

Genetic and phenotypic characterisation of commercial dusky kob (*Argyrosomus japonicus*) cohorts

by

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Abstract

Dusky kob, *Argyrosomus japonicus*, is a large Sciaenid finfish that has been identified as an emergent aquaculture species in South Africa. Current production practices for dusky kob are based on mass spawning of genetically unimproved (wild-caught) broodstock. In recent years, considerable efforts have been initiated to retain first filial (F_1)-generation animals with fast growth rate as potential broodstock for a selective breeding programme. Although a few studies have been conducted on the species, previous studies have not addressed fundamental questions related to the effects of mass spawning production practices for the development of a selective breeding programme for dusky kob. This study aimed not only to bridge this gap, but also to investigate, for the first time, the potential for selection for increased growth rate in dusky kob. By using 14 microsatellite markers, the genetic properties of a wild population ($n = 34$) were compared to an F_1 cohort that represents three temporal groups that were sampled throughout the production cycle (*i.e.* from weaning to market size). Despite a heterozygote excess, likely as a result of a genetic bottleneck, the F_1 cohort displayed comparatively low levels of allelic diversity with respect to the wild population ($P < 0.01$). This was attributed primarily to the establishment of a small founder breeding population ($n = 12$), but also to low participation amongst females during the spawning event. Parentage analyses indicated only five (full-sib) F_1 families. Families with low starting contributions were not eliminated following removal of the smallest animals by culling. Culling, however, did contribute to a significant increase in genetic relatedness and a single family represented 88% of the market-sized group, suggesting that these practices may have the potential to further complicate the selection of unrelated broodstock in commercial mass spawning species. Pedigree relations were inferred for an additional three F_1 cohorts each produced from a breeding population comprising no more than five wild captive broodstock. Averaged relatedness amongst the three F_1 cohorts was comparatively higher than that detected for the F_1 animals of the first spawning event analysed. Furthermore, estimates of direct heritability (h^2) were 0.34 ± 0.25 and 0.36 ± 0.27 for juvenile weight and length, respectively, and the genetic correlation between the traits was 0.98 ± 0.03 . Although estimates of h^2 are likely

biased due to small sample sizes, and possibly maternal and/or competition effects, it is concluded that selective breeding for increased growth rate can be successful in juvenile dusky kob. However, the current analysis indicates that F₁ broodstock candidates are likely to be related and when bred will lead to excessive inbreeding. As this could have grave consequences for the profitability of dusky kob production, it is advisable that a selection programme for the species will need to consider both individual growth performance and genetic relatedness, e.g. using walk-back selection. Continued monitoring is therefore advised.

Opsomming

Die Suid-Afrikaanse kabeljou, *Argyrosomus japonicus*, algemeen bekend as “dusky kob”, is 'n groot Sciaenied vinvis wat as 'n opkomende akwakultuurspesie geïdentifiseer is. Huidige produksiepraktyke vir kabeljou is gebaseer op die massateling van onverbeterde (wild gevang) populasies. Die afgelope jare is aansienlike pogings egter aangewend om F₁-generasie diere met 'n vinnige groeikoers as potensiële broeidiere te behou, met die spesifieke doel om 'n selektiewe teelprogram te begin. Vorige studies het nie fundamentele vrae wat verband hou met die uitwerking van massaproduksiepraktyke op die ontwikkeling van 'n teelprogram vir dusky kob aangespreek nie. Hierdie studie het gemik om nie net hierdie gaping te oorbrug nie, maar ook om vir die eerste keer die potensiaal vir seleksie vir verhoogde groeikoers in kabeljou te ondersoek. Deur 14 mikrosatelliet-merkers te gebruik, is die genetiese eienskappe van 'n wilde populasie (van 34 individue) met dié van drie temporale F₁-kohorte wat gedurende die produksiesiklus (dit wil sê van speen tot markgrootte) gemonster is, vergelyk. Algeheel het die F₁ diere 'n aansienlike hoeveelheid alleliese diversiteit verloor in vergelyking met die wilde individue ($P < 0.01$). Dit is hoofsaaklik toegeskryf aan die totstandkoming van 'n klein stammende broeipopulasie (van 12 individue), maar ook tot 'n lae deelname onder vroulike broeivisse tydens die broeigeleentheid. Slegs vyf nageslagfamilies is aangedui. Families met 'n lae aanvangsbydrae is nie uitgeskakel na die verwydering van die kleinste diere deur uitdunning nie. Uitdunning het egter bygedrae tot 'n beduidende toename in genetiese verwantskap en in enkele nageslagfamilie het 88% van die markgrootte groep verteenwoordig. Dit dui daarop dat hierdie praktyke die potensiaal kan hê om die seleksie van onverwante broeiviskandidate in kommersiële massa broeispesies verder te bemoeilik. Stamboomverhoudings is afgelei vir 'n addisionele drie F₁-kohorte wat elk geproduseer was van 'n broeipopulasie van nie meer as vyf wild-gevangene diere nie. Die genetiese verwantskap tussen die drie F₁-kohorte was aansienlik hoër as dié wat vir die vorige broeigeleentheid bespeur is. Verder is genetiese parameters vir liggaamsgewig en standaardlengte bereken. Oorerflikheid (h^2) was 0.34 ± 0.25 en 0.36 ± 0.27 vir die twee groeiverwante eienskappe, respektiewelik, en die genetiese korrelasie tussen die

twee eienskappe was 0.98 ± 0.03 . Alhoewel ramings van h^2 waarskynlik bevooroordeel is as gevolg van klein steekproefgroottes, en moontlik moederlike- en/of kompetisie effekte, word daar tot die gevolgtrekking gekom dat selektiewe teling vir verhoogde groeikoers suksesvol kan wees in kabeljou. Uit die resultate in die huidige studie is dit egter duidelik dat F_1 -broeiviskandidate verwant sal wees en dus sal inteel. Aangesien dit ernstige gevolge kan hê vir die produktiwiteit van akwakultuur, is dit raadsaam dat 'n seleksieprogram vir die spesie beide individuele groeiprestasie en genetiese verwantskap in ag moet neem, *bv.* deur van terugloop seleksie gebruik te maak. Voortgesette monitering word dus aangeraai.

