeding and measurement during each test period, all tanks were 'blacked-out' following feeding on the afternoon prior to handling, and remained in darkness until harvest and measurement. The following test phase began on the afternoon of measurement and at least two hours following all fish being fully recovered and showing normal activity.

Analysis

The effect of sire on weight gain and feed intake was estimated at 14, 28, 42 and 56 days using a one-way analysis of variance, with sire as a random effect and initial (i.e. day 0) weight as a covariate (see Valente *et al*. 2001; Ogata *et al*. 2002).

Two composite measures of FCE were used. Feed efficiency was calculated for each replicate full-sib group as (final weight – initial weight)/(dry feed intake), and residual feed intake was calculated as the residual from the linear model:

$$Y_{ijk} = \mu + T_i + G_j + e_{ijk}$$

where Y_{ijk} is test period feed intake, μ is the overall mean, T_i is the initial test weight, G_j is the test period weight gain, and e_{ijk} is the residual error. Genetic correlations (± SE) were calculated from Becker (1984).

Results and discussion

Experimental management was kept uniform so that any environmental deviations were assumed to have a random distribution across all tanks and treatments, and stocking densities remained constant with only a single mortality on day 37. The 56-day test period family mean for weight gain was 18.86 ± 2.20 g (range: 14.17 – 22.72 g; CV = 11.6), feed intake was 44.55 ± 2.66 g (range: 40.82 – 50.22 g; CV = 5.9), feed efficiency was 0.41 (range: 0.33 – 0.48) and the median residual feed intake was –0.15 ± 0.97 g (range: –1.81 – 3.70 g). A summary of trait measurements for these groups is provided in Appendix 5.

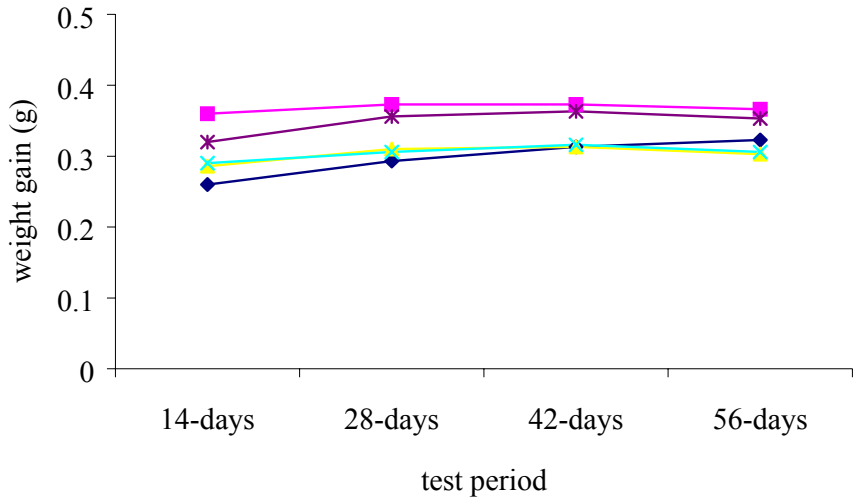
Evidence for genetic variation in feed conversion efficiency

There were no significant effects of sire on either weight gain or feed intake until day 42 of the test (weight gain: $F = 3.55$, $P = 0.05$; feed intake: $F = 3.76$, $P = 0.04$), and again at day 56 (weight gain: $F = 3.04$, $P = 0.07$; feed intake: $F = 5.33$, $P = 0.01$). After day 42, 57% of the variance in weight gain and 55% of the variance in feed intake were due to differences among sires. These variance component estimates were not suitable for estimating heritabilities because my experimental design artificially reduced within-sire variance. Most importantly, between-individual genetic variance was not estimated because measurements (of feed intake) were made at a group (tank) level.

Support for a minimum test period of 42 days to detect genetic variation in component traits of FCE can be seen in the relationships between the test duration and each of mean family group weight gain (Fig. 6.1a), and feed intake (Fig. 6.1b). In either figure, a minimum test period of 42 days is required for the trait to stabilise.

There were significant positive phenotypic correlations between weight gain and feed intake after both 42 days ($r_P = 0.78$, $P < 0.005$) and 56 days ($r_P = 0.69$, $P < 0.005$). Genetic correlations were also high and positive at both times (42 days: $r_G = 0.96 \pm 0.34$; 56 days: $r_G = 0.84 \pm 0.12$), but should not be regarded as any more than indicative for the same reasons as outlined above for heritabilities.

(a)



(b)

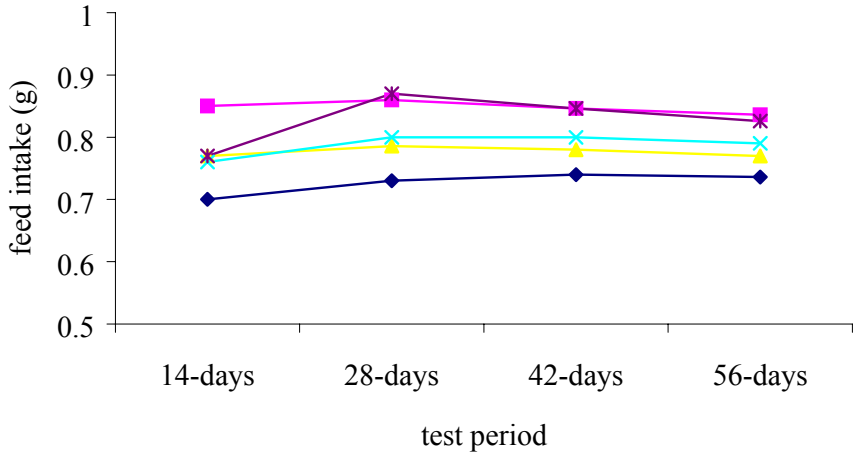


Figure 6.1 Relationships between length of test period and mean family weight gain (a), and mean family feed intake (b). Different full-sib families are represented by the different symbols