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List of Abbreviations

%	Percentage
(Pty)	Property Limited
>	Greater than
<	Less than
≥	Greater than or equal to
~	Approximately
∞	Infinity
±	Plus-minus
5'	Five prime
3'	Three prime
A	Adenine
A _e	Effective number of alleles
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
A _n	Number of alleles
BD	Body depth
BLUP	Best Linear Unbiased Prediction
bp	Base pair
C	Cytosine
°C	Degree Celsius
CI/s	Confidence interval/s
cm	Centimetre
CV	Coefficient of variance
DAFF	Department of Agriculture, Forestry and Fisheries
dph	days post hatch
DNA	Deoxyribonucleic acid

dNTP	Deoxynucleotide triphosphate
EBV	Estimated breeding values
<i>e.g.</i>	<i>exempli gratia</i> (for example)
EST	Expressed sequence tag
EtBr	Ethidium bromide
<i>et al.</i>	<i>et alii</i> (and others)
F ₁	First-generation
FAO	Food and Agriculture Organisation
F _{IS}	Wright's fixation index (individual relative to the sub-population, equal to inbreeding coefficient - <i>f</i>)
F _{IT}	Wright's fixation index (individual relative to the total population)
F _{ST}	Wright's fixation index (subpopulation relative to the total population)
G	Guanine
g	Grams
H	Body shape index
h ²	(narrow-sense) heritability
H _o	Observed heterozygosity
HW	Hardy-Weinberg
IAM	Infinite alleles model
<i>i.e.</i>	<i>id est</i> (that is to say)
K	Fulton's conditioning factor
KW	Kruskal-Wallis
LG	Large-grade tank
LD	Linkage disequilibrium
L _s	Standard length
MAS	Marker-assisted selection
m	meters
mm	millimeters
N	Sample size

N_e	Effective population size
N tank	Number of broodstock in tank
N_d	Number of dames
N_{ed}	Effective number of dames
N_{es}	Effective number of sires
NG	Non-grade tank
N_s	Number of sires
PA_r	Private allelic richness
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PIC	Polymorphism information content
P-value	Probability value
QTL/s	Quantitative trait locus/loci
r	Relatedness
r	Correlation coefficient
R^2	Squared correlation coefficient
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
r_g	Genetic correlation
r_p	Phenotypic correlation
RNA	Ribonucleic acid
s.d.	Standard deviation
s.e.	Standard error
SG	Small-grade tank
SNP	Single nucleotide polymorphism
T	Thymine
T_a	Annealing temperature
uH_e	Unbiased expected heterozygosity
W	Bodyweight

CHAPTER 1

Introduction: Literature Survey, Aims and Objectives

1.1) Species biology: An introduction to dusky kob (*Argyrosomus japonicus*)

1.1.1) *Argyrosomus japonicus*, a confusing taxonomy

Argyrosomus is a genus of fish represented by at least nine recognised species within the family Sciaenidae (order: Perciformes) (Griffiths and Heemstra, 1995). Sciaenid species of the genus *Argyrosomus* exhibit a high degree of interspecific morphology, which in turn has led to misidentification of many species within the genus, particularly those that inhabit a wide range of coastal areas. *Argyrosomus japonicus* has been known by at least 13 different synonyms throughout its distribution, *i.e.*, Northern Indian, North Pacific, Southern Africa and Australia (Trewavas, 1977; Kailola *et al.*, 1993; Griffiths and Heemstra, 1995) (Figure 1.1). In 1990, an in-depth comparison of habitat distribution, morphometrics, otolith and anatomical structure indicated that *A. japonicus* had recently been referred to as *A. hololepidotus* (a species endemic to Madagascar) in both Australia and South Africa. In South Africa, *A. japonicus* was also confused with *A. inodorus* (Griffiths and Heemstra, 1995) - a species with which *A. japonicus* may occasionally hybridise (Mirimin *et al.*, 2014).

Following the revision of the genus *Argyrosomus* by Griffiths and Heemstra, (1995), wild populations of *A. japonicus* in South Africa and Australia could not be separated and thus were considered conspecific. The biology of *A. japonicus* is well studied in South Africa (Griffiths and Hecht, 1995b; Griffiths, 1996), and more recently studied in Australia (Silberschneider and Gray, 2007; Silberschneider *et al.*, 2009; Ferguson *et al.*, 2014). These studies show significant differences in the life-history traits (*e.g.* growth, age at sexual maturity, time of spawning) amongst the geographical locations. Moreover, strong genetic differentiation between wild populations of *A. japonicus* in Australia and South Africa, revealed by mitochondrial DNA analysis,

suggested that these populations have been isolated for a long period of time and could potentially represent two different species (Farmer, 2008). A revision of the taxonomy *A. japonicus* is, therefore, justified. This thesis will focus mainly on the South African *A. japonicus*, commonly known as dusky kob. Aspects of the species' natural life-history and distribution in South Africa are described below.



Figure 1.1: World distribution of *Argyrosomus japonicus*. The figure was adapted from the original by Silberschneider and Gray (2007).

1.1.2) Ecology, life-history and distribution in South Africa

Dusky kob are voracious predatory fish that hunt mainly by using lateral line senses and smell instead of sight. They are essentially ambush (sit-and-wait) predators, especially as adult fish, when they feed mostly near the bottom, but also throughout the water column. Juveniles feed mainly on crustaceans and smaller fish whereas adults are mainly piscivorous, but it is known that they may feed on squid and octopus as well (Griffiths, 1997; Bergamino *et al.*, 2014). Adaptive traits, such as a large mouth, sharp teeth for gripping, widely spaced gill rakers and a large rigid distensible stomach render them well-suited for this feeding mode (Kailola *et al.*, 1993). Similar to other members of the family Sciaenidae, dusky kob produces

drumming sounds by vibrating its swim bladder. This phenomenon is linked to territorial display and spawning behaviour, and may reflect adaptation to spawning at night and in habitats that are turbid (Blaber, 2000). A distinctive feature of dusky kob is its possession of drumming muscles in both sexes (Griffiths and Heemstra, 1995), similar to *A. regius* (Lagardere and Mariani, 2006), and the ability to produce several call variations (Parsons and McCauley, 2017).

The life history of dusky kob is extremely similar to that of two Sciaenid species from North America; black drum, *Pogonias cromis*, and red drum, *Scianops ocellatus* (Jones and Wells; 1998; Craig *et al.*, 2000), but differs remarkably from sympatric species' silver kob and *A. thorpei* (squaretail kob), two species from South Africa. Dusky kob is the largest South African (and Australian) sciaenid reaching 2 m in length (Griffiths, 1997) with a maximum record age of 42 years (Griffiths and Hecht, 1995b). The dusky kob reaches (50%) sexual maturity at an age and size nearly doubled that of silver- and squaretail kob - being 1.0 m at 5 years of age for males and 1.1 m at 6 years for females of dusky kob - where after growth rate declines dramatically (Griffiths, 1996).

In Southern Africa, dusky kob is prevalent on the east coast from Cape Point to Mozambique [where it represents a homogenous genetic stock (Mirimin *et al.*, 2015)], but is especially abundant between Cape Agulhas and KwaZulu-Natal (Griffiths and Heemstra, 1995) (Figure 1.2). At the commencement of the rainy season (between August and October) each year, a large proportion of the adult population migrates northward to the warmer waters of KwaZulu-Natal to spawn. Spawning generally continues up until January in the southern and southern-eastern Cape Regions when adults return from KwaZulu-Natal (Griffiths, 1996). However, some adult fish do not migrate to KwaZulu-Natal, but remain in the southern and southern-eastern Cape Regions to spawn. Spawning occurs on shallow inshore reefs, pinnacles and wrecks at depths of 10-15 m, and at night - an adaptation which reduces predation on eggs by zooplanktivores (Griffiths, 1996, 1997a; Connell, 2007). Dispersal of eggs and larvae along the South African coastline is facilitated by the southward movement of the Agulhas Current (Beckley, 1995).



Figure 1.2: Areas of distribution and abundance of dusky kob in South African waters. The figure was adapted and modified from Mirimin *et al.*, (2015).

In contrast to sympatric species' squaretail kob and silver kob, juveniles of dusky kob are estuarine-dependent and are euryhaline. Early juveniles (< 1.5 cm in length) are predominantly found in the upper reaches of estuaries, preferring estuaries that are turbid, as they provide adequate food supplies and increased protection from predators (Griffiths, 1996; Whitfield *et al.*, 2002; van Niekerk *et al.*, 2012). As juveniles grow, they migrate to lower reaches of estuaries, into the inshore marine environment where they generally remain until maturation and eventually offshore into deeper water. Larger juveniles and adults also frequent deeper areas of the estuaries. It has been suggested that the life-history strategy of dusky kob (*i.e.* longevity, late maturity and high fecundity), referred to as periodic strategy or 'bet-hedging' (Winemiller and Rose, 1992), has evolved in conjunction with low juvenile mortality in protected estuarine habitats (Griffiths, 1996).

1.2) Dusky kob, an emerging aquaculture finfish species

Dusky kob have sustained both commercial and recreational fisheries for decades (Brouwer *et al.*, 1997; Pradervand *et al.*, 2007; Childs and Fennessy, 2013). Consequently, wild stocks have come under extreme pressure. Spawner biomass-per recruit was last estimated to be between 1.0 and 4.5% of pristine levels, with levels below 20% considered unsustainable (Griffiths, 1997; Otgaar, 2012). Although the taxonomic confusion within the genus was rectified (Griffiths and Heemstra, 1995), dusky- and silver kob were still managed as “*A. holopidotus*” (with legal size set at 40 cm) until the year 2004, when regulations for recreational fishers were changed (Sauer *et al.*, 2003). In 2005, semi-commercial participants were removed; however, as this has shifted fishing efforts towards estuarine nursery areas, wild stocks have since diminished even further (Griffiths, 2000; Dunlop and Mann, 2012; Cowley *et al.*, 2013). Today dusky kob is considered a threatened species and is listed on the South African Sustainable Seafood Initiative’s Customer Seafood List as Red if caught from linefish or trawl.

Following the decline of wild stocks and growing seafood demand, dusky kob farming has been established. Since the commencement of dusky kob culture in South Africa, a number of research efforts have been launched to gain a better understanding of the species’ biology (Daniel, 2004; Collett, 2007; Bernatzeder *et al.*, 2007; Kaiser *et al.*, 2011; Musson and Kaiser, 2014). Dusky kob compares well to red drum, an established Sciaenid species cultured in China (Hong and Zhang, 2003) and in the United States (Lee and Ostrowski, 2001), with fast initial growth rate, good feed conversion ratio, tolerance to low salinity and low oxygen levels, high crowding densities and disease resistance all favouring its choice as a suitable candidate for aquaculture (Griffiths, 1996; Whitfield 1998; Fitzgibbon *et al.*, 2007; Collett *et al.*, 2008, 2011; Fielder and Heasman, 2011).

In line with global trends, aquaculture production in South Africa is experiencing a steady growth (of 6% per year) whilst marine catch is plateauing (DAFF, 2016; FAO, 2016). The South African marine finfish industry, which is currently centred around dusky kob and yellowtail (*Seriola lalandii*), is an infant, but growing sector. In 2011, there was a significant portion of capital investment into the farming of marine finfish in South Africa (*i.e.* 42% of total aquaculture investment; DAFF, 2012). The industry

has subsequently experienced a dramatic increase in production, reaching nearly 50 tons in 2012 and 160 tons in 2014 (DAFF, 2016). Cage culture of dusky kob is still under development in South Africa. There are currently three commercial hatcheries of dusky kob in operation in South Africa: these include two recirculation facilities situated in the Eastern Cape Province, which will be the focus of the present study, and a pond culture facility in KwaZulu-Natal (which seed supply comes from one of the above-mentioned producers).

Current production practices for dusky kob rely on mass spawning of broodstock (*i.e.* each male reproducing with many females and each female reproducing with many males in a single tank). Broodstock are held under photoperiod control to produce eggs throughout the year. Prior to spawning, female broodstock are sedated and cannulated, and oocytes are collected using a catheter. Generally, oocytes of a diameter of 0.5 mm or more are considered appropriate for successful spawning. Together with a rise in water temperature ($> 22\text{ }^{\circ}\text{C}$), male- and female brooders are hormonally induced to commence the spawning process. Individual female broodstock provides anything between 2 - and 12 million eggs. The production cycle begins with the collection of viable (floating) fertilised eggs, which are then placed into incubating tanks for hatching. Hatching occurs at approximately 24-30 hours after spawning. Larvae feed from their yolk sac for the first 48 hours, after which they are transferred to a larval rearing system which consists of circular tanks that are on a partial recirculation system. After this period live feeds are introduced beginning with *Branchionus spp.* (Rotifers), followed by *Artemia* (Brine shrimp) until the larvae are fully weaned and then transferred to the nursing tanks (juvenile stage).