Practical implications for the genetic improvement of feed conversion efficiency

I propose two options for how these findings might be practically applied to the genetic improvement of FCE in cultured fish species. The first is to use a measure of either gross FCE (e.g. feed efficiency) or net FCE (e.g. residual feed intake or RFI). Both composite measures of FCE include the same component traits of weight gain and feed intake. They differ in that RFI partitions feed intake into components due to body maintenance and growth rate, whereas feed efficiency does not. This distinction should be less important for poikilothermic than homeothermic animals (Gjedrem 1983), and we might therefore expect the two traits to be highly correlated in fish. At 42 days, however, I found only a moderate phenotypic correlation ($r_P = -0.513$, $P = 0.05$), and wide family group variation for both feed efficiency and RFI (Fig. 6.2).

If we were to choose one of these composite measures to evaluate FCE in fish species, which should we use? There are at least three points supporting the use of RFI over feed efficiency. First, unlike feed efficiency, RFI is phenotypically independent of growth rate, although it is not necessarily genetically independent (Kennedy *et al*. 1993). Second, Gunsett (1984) argued that because feed efficiency is a ratio, its use as a selection criterion does not allow an accurate prediction of the response to selection, and may result in different responses in the component traits (see also Campo & Turrado 1998). Third, RFI is thought to better reflect production system efficiency (Archer *et al*. 1999). This is an important point because the high fecundity of most aquaculture species means that particular aquaculture enterprises usually specialise in specific parts of the production cycle. This is especially true for finfish species, where the proportion of growing animals to breeding animals is very high and FCE in the growing phase of the production cycle is more likely to be closely correlated with efficiency over the whole production system (Chapter 5).

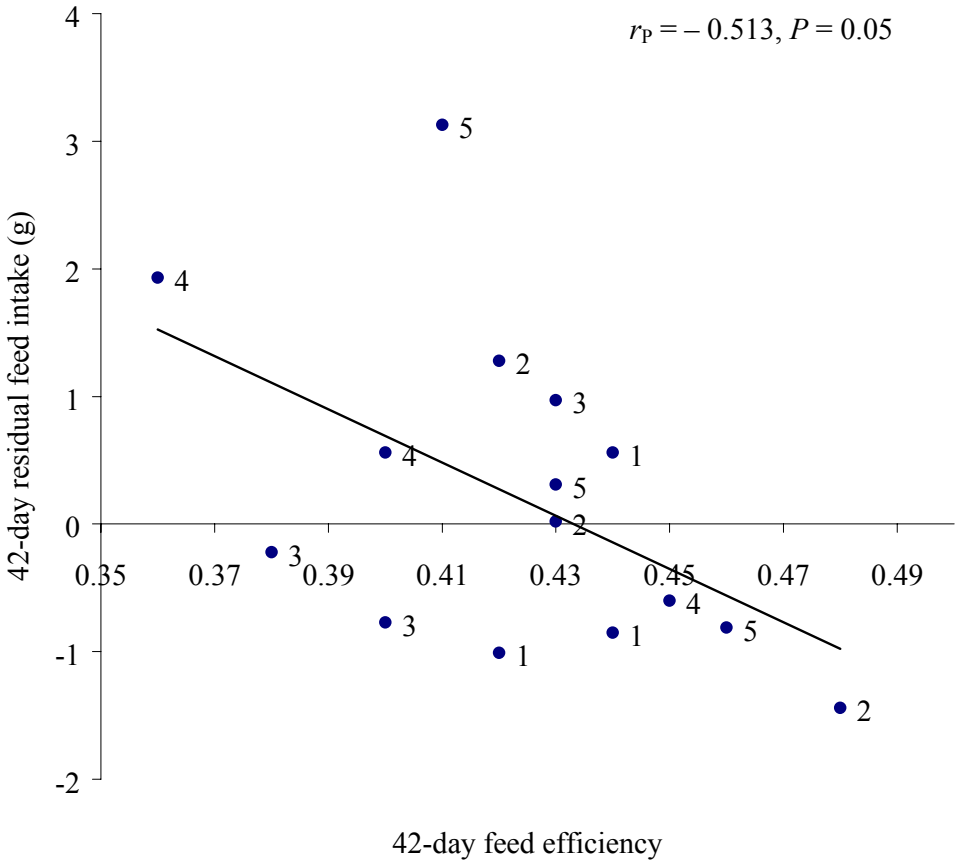


Figure 6.2 Correlation between residual feed intake and feed efficiency at 42 days. • indicates full-sib family group by sire; points with the same number are replicate full-sib groups

Despite the widespread use of FCE measures, such as feed efficiency and RFI, there are still problems in their implementation in genetic improvement programs. It is particularly difficult to obtain unbiased estimates of genetic parameters (heritabilities and genetic correlations) from composite traits, such as feed conversion ratio or residual feed intake (Kennedy *et al*. 1993). Genetic variation in either feed efficiency or RFI includes genetic variation in their component traits and in the relationships between them (Campo & Turrado 1998). Whilst I found additive genetic variation in both feed intake and weight gain at my

proposed optimal test period of 42 days, there was no significant additive genetic variation for either feed efficiency ($F = 0.600$, $P = 0.67$) or residual feed intake ($F = 0.167$, $P = 0.94$).