After several months of rearing, juveniles of similar age are pooled and divided into two or more independent size grades, depending on body weight. Slower-growing juveniles are often culled before and/or after grading, or as an alternative to grading when tanks are limited. These practices are necessary to maintain standard growth rates throughout to harvest (which can range from 400 g to 3 kg), and can also help to minimise detrimental social/behavioural effects. Aggressive behaviour leading to cannibalism is a common occurrence in dusky kob aquaculture (Figure 1.3) and may occur as soon as 18 dph (O'Sullivan and Ryan, 2001). Cannibalism has also been reported for other aquaculture species, including barramundi (Loughnan *et al.*,

2013), giant grouper (Hseu *et al.*, 2007), sharptooth catfish (Baras, 2001), Japanese flounder (Dou *et al.*, 2004) and red drum (Liao and Chang, 2002). Cannibalism may be more pronounced in cases where offspring from multiple families - with differential growth rates - are raised in a communal environment (Baras and Jobling, 2002; Liu *et al.*, 2017), but can regardless arise or be altered through environmental factors, such as inadequate food source, low feeding frequency, stocking and crowding density and light intensity (Hecht and Pienaar, 1993; Kestemont *et al.*, 2003; Qin *et al.*, 2004; Fessehaye *et al.*, 2006a; Collett *et al.*, 2008; Timmer and Magellan, 2011). Typically, the largest animals pose the greatest potential threat for cannibalistic behaviour.



Figure 1.3: Juvenile cannibalism observed in dusky kob.

Whereas an increasing understanding of the biology and culture of dusky kob has contributed to a developing South African marine finfish industry, hatchery managers still rely on unimproved or wild-caught broodstock where inconsistency in production performance and uncertainty of long-term survival are commonly observed. For these reasons, dusky kob selective breeding programmes are being considered in South Africa.

1.3) Selective breeding in aquaculture

The increased productivity achieved in response to genetic improvement with selective breeding has been a key factor facilitating the development of major aquaculture industries [e.g. salmonids, tilapias, oysters, and shrimps (Fjalestad *et*

al., 1997; Eknath and Hulata, 2009; Thodesen *et al.*, 2011; Gjedrem, 2012; Zak *et al.*, 2014)]. The genetic progress achieved per generation for growth rate and disease resistance has been four to five times higher than that obtained for terrestrial livestock (Gjerde and Korsvoll, 1999; Gjerde *et al.*, 2012), thanks to the extremely high fecundities of aquatic organisms and the existence of broad genetic variation for production traits: both of which allow for high selection intensities (and thus high selection responses). In addition, strong correlations between fish growth and feed conversion efficiency indicated that aquaculture organisms selected for fast growth rate better utilised feed resources compared to terrestrial livestock (Thodesen *et al.*, 1999; Ytrestøyl *et al.*, 2011).

Genetic improvement of aquatic species further demonstrated that sustainable aquaculture production could be achieved through the implementation of family-based breeding programmes (*i.e.* within- and between family selection). In these programmes, individual family relationships can be monitored through single pair spawns and/or strip spawning (followed by physical tagging and communal rearing). Records can thus be obtained, and parental breeding values estimated for a variety of different traits [using best linear unbiased prediction (BLUP) methodology], including traits with low heritability and/or traits that require sacrifice of the animal – for which records can only be obtained from relatives (Meuwissen, 1997; Gjedrem, 2010). Family-based breeding programmes also provide an effective means to preserve the genetic gains that have been made as inbreeding and loss of “general” genetic diversity can be limited. Inbreeding, *i.e.* mating between individuals that share common ancestry, increases homozygosity and leads to expression of deleterious recessive alleles, resulting in depression of fitness-related traits [*e.g.* growth rate, survival, age at sexual maturity, reproductive success (Su *et al.*, 1996; Pante *et al.*, 2001; Fessehaye *et al.*, 2009)]. To maximise genetic gains and limit inbreeding, however, the number of test families, and thus tanks required, must be large. Physical tagging is also labour intensive and can only be performed once individuals are of sufficient size. What is more, this early rearing system introduces environmental (*i.e.* tank) effects common to full-sib families, which can create bias in BLUP procedures and slow genetic improvement (Martinez *et al.*, 1999; Vandeputte *et al.*, 2011).

For many aquaculture ventures separate rearing is impractical, not only in financial terms, but also because of biological constraints (as is the case with natural mass spawning species, such as dusky kob). Alternatively, mass selection is practiced. Mass selection is based solely on individual phenotypic performance and it is therefore particularly suited for traits that can be easily recorded, such as growth rate, which is the trait with most impact on profit (Gjedrem *et al.*, 2012). Although more effective in improving growth rate than family-selection (Volckaert and Hellemans, 1999; Vandeputte *et al.*, 2009), mass selection inevitably increases the rate of inbreeding as individual relatedness is not taken into consideration at the time of selection (Huang and Liao, 1990; Blonk *et al.*, 2009; Knibb *et al.*, 2014). Selection of unrelated broodstock candidates under mass spawning conditions is further hampered by the occurrence of limiting- and unequal parental contributions, which is accentuated if using small breeding populations, due to increased reproductive competition, differential spawning timings, and several others (Bekkevold *et al.*, 2002; Campton, 2004; Wedekind *et al.*, 2007; Fessehayee *et al.*, 2009; Bright *et al.*, 2016). Using a restricted number of breeding individuals can therefore exacerbate the effects of random genetic drift as it leads to substantial fluctuations in allele frequencies between generations, which in turn contribute to a loss of rare alleles, further increasing population homozygosity and relatedness.

To attain sufficient genetic variation for selective breeding of commercial mass spawning species, the base population should therefore comprise several unrelated broodstock [e.g. 50 pairs (Gjerde *et al.*, 1996; Bentsen and Olesen, 2002; Sonesson *et al.*, 2005; Fjalestad, 2005)]. However, selection of such numbers is not always possible for start-up aquaculture facilities, especially for those facilities that rely on species with delayed maturity (*i.e.* large adult size), such as dusky kob. It is also a major issue as the turn-over from one improved generation to the next is greatly increased. Consequently, the implementation of selective breeding programmes for such species lags behind those species that have shorter generation intervals and, especially, to those where loss of genetic diversity can be limited through single pair mating's and/or strip spawning (Gjerde, 2005; Rye *et al.*, 2010). Fortunately, for such species, the potential of merging new molecular marker technologies now exists to study genetic variation and aid conventional breeding strategies.