The second option for the practical genetic improvement of FCE in fishes is to avoid composite measures and use the component traits of feed intake and weight gain in either a weighted selection index, or a two-step selection process. In a selection index, the relative merits of either trait need to be weighted in a selection index along the lines of, $I = b_1 y_{WG} b_2 y_{FI}$, where b_1 and b_2 are index weights derived from the economic weights for weight gain (y_{WG}) and feed intake (y_{FI}) traits respectively (van der Werf 2001). Weight gain and feed intake are positively genetically correlated and therefore act antagonistically during selection, because we wish to increase the former and decrease the latter; it is therefore very important that we obtain accurate estimates of their genetic correlations.

Accurate estimates of genetic (co)variation could be achieved most effectively by measuring weight gain and feed intake on individual fish, but that requires some form of specific marking system. Physical marking of young fish is notoriously difficult (Doupé *et al*. 2003c), and the most promising option for the future is genetic fingerprinting (Fishback *et al*. 2002). Nevertheless, even with a specific marking system there are still technical difficulties with measuring feed intake on individual fish (Chapter 5). The alternative is to measure feed intake on a group basis, as in the current design, but with much greater replication. This soon becomes prohibitively expensive in space and equipment.

A two-step selection process would involve, for example, selecting for the fastest growing families based on achieving a minimum level of performance in weight gain, and then selecting for families displaying the most efficient feed intake. Such tandem selection is

not only time consuming, but it may result in a correlated response in one or more other traits either in or outside of the selection index (Tave 1986).

Chapter 7

Epilogue

An increase in growth rate of about 33% is required for black bream to become a profitable species for inland saline aquaculture, irrespective of whether fish production is an independent commercial operation or that it is incorporated within an existing farm enterprise. Growth rate may be genetically improved by crossbreeding among populations and/or by selection within populations. I found no evidence that crossbreeding could provide an increase in growth rate through heterosis. There was, however, substantial additive genetic variation for selection. At typical selection intensities, we could expect a rate of genetic improvement in growth rate of about 10% per generation.

The high genetic correlations among standard and total length and wet weight, indicates that any or all growth traits could serve as useful selection criteria between 90 and 130 days of age, when fish are either kept as broodstock or sold for grow-out. Length is simpler and cheaper to measure than weight and may be the preferred criterion in the implementation of a hatchery genetic improvement program. Weight gain in black bream, however, also appears to be negatively genetically correlated to feed intake. The implication of this finding is that many of the gains to be made by selection for weight gain might be nullified if this (co)variation is not accounted for by appropriate weighting in a selection index.

We are still not at a stage to derive suitable trait weights for a selection program to improve black bream growth rate, because the breeding objective is improved on-farm growth rate between the time that the fish are stocked and 12 months later, when they are harvested. This

project is currently underway and will enable us to predict how much genetic (co)variation exists between the grow-out and other production traits, and to confirm the suitability and accuracy of selection decisions that we made in the hatchery 12 months earlier.

Chapter 8

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Appendix 1 Summary population and growth parameters for selected straight-bred and crossbred lines of black bream at age 90 days

Breed [1]	Population size (n)	Stocking density (Fish.L^{-1})	Wet weight (g) ($\bar{x} \pm$ S.D.; C.V.)	Standard length (mm) ($\bar{x} \pm$ S.D.; C.V.)	Total length (mm) ($\bar{x} \pm$ S.D.; C.V.)
Straight-bred sire lines					
S x S	355	1.18	2.44 ± 1.32; 54.0	40.48 ± 6.63; 16.0	46.20 ± 7.15; 15.0
S x S	932	3.11	1.99 ± 0.92; 46.0	37.33 ± 5.89; 15.0	43.10 ± 6.25; 14.5
S x S	813	2.71	2.15 ± 1.00; 46.5	37.92 ± 6.24; 16.4	44.05 ± 6.73; 15.2
S x S	644	2.15	2.34 ± 0.85; 36.3	39.70 ± 5.02; 12.6	46.04 ± 5.38; 11.6
S x S	440	1.46	3.26 ± 1.02; 31.2	44.04 ± 5.08; 11.5	50.70 ± 5.53; 10.9
S x S	968	3.22	1.65 ± 0.69; 41.8	34.84 ± 5.09; 14.6	40.40 ± 5.60; 13.8
S x S	1405	4.68	1.66 ± 0.60; 36.1	34.32 ± 4.56; 13.2	39.00 ± 4.78; 12.2
B x B	52	0.17	3.63 ± 1.27; 34.9	47.80 ± 6.00; 12.5	54.52 ± 6.75; 12.3
B x B	67	0.22	4.66 ± 0.98; 21.0	51.64 ± 3.78; 7.31	58.48 ± 3.99; 6.82
Crossbred sire lines					
B x S	2099	6.99	1.13 ± 0.40; 35.3	31.28 ± 3.83; 12.2	36.20 ± 4.19; 11.5
B x S	230	0.76	3.61 ± 1.39; 38.5	46.69 ± 6.60; 14.1	53.01 ± 7.32; 13.8
B x S	737	2.45	2.13 ± 0.71; 33.3	38.82 ± 4.78; 12.3	44.73 ± 5.21; 11.6
B x S	645	2.15	2.64 ± 0.81; 30.6	40.62 ± 5.52; 13.5	46.52 ± 6.14; 13.1
B x S	233	0.77	3.36 ± 1.50; 44.6	43.90 ± 6.74; 15.3	50.49 ± 7.34; 14.5
B x S	2130	7.14	1.09 ± 0.35; 32.1	31.16 ± 3.56; 11.4	36.20 ± 3.81; 10.5
B x S	793	2.64	2.77 ± 0.82; 29.6	42.40 ± 4.28; 10.0	48.44 ± 4.72; 9.74
S x B	14	0.04	2.40 ± 0.84; 35.0	43.00 ± 5.15; 11.9	49.07 ± 5.74; 11.6
S x B	35	0.11	2.92 ± 1.42; 48.6	44.88 ± 6.69; 14.9	51.60 ± 7.46; 14.4

[1] S is Swan River and B is Blackwood River

Appendix 2 Summary population and growth parameters for straight-bred lines of black bream at age 75 days

Family		Population size	Stocking density	Wet weight (g)	Standard length (mm)	Total length (mm)
Sire	Dam	(n)	(Fish.L^{-1})	($\bar{x} \pm$ S.D.; C.V.)	($\bar{x} \pm$ S.D.; C.V.)	($\bar{x} \pm$ S.D.; C.V.)
2	A	200	0.66	0.64 ± 0.25; 39.0	25.43 ± 3.97; 15.6	29.90 ± 4.26; 14.2
3	A	205	0.68	0.72 ± 0.26; 36.1	27.10 ± 3.69; 13.6	31.46 ± 4.04; 12.8
4	A	124	0.41	0.60 ± 0.26; 43.3	25.63 ± 4.45; 17.3	30.36 ± 4.99; 16.4
5	A	348	1.16	0.64 ± 0.24; 37.5	25.86 ± 3.30; 12.7	30.20 ± 3.69; 12.2
6	A	135	0.45	0.62 ± 0.28; 45.1	25.83 ± 4.28; 16.5	30.43 ± 4.76; 15.6
1	B	1034	3.44	0.58 ± 0.19; 32.7	24.66 ± 3.61; 14.6	28.86 ± 3.90; 13.5
2	B	1083	3.61	0.52 ± 0.18; 34.6	24.10 ± 3.55; 14.7	28.60 ± 4.58; 16.0
3	B	911	3.03	0.49 ± 0.21; 42.8	23.40 ± 3.95; 16.8	27.33 ± 4.28; 15.6
4	B	985	3.28	0.48 ± 0.17; 35.4	23.33 ± 3.56; 15.2	27.43 ± 3.83; 13.9
5	B	972	3.24	0.65 ± 0.26; 40.0	26.73 ± 4.02; 15.0	30.90 ± 4.52; 14.6
6	B	1006	3.35	0.53 ± 0.19; 35.8	24.03 ± 3.45; 14.3	27.83 ± 3.99; 14.3
1	C	888	2.96	0.57 ± 0.18; 31.5	25.46 ± 2.88; 11.3	29.76 ± 3.33; 11.1
2	C	1033	3.44	0.54 ± 0.18; 33.3	24.90 ± 3.52; 14.1	29.03 ± 3.69; 12.7
4	C	956	3.18	0.56 ± 0.22; 39.2	25.36 ± 3.52; 13.8	29.80 ± 4.04; 13.5
5	C	886	2.95	0.54 ± 0.18; 33.3	25.43 ± 2.62; 10.3	29.83 ± 3.07; 10.2
6	C	1125	3.75	0.53 ± 0.26; 49.0	24.40 ± 4.85; 19.8	28.33 ± 4.72; 16.6
1	D	474	1.58	0.70 ± 0.24; 34.2	26.72 ± 3.72; 13.9	31.10 ± 4.13; 13.2
2	D	392	1.30	0.85 ± 0.26; 30.5	28.70 ± 3.62; 12.6	33.23 ± 3.87; 11.6
3	D	391	1.30	0.73 ± 0.24; 32.8	26.90 ± 3.29; 12.2	31.50 ± 3.55; 11.2
4	D	402	1.34	0.78 ± 0.24; 30.7	28.23 ± 3.30; 11.6	32.93 ± 3.53; 10.7
5	D	87	0.29	0.70 ± 0.25; 35.7	27.56 ± 3.52; 12.7	32.26 ± 3.70; 11.4
6	D	100	0.33	0.74 ± 0.25; 33.7	27.66 ± 3.35; 12.1	32.26 ± 3.77; 11.6
1	E	733	2.44	0.56 ± 0.20; 35.7	24.86 ± 3.32; 13.3	29.03 ± 3.64; 12.5
2	E	1390	4.63	0.39 ± 0.17; 43.5	21.66 ± 3.83; 17.6	25.66 ± 4.20; 16.3
3	E	975	3.25	0.66 ± 0.23; 34.8	26.00 ± 3.89; 14.9	30.46 ± 4.53; 14.8
4	E	1321	4.40	0.38 ± 0.18; 47.3	21.43 ± 3.55; 16.5	25.23 ± 4.10; 16.2
5	E	170	0.56	0.58 ± 0.33; 56.8	25.03 ± 5.94; 23.7	29.40 ± 6.46; 21.9
6	E	1127	3.75	0.50 ± 0.20; 40.0	23.53 ± 3.62; 15.3	27.56 ± 3.87; 14.0

Appendix 3 Summary population and growth parameters for straight-bred lines of black bream at age 130 days