1.4) Molecular markers in aquaculture

Early molecular work on aquaculture species was based on allozymes (enzyme products of genes) followed by DNA markers, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and minisatellites (Vos *et al.*, 1995; Carvalho and Pitcher, 1995; Clifford *et al.*, 1998). Allozymes are not ideal for population genetic research and parentage studies as they have limited power in detecting genetic variability (or polymorphism), and require large amounts of tissue from organs (*i.e.* liver and heart) for their assay, thus causing the death of the animal. The main disadvantages of RAPDs and AFLPs are that they are dominantly expressed, difficult to interpret and inconsistent (Liu and Cordes, 2004). One of the criticisms levelled against minisatellites is that allele frequencies for a given locus can not be determined as multiple loci are assayed simultaneously (Magoulas *et al.*, 1998).

Molecular genetic studies on aquaculture species have consequently extended to employ mitochondrial DNA (mtDNA) markers, microsatellites, and more recently single nucleotide polymorphisms (SNPs). Due to the maternal inheritance and small effective population size of mtDNA, it is especially effective in evaluating genetic variability at the species or intra-specific level, but not as effective for assessing genetic variability within commercial stocks (Meyer, 1993; Hillis *et al.*, 1996). In addition, mtDNA represents only a single locus. Microsatellites and SNP markers are the most widely used molecular markers in aquaculture genetics at present. Both markers are co-dominant and ubiquitous throughout the genome, especially SNP'-s; however, microsatellites are favoured due to their multi-allelic nature (higher polymorphism) and, currently, they are less expensive than SNPs (Liu and Cordes, 2004; Yue and Xia, 2014). Microsatellite markers are essentially type 2- selectively neutral- markers, but may also occur in genic (type 1) regions (*e.g.* Expressed Sequence Tags, ESTs) where they may serve functional roles as coding or regulatory elements (review by Li *et al.*, 2002). The versatility of microsatellite markers has made them suitable for a wide range of applications in aquaculture genetics.

1.5) Application of microsatellite markers in aquaculture

1.5.1) Assessing genetic diversity

Microsatellite markers can provide valuable information on population genetic structure, population bottlenecks, effective population size (N_e), gene flow and several other population genetic parameters such as population differentiation and inbreeding (Archangi, 2008; André *et al.*, 2011; Senanan *et al.*, 2015; Li *et al.*, 2017). This is essential in understanding demographic history as well as to reveal geographical centres of genetic diversity from which broodstock can be sourced. By utilising microsatellite markers, it is possible to assess levels of neutral genetic diversity amongst broodstock candidates. Genetic relatedness can thus be lowered significantly to expedite the establishment of a genetically diverse base population (Sekino *et al.*, 2004; Sriphairoj *et al.*, 2007; Loughnan *et al.*, 2015). Increasing the genetic variation at the commencement of a selective breeding programme presents the possibility of both adapting to changing environments (*e.g.* rearing conditions, diseases), and providing unique genetic variants for traits of current and future interest (Ballou and Lacy, 1995; Elliott, 2000; Hayes *et al.*, 2006).

Microsatellite markers can further be used to assess differences in estimates of genetic diversity (*via* changes in allele frequencies) between broodstock and subsequent cultured generations and, consequently, reveal processes that determine the observed differences. Differential broodstock contributions, high fecundity, and low survival rates are typical factors that can result in a reduction in the genetic variance of F_1 -generation cultured populations. This phenomena has been reported for a number of fish species, including brown trout (Hansen, 2002; Was and Wenne, 2002), Japanese flounder (Sekino *et al.*, 2002; 2004), common carp (Bártfai *et al.*, 2003), barramundi (Loughnan *et al.*, 2013; Domingos *et al.*, 2014), and also for dusky kob (Mirimin *et al.*, 2015). Considering that breeding candidates are generally selected at later stages in the production cycle particularly closer to harvest, it is also important to assess if and how levels of genetic variability are maintained throughout the production cycle. This is particularly evident for commercial mass spawning species where families with variable and unknown numbers of offspring are routinely subjected to grading and culling practices. Frost *et*

al., (2006) reported a reduction in genetic diversity following culling of cultured barramundi in the short time span of 2 days-post hatch (dph) to 27 dph. This study consequently suggested that greater losses may occur over time.

1.5.2) *Inferring pedigrees*

A major application of microsatellite markers is in the evaluation of pedigree relationships, particularly under communally reared aquaculture conditions (Gheyas *et al.*, 2009; Liu *et al.*, 2012; Vandeputte *et al.*, 2014; Vandeputte and Haffray, 2014). By assessing offspring relations based on similarity of alleles, it is possible to determine the effective number of breeders that participated to the next generation (and even reconstruct unknown parental genotypes *via* Maximum Likelihood methods). In particular, parentage analysis can be performed to reveal differences in gender reproductive performance (*i.e.* participation and levels of contribution) across mating designs (*e.g.* Selly *et al.*, 2014; LaCava *et al.*, 2015; Bright *et al.*, 2016), which in turn will aid in the development of effective mating strategies, so as to boost genetic variability for selective breeding programmes.

Microsatellite markers can also aid in the selection of unrelated broodstock candidates after mass selection in a two-step selection process called walk-back selection (Doyle and Herbingler, 1994). According to the strategy superior animals are physically tagged and held separately for DNA profiling. When pedigree information becomes available (through genetic testing), the best performing animals are retained for continued breeding only if they bear no relation to an individual already selected. Walk-back selection therefore, presents an improvement to conventional within-family selection, as pedigree records can be retained in a communal environment without the requirement to physically tag offspring populations, thus improving both space and labour, whilst minimising potential influences of non-heritable variation on trait expression (Waldbieser and Wolters, 1999; Robinson and Jerry, 2009; Robinson *et al.*, 2010).

Utilising pedigree information inferred from marker-assisted parentage assignment and provided phenotypic measurements of production traits, heritability (h^2 , the phenotypic variation that is genetic in origin) for the traits can be estimated.