Family		Family size	Survival	Stocking density	Wet weight (g)	Standard length (mm)	Total length (mm)
Sire	Dam	(n)	(%)	(Fish.L^{-1})	($\bar{x}$ ± S.D.; C.V.)	($\bar{x}$ ± S.D.; C.V.)	($\bar{x}$ ± S.D.; C.V.)
2	A	92	92	0.306	4.60 ± 2.14; 46.5	50.16 ± 8.68; 17.3	57.13 ± 9.18; 16.0
3	A	94	94	0.313	5.05 ± 2.58; 51.0	50.60 ± 8.63; 17.0	57.63 ± 9.11; 15.8
4	A	53	53	0.176	3.17 ± 1.69; 53.3	43.50 ± 8.91; 20.4	50.10 ± 9.94; 19.8
5	A	93	93	0.31	4.13 ± 1.80; 43.5	47.83 ± 8.23; 17.2	55.60 ± 7.76; 13.9
6	A	89	89	0.296	5.61 ± 2.06; 36.7	52.93 ± 7.45; 14.0	60.10 ± 8.06; 13.4
1	B	99	99	0.33	4.33 ± 2.19; 50.5	48.76 ± 10.77; 22.0	56.06 ± 11.85; 21.1
2	B	96	96	0.32	5.08 ± 1.88; 37.0	52.30 ± 7.23; 13.8	59.36 ± 7.59; 12.7
3	B	100	100	0.333	4.54 ± 2.01; 44.2	49.66 ± 9.80; 19.7	57.00 ± 9.97; 17.4
5	B	96	92	0.32	4.05 ± 2.13; 52.5	48.23 ± 9.65; 20.0	55.06 ± 10.41; 18.9
6	B	100	100	0.333	5.14 ± 1.74; 33.8	53.36 ± 8.18; 15.3	60.30 ± 9.02; 14.9
1	C	100	100	0.333	4.76 ± 2.01; 42.2	50.73 ± 9.01; 17.7	57.83 ± 9.68; 16.7
2	C	96	96	0.32	5.26 ± 2.00; 38.0	53.00 ± 7.26; 13.6	60.23 ± 7.70; 12.7
4	C	100	100	0.333	5.48 ± 2.42; 44.1	52.63 ± 9.59; 18.2	59.80 ± 10.50; 17.5
5	C	95	95	0.316	4.41 ± 2.79; 63.2	47.90 ± 12.05; 25.1	54.96 ± 13.52; 24.5
6	C	95	95	0.316	5.67 ± 2.56; 45.1	53.13 ± 8.85; 16.6	60.36 ± 9.56; 15.8
1	D	99	99	0.33	5.25 ± 2.28; 43.4	51.73 ± 8.85; 17.1	59.13 ± 9.38; 15.8
2	D	96	96	0.32	6.13 ± 1.81; 29.5	56.96 ± 5.09; 8.9	64.33 ± 5.42; 8.4
3	D	98	98	0.326	6.48 ± 1.92; 29.6	56.86 ± 6.13; 10.7	64.83 ± 6.72; 10.3
4	D	92	92	0.306	5.93 ± 2.42; 40.8	54.16 ± 8.53; 15.7	61.86 ± 9.36; 15.1
5	D	80	92	0.266	5.37 ± 2.62; 48.7	52.50 ± 10.59; 20.1	59.66 ± 11.52; 19.3
6	D	94	94	0.313	5.90 ± 1.85; 31.3	55.20 ± 6.58; 11.9	62.86 ± 7.27; 11.5
1	E	99	99	0.33	5.10 ± 1.71; 33.5	52.46 ± 5.53; 10.5	60.03 ± 5.90; 9.8
2	E	99	99	0.33	4.06 ± 1.66; 40.8	48.50 ± 6.62; 13.6	55.43 ± 7.11; 12.8
3	E	97	97	0.323	4.90 ± 2.29; 46.7	50.70 ± 9.16; 18.0	58.40 ± 9.90; 16.9
4	E	95	95	0.316	3.43 ± 1.96; 57.1	44.50 ± 10.02; 22.5	51.50 ± 10.87; 21.1
5	E	44	44	0.146	4.88 ± 2.59; 53.0	50.70 ± 11.06; 21.8	57.70 ± 12.26; 21.2
6	E	98	98	0.326	5.18 ± 2.04; 39.3	51.33 ± 7.90; 15.3	58.63 ± 8.82; 15.0

Appendix 4 Summary population and growth parameters for straight-bred lines of black bream at age 180 days