Estimation of heritability allows evaluating expected genetic gains and is therefore, a prerequisite for initiating efficient selective breeding programmes. Genetic correlations between traits should also be estimated to evaluate the possibility of their simultaneous selection. Moderate to high heritability for fish growth traits, body weight and length, and strong genetic correlations between these traits, have been reported (Kause *et al.*, 2003; Vandeputte *et al.*, 2004; Saillant *et al.*, 2006; 2007; Wang *et al.*, 2008; Domingos *et al.*, 2013). Heritabilities have also been estimated for other body traits, such as Fulton's conditioning factor – an indicator of the “well-being” of a fish - (Kause *et al.*, 2003, 2007; Saillant *et al.*, 2007; Domingos *et al.*, 2013) as well as for processing traits (Navarro *et al.*, 2009; Saillant *et al.*, 2006), deformities (Bardon *et al.*, 2009), disease resistance (Antonello *et al.*, 2009) and flesh colour (Norris and Cunningham, 2004). Due to absence of between-family environmental variance in communal rearing, heritabilities obtained are often higher and more accurate than that obtained in separate rearing conditions (Herbinger *et al.*, 1999; Ninh *et al.*, 2011, 2013), although competition effects on expression of growth traits should not be ruled out (Vollestad and Quinn, 2003; Muir, 2005). Large sample sizes are required to avoid bias in estimates, especially if family sizes are highly skewed. In addition, grading of untagged juveniles can result in under estimation of heritability estimates, especially if the sampling cohort is not collected from all the available tanks (Blonk *et al.*, 2010) as slower-growing families, for instance, are more likely to end up in the same tank. Furthermore, by using microsatellite markers, genetic correlations amongst traits in disparate environments can be estimated to measure genotype by environment (G x E) interactions, and evaluate expected genetic gains across e.g. farming systems (Dupont-Nivet *et al.*, 2008; Domingos *et al.*, 2013; Vandeputte *et al.*, 2014), densities or rearing temperatures (Saillant *et al.*, 2006) and feeding regimes (Pierce *et al.*, 2008; Bestin *et al.*, 2014).

1.5.3) Quantitative Trait Loci Mapping and Marker-Assisted Selection

Microsatellites are routinely used in association with other markers for genome mapping and quantitative trait loci (QTL) analyses (Sakamoto *et al.*, 1999; Nichols *et al.*, 2003; Gilbey *et al.*, 2004; Sun and Liang, 2004; Yue, 2014). The ultimate

application of QTL mapping (*i.e.* detecting the most relevant genes for a phenotype) is in marker-assisted selection (MAS). The major advantage of MAS is that high performing animals can be selected at an early stage and with little to no harm to the animal itself (Yue, 2014). Rates of genetic gain are therefore, expected to be much higher for traits for which breeding value predictions rely solely on measurement of relatives (as is the case with family-selection), and also for traits that are only measurable after sexual maturity or only observed in a particular sex. However, MAS also presents a potentially powerful tool for improving growth rate in fish, especially species with extremely long generation intervals, particularly those that are currently produced from unimproved (wild-caught) broodstock.

For those aquaculture species where QTL analyses have not yet been conducted to assist in marker-assisted selection, an alternative means to assess parental breeding values would be through progeny testing. Progeny testing is considered more accurate than individual, family or combined selection for estimating parental breeding values, given that a number of half-sib families could be tested under communal rearing settings (Gjerde, 1991, 2005; Bourdon, 2000). Such a method of selection may therefore, be particularly suited for species such as dusky kob, where broodstock with unknown phenotypic performance are repeatedly spawned over several years and where selective breeding programmes are being considered and/or implemented. However, considering that these progeny cohorts present potential future breeding candidates, it is also important to evaluate the numbers and sizes of the families that are produced from mass spawning and, most importantly, how families perform relative to each other across the production cycle, closer to the time when new broodstock candidates are selected.

1.6) Study rationale, aims and objectives

1.6.1) Problem statement

Dusky kob is an estuarine-dependent Sciaenid finfish indigenous to South Africa. For decades, the species has supported a lucrative fisheries sector; however, due to unsustainable harvesting, poor management and subsequent collapse of natural populations, the burden of meeting the growing demand for the species has now

shifted to aquaculture production. The South African marine finfish industry has long appreciated the potential for improving growth rate of dusky kob through the implementation of a selective breeding programme. However, dusky kob are commercially mass spawned and, therefore, pedigree information is needed to record individual family relations and phenotypic performance and, consequently, to calculate genetic parameters.

Several authors have now developed microsatellite loci for dusky kob to aid population genetic data and parentage studies (Archangi *et al.*, 2009; Mirimin *et al.*, 2013; Barnes *et al.*, 2014). Recently, Mirimin *et al.*, (2015) assessed parental contribution from mass spawning broodstock to first-generation (F₁) individuals of dusky kob and also compared estimates of genetic diversity to that of the wild progenitors. This study was however limited in terms of the F₁ cohort investigated, which represented approximately 50 individuals that originated from breeding populations with variable, albeit unknown sizes and sex ratios. Some questions, therefore, remain unanswered. For instance, are there differences in sex reproductive performance across mating designs? How will this affect levels of genetic diversity and family compositions in the resulting progeny cohorts?, and will grow-out rearing practices further impact on initial compositions and phenotypic performance of families that will be available for selective breeding? Key knowledge gaps further impeding the development of a selective breeding programme for dusky kob, such as the heritability and genetic correlations for juvenile growth traits, have not yet been addressed.

1.6.2) Aims and objectives

The aim of this study was thus to genetically and phenotypically characterise commercial populations of dusky kob within the context of implementing a selective breeding programme. A population genetic analysis, coupled with DNA parentage analysis, will be performed (in *Chapter 2*) to investigate how levels of genetic diversity and family compositions are represented and maintained within a mass spawned F₁ cohort of dusky kob. An additional three F₁ cohorts will be characterised (in *Chapter 3*), to investigate further the effects of mass spawning and associated grow-out rearing practices on offspring family compositions and phenotypic

performance. Additionally, so as to exploit the potential for selection for dusky kob growth rate and body shape (*i.e.* Fulton's K-index), the use of modern quantitative genetic theory (*via* linear mixed models and Restricted Maximum Likelihood) will be employed to estimate phenotypic and genetic parameters (*i.e.* heritability and genetic correlations). The obtained results will then be interpreted and (in *Chapter 4*) discussed in terms of broad managerial recommendations related to the development of genetic improvement strategies for the South African dusky kob.

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CHAPTER 2

Broodstock contribution and genetic diversity in a commercial population of mass spawning dusky kob

Abstract

In the present study, 14 microsatellite markers were used to monitor changes in family compositions throughout the production cycle of three F₁ dusky kob (*Argyrosomus japonicus*) groups, and to compare estimates of genetic diversity with that of a wild progenitor population. Overall, the F₁ animals experienced a substantial reduction in allelic content and presented statistically significant differentiation from the wild, with a global F_{ST} of 0.03 ($P < 0.01$) detected. Parentage analyses indicated an effective broodstock population size of only seven (out of 12) individuals and five (full-sib) offspring families. Two families each had a starting contribution of less than 5%, though neither was eliminated following removal of the smallest animals by culling. Culling, however, did contribute to a significant increase in genetic relatedness in the oldest (*i.e.* market-sized) group analysed and a single family represented 88% of the resulting stock, suggesting that these individuals were on average faster growing than others. The obtained results demonstrate that culling practices do have the potential to create bias in the representation of families, further complicating the selection of unrelated broodstock candidates in selective breeding programmes.

2.1) Introduction

Mass spawning is a method commonly utilised in aquaculture for those species that naturally spawn in groups. As a result of infrastructure constraints and high fecundity, small breeding populations are typically used to produce large offspring populations. Whilst this reproductive strategy potentially provides the advantage of increasing production output in the short-term, indirectly, it creates a particular challenge for the implementation of selective breeding programmes. Captive broodstock only represents a small proportion of the genetic variation available in the wild progenitor individuals and therefore, the establishment of a founder broodstock population creates a genetic bottleneck, significantly contributing to a loss of rare or unique alleles. This founder effect subsequently increases the possibility for genetic deterioration in later generations through inbreeding and random genetic drift (Frankham *et al.*, 2002; Aho *et al.*, 2006; Lind *et al.*, 2012). Monitoring pedigree relationships is the key to circumventing these problems and for many aquaculture species this is possible through individual family spawning in separate tanks. For commercial mass spawning species, such as dusky kob (*Argyrosomus japonicus*), single pair mating designs are not conducted and thus, hatchery managers do not have control in the contribution of broodstock within a spawning event. In a typical spawn, some broodstock will not contribute and if the breeding population is small, contribution to offspring numbers is likely to be skewed (Sekino *et al.*, 2004; Brown *et al.*, 2005; Herlin *et al.*, 2008; Hillen *et al.*, 2017). Consequently, selected fish for the next generation may yield from a limited number of families and when bred will lead to excessive inbreeding. The detrimental effect of inbreeding is reduced fitness and, consequently, a reduction in aquaculture productivity, which are well documented for commercial mass spawning species, e.g. rainbow trout (Pante *et al.*, 2001), Nile Tilapia (Fessehaye *et al.*, 2009), abalone (Kobayashi and Kijima, 2010), shrimp (Moss *et al.*, 2007).

What is not as widely recognised within mass spawning operations, however, is that relative broodstock contributions can be altered even further as a consequence of grow-out rearing practices that impact on offspring family sizes (Frost *et al.*, 2006). Differential family growth and survival are commonly observed in the communal tank and for many mass spawning species this issue is addressed by dividing individuals

into separate tanks, depending on body size, which can be labour intensive, and often separate rearing under commercial settings is impractical. Alternatively, or in addition to this, slower-growing juveniles are frequently culled to maintain standard growth across the production cycle (Macbeth *et al.*, 2002). It is however likely that unfavourable phenotypes may contain unique (family-specific) genetic variants, which are then excluded from the cohorts. Frequent culling of such individuals may therefore lead to further contractions in the sizes of poorly represented families by the time of harvest and before new broodstock candidates are selected for the next generation of breeders. Thus, when selecting phenotypically superior F₁ animals for broodstock replacement, the probability of inbreeding increases.

Microsatellite markers are ideal for assessing genetic diversity and relatedness in the absence of pedigree data (Norris *et al.*, 1999; Jerry *et al.*, 2004; Van den Bergh and Roodt-Wilding, 2010; Liu *et al.*, 2012; Vandeputte *et al.*, 2014). Recently, Mirimin *et al.*, (2015) assessed genetic diversity in mass spawned cohorts of dusky kob and compared it to wild populations of the species. This study was however limited in terms of the cultured cohorts and the breeding populations investigated. For most commercial mass spawning species, and certainly for dusky kob, there is also a need to know whether grow-out rearing practices can further impact on family compositions and levels of genetic diversity that will be available for selective breeding. Against this background, the objective of this study is to evaluate the genetic properties of F₁-generation cultured animals, sampled across the culture period, and to compare estimates with wild broodstock individuals currently held at the hatchery for dusky kob.

2.2) Materials and methods

2.2.1) Study populations and genotyping

Three commercially produced F₁-generation groups of 100 individuals each (referred to as cultured groups hereafter) were collected at random over a two year-duration, from weaning to marketable-size. The first and second groups (cul_1 and cul_2) were collected at approximately one month- and five months of age, respectively. The former group was sampled from four different tanks during weaning procedures

whilst individuals from the latter group, representatives of the first group that survived weaning procedures, were collected from a single tank. The third group (cul_3), representatives of the second group that survived culling practices, was collected from the same tank at approximately two years of age (*i.e.* at marketable-size). All cultured animals were produced from a single spawning event through random mating of twelve broodstock individuals. Prior to spawning, eight females were checked for reproductive readiness and hormonally induced along with four males to induce final gamete maturation and spawning. Furthermore, a wild-caught broodstock database ($n = 34$), representative of the progenitor population, and under which the aforementioned 12 broodstock individuals were included, was also included for the analysis (Mirimin *et al.*, 2013).