Family		Family size	Survival	Stocking density	Wet weight (g)	Standard length (mm)	Total length (mm)
Sire	Dam	(n)	(%)	(Fish.L^{-1})	($\bar{x}$ ± S.D.; C.V.)	($\bar{x}$ ± S.D.; C.V.)	($\bar{x}$ ± S.D.; C.V.)
2	A	92	92	0.306	18.67 ± 4.29; 22.9	78.70 ± 5.80; 7.3	88.36 ± 6.34; 7.1
3	A	93	93	0.313	16.35 ± 6.88; 42.0	73.70 ± 10.12; 13.7	83.33 ± 10.92; 13.1
5	A	92	92	0.31	15.14 ± 5.80; 38.3	73.10 ± 10.35; 14.1	82.63 ± 11.07; 13.3
6	A	89	89	0.296	16.57 ± 4.76; 28.7	74.80 ± 7.05; 9.4	84.60 ± 7.31; 8.6
1	B	93	93	0.31	16.43 ± 6.62; 40.2	76.40 ± 10.69; 13.9	86.36 ± 11.54; 13.3
2	B	95	95	0.316	15.47 ± 4.80; 31.0	74.83 ± 7.65; 10.2	84.13 ± 8.60; 10.2
3	B	94	94	0.313	15.99 ± 7.61; 47.5	75.33 ± 13.72; 18.2	85.00 ± 14.87; 17.4
5	B	93	93	0.31	16.22 ± 6.52; 40.1	75.56 ± 11.20; 14.8	85.16 ± 12.17; 14.2
6	B	94	94	0.313	17.09 ± 5.47; 32.0	77.56 ± 8.77; 11.3	87.53 ± 9.11; 10.4
1	C	98	98	0.326	17.66 ± 6.51; 36.8	77.10 ± 10.78; 13.9	87.06 ± 11.50; 13.2
2	C	94	94	0.313	18.06 ± 6.27; 34.7	78.80 ± 9.93; 12.6	88.30 ± 10.59; 11.9
4	C	89	89	0.296	17.92 ± 6.19; 34.5	78.76 ± 9.39; 11.9	88.70 ± 10.06; 11.3
5	C	98	89	0.296	17.47 ± 7.08; 40.5	77.96 ± 11.14; 14.2	87.70 ± 11.96; 13.6
6	C	93	93	0.31	19.37 ± 5.59; 28.8	80.76 ± 7.38; 9.1	90.83 ± 7.90; 8.6
1	D	98	98	0.32	17.91 ± 5.87; 32.7	77.66 ± 8.79; 11.3	87.76 ± 9.42; 10.7
2	D	96	96	0.32	20.62 ± 5.36; 25.9	82.03 ± 6.41; 7.8	92.53 ± 7.01; 7.5
3	D	96	96	0.32	17.48 ± 5.41; 30.9	78.90 ± 7.73; 9.7	89.16 ± 8.35; 9.3
4	D	90	90	0.3	17.44 ± 5.17; 29.6	77.06 ± 7.52; 9.7	87.46 ± 8.18; 9.3
5	D	80	92	0.266	16.90 ± 6.74; 39.8	76.43 ± 10.63; 13.9	86.46 ± 11.58; 13.3
6	D	91	91	0.303	18.60 ± 5.78; 31.0	79.20 ± 8.80; 11.1	89.43 ± 9.66; 10.8
1	E	93	93	0.31	16.39 ± 4.55; 27.7	75.50 ± 6.14; 8.1	85.43 ± 6.63; 7.7
2	E	98	98	0.326	15.40 ± 7.28; 47.2	73.73 ± 11.99; 16.2	83.86 ± 13.15; 15.6
3	E	93	93	0.31	19.03 ± 7.41; 38.9	79.00 ± 11.07; 14.0	89.20 ± 12.13; 13.5
4	E	95	95	0.316	14.27 ± 6.27; 43.9	72.56 ± 9.87; 13.6	82.33 ± 10.72; 13.0
6	E	91	91	0.303	17.38 ± 6.17; 35.5	76.76 ± 8.95; 11.6	87.16 ± 9.88; 11.3

Appendix 5 Summarised test results for feed intake and growth traits in replicated full-sib families of black bream at 14, 28, 42 and 56 days

Family Sire	Dam	(replicate)	Feed intake (g) (Total; $\bar{x} \pm$ S.D.; C.V.)	Wet weight (g) ($\bar{x} \pm$ S.D.; C.V.)	Standard length (mm) ($\bar{x} \pm$ S.D.; C.V.)	Total length (mm) ($\bar{x} \pm$ S.D.; C.V.)
Day 0						
1	D	a		33.78 ± 9.29; 27.5	97.25 ± 8.05; 8.2	110.30 ± 8.81; 7.9
1	D	b		32.34 ± 10.66; 32.9	96.50 ± 9.23; 9.5	109.80 ± 10.09; 9.1
1	D	c		35.37 ± 7.70; 21.7	99.90 ± 6.67; 6.6	112.40 ± 7.11; 6.3
2	D	a		42.15 ± 12.95; 30.7	104.15 ± 9.29; 8.9	117.65 ± 9.94; 8.4
2	D	b		40.46 ± 8.18; 20.2	104.25 ± 5.95; 5.7	117.30 ± 6.58; 5.6
2	D	c		43.83 ± 11.53; 26.3	105.60 ± 8.60; 8.1	119.40 ± 9.34; 7.8
3	D	a		44.72 ± 7.64; 17.0	107.55 ± 5.62; 5.2	121.70 ± 6.07; 4.9
3	D	b		41.40 ± 7.65; 18.4	105.55 ± 5.74; 5.4	118.95 ± 6.38; 5.3
3	D	c		43.37 ± 10.10; 23.2	106.20 ± 7.31; 6.8	120.15 ± 7.84; 6.5
4	D	a		39.80 ± 8.58; 21.5	102.55 ± 8.01; 7.8	116.10 ± 8.74; 7.5
4	D	b		44.88 ± 7.63; 17.0	107.80 ± 5.56; 5.1	121.75 ± 5.99; 4.9
4	D	c		31.51 ± 9.19; 29.1	97.05 ± 7.52; 7.7	110.00 ± 8.37; 7.6
5	D	a		36.37 ± 9.32; 25.6	100.00 ± 7.82; 7.8	113.35 ± 8.56; 7.5
5	D	b		39.69 ± 9.85; 24.8	103.25 ± 7.18; 6.9	116.75 ± 7.83; 6.7
5	D	c		41.64 ± 7.14; 17.1	105.45 ± 4.74; 4.4	119.30 ± 5.16; 4.3
Day 14 Test						
1	D	a	182.29; 13.02 ± 2.87; 22.0	36.78 ± 9.97; 27.1	101.80 ± 8.83; 8.6	114.55 ± 9.25; 8.0
1	D	b	201.08; 14.36 ± 3.43; 23.8	36.31 ± 11.80; 32.4	100.65 ± 10.04; 9.9	114.50 ± 10.84; 9.4
1	D	c	207.84; 14.84 ± 3.72; 25.0	39.48 ± 8.58; 21.7	103.25 ± 6.56; 6.3	116.75 ± 7.45; 6.3
2	D	a	240.03; 17.14 ± 4.14; 24.1	47.66 ± 14.62; 30.6	108.80 ± 9.18; 8.4	122.95 ± 10.03; 8.1
2	D	b	231.59; 16.54 ± 3.82; 23.0	45.02 ± 8.91; 19.7	107.65 ± 6.19; 5.7	121.55 ± 6.80; 5.5
2	D	c	247.46; 17.67 ± 4.11; 23.2	49.09 ± 12.84; 26.1	110.05 ± 9.08; 8.2	124.40 ± 9.99; 8.0
3	D	a	218.61; 15.61 ± 3.50; 22.4	48.71 ± 7.74; 15.8	111.90 ± 5.62; 5.0	126.65 ± 6.02; 4.7
3	D	b	219.34; 15.66 ± 4.02; 25.6	45.92 ± 8.37; 18.2	108.75 ± 6.10; 5.6	123.15 ± 6.49; 5.2
3	D	c	210.81; 15.05 ± 3.91; 25.9	47.12 ± 11.06; 23.4	109.95 ± 7.49; 6.8	124.45 ± 8.26; 6.6

4	D	a	214.29; 15.30 ± 3.78; 24.7	43.91 ± 10.00; 22.7	105.40 ± 8.55; 8.1	119.55 ± 9.50; 7.9
4	D	b	233.43; 16.67 ± 4.00; 23.9	49.74 ± 8.63; 17.3	112.35 ± 5.65; 5.0	127.15 ± 6.08; 4.7
4	D	c	195.60; 13.97 ± 3.24; 23.1	34.90 ± 9.92; 28.4	100.30 ± 8.06; 8.0	114.15 ± 9.02; 7.9
5	D	a	212.64; 15.18 ± 3.36; 22.1	41.03 ± 9.98; 24.3	105.00 ± 8.93; 8.5	119.25 ± 9.50; 7.9
5	D	b	211.00; 15.07 ± 3.79; 25.1	43.84 ± 11.08; 25.2	106.70 ± 7.51; 7.0	121.25 ± 8.19; 6.7
5	D	c	227.22; 16.23 ± 3.78; 23.2	46.54 ± 8.29; 17.8	109.80 ± 5.22; 4.7	124.10 ± 5.60; 4.5
Day 28 Test						
1	D	a	214.15; 15.29 ± 3.23; 21.1	41.51± 10.86; 26.1	104.80 ± 8.61; 8.2	119.30 ± 9.31; 7.8
1	D	b	215.74; 15.41 ± 3.29; 21.3	40.94 ± 13.38; 32.6	104.90 ± 10.52; 10.0	119.50 ± 11.51; 9.6
1	D	c	218.20; 15.58 ± 3.39; 21.7	44.25 ± 9.71; 21.9	107.85 ± 7.19; 6.6	122.00 ± 7.67; 6.2
2	D	a	243.25; 17.37 ± 3.88; 22.3	53.90 ± 16.91; 31.3	114.25 ± 10.08; 8.8	128.50 ± 10.38; 8.0
2	D	b	240.41; 17.17 ± 4.06; 23.6	50.50 ± 9.88; 19.5	112.75 ± 6.49; 5.7	127.55 ± 6.80; 5.3
2	D	c	260.06; 18.57 ± 4.14; 22.2	54.16 ± 14.56; 26.8	114.65 ± 9.66; 8.4	129.35 ± 10.04; 7.7
3	D	a	225.86; 16.13 ± 3.41; 21.1	53.41 ± 8.39; 15.7	115.40 ± 5.83; 5.0	130.60 ± 6.08; 4.6
3	D	b	233.01; 16.64 ± 3.80; 22.8	51.00 ± 8.95; 17.5	113.25 ± 6.09; 5.3	128.35 ± 6.35; 4.9
3	D	c	224.86; 16.06 ± 3.33; 20.7	51.31 ± 11.96; 23.3	113.35 ± 8.00; 7.0	128.65 ± 8.45; 6.5
4	D	a	230.64; 16.47 ± 3.68; 22.3	48.10 ± 11.20; 23.2	110.25 ± 9.49; 8.6	124.85 ± 10.29; 8.2
4	D	b	249.81; 17.84 ± 4.17; 23.3	55.59 ± 9.55; 17.1	116.90 ± 5.89; 5.0	132.05 ± 6.31; 4.7
4	D	c	226.15; 16.15 ± 3.48; 21.5	38.71 ± 11.43; 29.5	103.65 ± 8.49; 8.1	118.20 ± 9.24; 7.8
5	D	a	234.18; 16.72 ± 3.56; 21.2	46.58 ± 10.98; 23.5	109.20 ± 9.08; 8.3	124.00 ± 9.58; 7.7
5	D	b	236.43; 16.88 ± 3.70; 21.9	49.02 ± 12.34; 25.1	111.65 ± 7.96; 7.1	126.70 ± 8.54; 6.7
5	D	c	248.89; 17.77 ± 3.66; 20.5	52.41 ± 9.65; 18.4	115.10 ± 5.91; 5.1	129.95 ± 6.33; 4.8
Day 42 Test						
1	D	a	208.18; 14.87 ± 3.84; 25.8	46.34 ± 12.29; 26.5	108.70 ± 8.82; 8.1	123.75 ± 9.60; 7.7
1	D	b	208.50; 14.89 ± 3.88; 26.0	46.16 ± 15.15; 32.8	109.00 ± 11.03; 10.1	123.75 ± 12.03; 9.7
1	D	c	221.06; 15.79 ± 4.05; 25.6	49.48 ± 10.48; 21.1	112.15 ± 7.05; 6.2	126.55 ± 8.10; 6.4
2	D	a	225.12; 16.08 ± 4.02; 25.0	59.20 ± 17.78; 30.0	117.25 ± 10.21; 8.7	132.65 ± 10.88; 8.2
2	D	b	220.31; 15.73 ± 3.61; 22.9	55.38 ± 10.74; 19.3	116.75 ± 7.91; 6.7	131.45 ± 7.10; 5.4
2	D	c	237.70; 16.97 ± 4.43; 26.1	59.61 ± 15.73; 26.3	118.25 ± 10.23; 8.6	133.70 ± 10.73; 8.0
3	D	a	213.01; 15.21 ± 3.64; 23.9	57.81 ± 8.97; 15.5	118.70 ± 5.84; 4.9	134.30 ± 6.27; 4.6
3	D	b	214.23; 15.30 ± 3.96; 25.8	55.82 ± 9.38; 16.8	117.20 ± 6.44; 5.4	132.60 ± 6.76; 5.0
3	D	c	214.62; 15.33 ± 3.59; 23.4	55.73 ± 13.00; 23.3	116.65 ± 8.02; 6.8	132.40 ± 8.49; 6.4

4	D	a	228.24; 16.30 ± 3.77; 23.1	53.24 ± 12.26; 23.0	113.90 ± 10.03; 8.8	129.05 ± 10.76; 8.3
4	D	b	231.76; 16.55 ± 4.04; 24.4	60.85 ± 10.37; 17.0	120.25 ± 6.06; 5.0	136.00 ± 6.65; 4.8
4	D	c	214.61; 15.32 ± 3.84; 25.0	43.07 ± 12.99; 30.1	107.15 ± 9.27; 8.6	121.85 ± 9.97; 8.1
5	D	a	221.45; 15.81 ± 3.89; 24.6	51.67 ± 11.88; 22.9	113.35 ± 9.10; 8.0	128.35 ± 9.72; 7.5
5	D	b	225.33; 16.09 ± 3.92; 24.3	54.47 ± 13.23; 24.2	115.40 ± 8.15; 7.0	131.00 ± 8.89; 6.7
5	D	c	223.13; 15.93 ± 4.39; 27.5	57.84 ± 10.86; 18.7	118.50 ± 6.18; 5.2	134.15 ± 6.69; 4.9

Day 56 Test

1	D	a	212.32; 16.33 ± 1.40; 8.5	51.72 ± 13.54; 26.1	112.50 ± 9.20; 8.1	127.50 ± 9.63; 7.5
1	D	b	199.13; 15.31 ± 1.35; 8.8	50.77 ± 16.58; 32.6	112.10 ± 11.58; 10.3	127.70 ± 12.33; 9.6
1	D	c	208.52; 16.04 ± 1.41; 8.7	54.28 ± 11.03; 20.3	115.45 ± 6.95; 6.0	130.50 ± 7.37; 5.6
2	D	a	227.77; 17.52 ± 1.49; 8.5	64.87 ± 19.17; 29.5	120.45 ± 10.23; 8.4	136.25 ± 11.11; 8.1
2	D	b	216.30; 16.63 ± 1.88; 11.3	59.87 ± 11.63; 19.4	119.40 ± 6.89; 5.7	134.70 ± 7.16; 5.3
2	D	c	229.48; 17.65 ± 2.30; 13.0	64.13 ± 16.79; 26.1	120.70 ± 9.72; 8.0	136.35 ± 10.78; 7.9
3	D	a	215.05; 16.54 ± 1.23; 7.4	62.64 ± 9.80; 15.6	121.35 ± 5.92; 4.8	137.45 ± 6.50; 4.7
3	D	b	211.35; 16.25 ± 2.14; 13.1	60.53 ± 9.92; 16.3	120.30 ± 6.73; 5.5	135.60 ± 6.73; 4.9
3	D	c	205.88; 15.83 ± 1.79; 11.3	58.35 ± 13.65; 23.3	119.00 ± 8.28; 6.9	135.25 ± 8.96; 6.6
4	D	a	212.81; 16.37 ± 2.03; 12.4	57.50 ± 13.26; 23.0	116.40 ± 9.91; 8.5	131.70 ± 11.31; 8.5
4	D	b	222.87; 17.14 ± 2.03; 11.8	65.54 ± 11.18; 17.0	123.00 ± 6.30; 5.1	139.05 ± 6.79; 4.8
4	D	c	206.82; 15.90 ± 1.70; 10.6	45.68 ± 17.57; 38.4	105.15 ± 26.20; 24.9	119.70 ± 29.60; 24.7
5	D	a	213.35; 16.41 ± 1.42; 8.6	56.21 ± 12.58; 22.3	116.15 ± 9.36; 8.0	132.05 ± 9.68; 7.3
5	D	b	222.98; 17.15 ± 1.27; 7.4	59.27 ± 14.36; 24.2	118.75 ± 8.39; 7.0	134.45 ± 8.92; 6.6
5	D	c	222.39; 17.10 ± 2.14; 12.5	62.90 ± 11.73; 18.6	121.55 ± 6.09; 5.0	137.70 ± 6.98; 5.0