Fin clip tissue from all wild and cultured individuals was preserved in 70% ethanol and stored at -20°C , after which genomic DNA extraction was performed on each specimen as a single extraction using a standard CTAB DNA extraction protocol (Saghai-Marooif *et al.*, 1984). DNA quantity and quality was evaluated with a NanoDropTM ND 1,000 spectrophotometer (Thermo Fisher Scientific) and normalized to a final concentration of $20\text{ng}/\mu\text{l}$. Multi-locus genotypic data were obtained by amplifying 17 microsatellite markers, previously developed for dusky kob (Archangi *et al.*, 2009; Mirimin *et al.*, 2013), in four multiplex reactions (Table 2.1; Table S2.1). Primer pairs for markers *Ajap34* and *Ajap37*, used in multiplex 3 and 2 respectively, amplified the same locus but differed in size by 24bp; therefore, *Ajap34* was only used as an internal control for genotyping accuracy. Microsatellite amplification was performed with a KAPA2GTM Fast Multiplex PCR Kit and all reactions were performed in a total volume of $10\mu\text{l}$. Each reaction mixture contained 2X KAPA2G Fast Multiplex Mix, $100\mu\text{M}$ stock solution of each primer and 20ng of DNA. PCR cycling parameters were identical for all multiplex reactions, and applied at an initial denaturing step at 95°C for 3 minutes, 30 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C (T_a) for 30 seconds, extension at 72°C for 20 seconds and a final extension step at 72°C for 7 minutes. PCR products were verified on 1.5% agarose gel electrophoresis and separated by means of capillary electrophoresis. Allele scoring was performed using GeneMapper v4.0 (Applied Biosystems).

Table 2.1: Seventeen microsatellite loci, grouped into four multiplex reactions, used for genotyping the wild and cultured specimens.

Multiplex 1	Multiplex 2	Multiplex 3	Multiplex 4
Ajap06	Ajap12	Ajap05	Ajap24
UBA05	Ajap14	Ajap34	UBA44
UBA06	Ajap37	UBA50	UBA853
UBA42	UBA40	UBA854	UBA53
	UBA851		

2.2.2) Genetic data analysis

Micro-checker v2.2.3 was used to evaluate the presence of potential genotypic errors and null alleles in the microsatellite data (1000 randomizations, Bonferroni-adjusted 95% confidence intervals) (Van Oosterhout *et al.*, 2004). The presence of null alleles was assessed with the Brookfield 1 estimator (Brookfield, 1996). The software program Microsatellite Toolkit v3.1 (Minch *et al.*, 1996) was used to test for the presence of redundant samples, calculate allele frequencies and to convert the molecular data to appropriate input file formats. To test whether genotypic frequencies across loci were consistent with those expected under Hardy-Weinberg (HW) equilibrium expectations, exact probability tests (under the Markov Chain method, 10000 dememorization, 500 batches and 5000 iterations per batch) were performed in GenePop v3.4 (Rousset, 2008). Marker neutrality was tested by means of an F_{st} -outlier test in Lositan v1.44 (50 000 permutations, implementing the infinite alleles model, assuming a correction for false discovery rate at 0.01 and statistical significance at the 5% nominal level) (Antao *et al.*, 2008).

Common indices of genetic diversity including: number of alleles (N_a), observed and unbiased expected heterozygosities (H_o and uH_e , respectively) and per locus F_{is} were estimated for each cultured group using GenAlEx v6.501 (Peakall and Smouse, 2012). Allelic richness and private allelic richness were also calculated for each group using HP-Rare v1.1 (Kalinowski, 2005), implementing the rarefaction

technique for a minimum of 66 alleles (33 diploid individuals) to standardize groups to a uniform sample size. Statistical differences in all the above mentioned measures of genetic diversity were tested within and between all groups by means of a Kruskal-Wallis test (KW; $P < 0.05$).

To evaluate population differentiation, and the partitioning of genetic variation between the three cultured groups and the wild population cohort, pairwise F_{ST} values (1000 permutations, significance: $P < 0.05$) were calculated and a locus-by-locus Analysis of Molecular Variance (AMOVA; 10000 permutations, significance: $P < 0.05$) performed in Arlequin v3.5.2.2 (Excoffier and Lischer, 2010). To visualise population distinctiveness, a principal coordinate analysis (PCoA; standardised variance method) was performed in GenAlEx. GenAlEx was also used to calculate mean relatedness (r) for each cohort, using the Queller and Goodnight, (1989) estimator (999 permutations at 95% confidence intervals). Effective population size (N_e) was calculated for the wild progenitors and the cultured population cohort using the linkage disequilibrium (LD) test (random mating, lowest allowed frequency: 0.05), in NeEstimator v2.01 (Do *et al.*, 2013). To check for signatures of recently reduced N_e within each cultured group, the Wilcoxon signed rank test (Cornuet and Luikart, 1996) for heterozygote excesses was used, assuming the infinite alleles model (IAM), applying 10000 iterations at 5% nominal level, in Bottleneck v1.2.02 (Piry *et al.*, 1999).

Broodstock contribution to each of the three cultured groups was evaluated through microsatellite based parentage analysis: firstly, by genotypic exclusion using all available broodstock genotypes, in Vitassign v8-2.1 (genotyping error rate: 0-10%) (Vandeputte *et al.*, 2006) and, secondly, using a full-pedigree likelihood method in Colony v2.0.5.0 (Jones and Wang, 2010). For the latter, allele frequencies were calculated for all the cultured individuals and their respective parents, implementing a genotypic rate of 0.01 for each marker, and assuming polygamous mating systems in both sexes. Lastly, chi squared analyses were used, with a threshold for significance of 0.05, to investigate changes in relative family sizes throughout the grow-out period and if these levels were significantly different between broodstock.

2.3) Results

2.3.1) Markers

Two of the markers (*Ajap24* and *UBA53*) in Multiplex 4 were excluded from subsequent analysis as they failed to amplify the target DNA fragments in all individuals. Microsatellite toolkit revealed no redundant samples within the data. No major genotypic errors due to stuttering and allelic dropout were identified; however, null alleles were evident in *Ajap12* within all three cultured groups. Across all loci, marker *Ajap12* displayed the highest F_{IS} coefficient, indicating a homozygote excess within each cultured group and therefore, this locus deviated significantly from HW equilibrium (Table S2.2). Lositan outlier-test also supported putative non-neutral behaviour as a possible candidate for directional selection (Figure 2.1): interestingly, sire S1 and dame D2 (Figure 2.5) were the only parents homozygous for this particular locus (data not shown). Removal of this locus did not change the outcome of analyses; therefore, results based on 14 loci are reported.

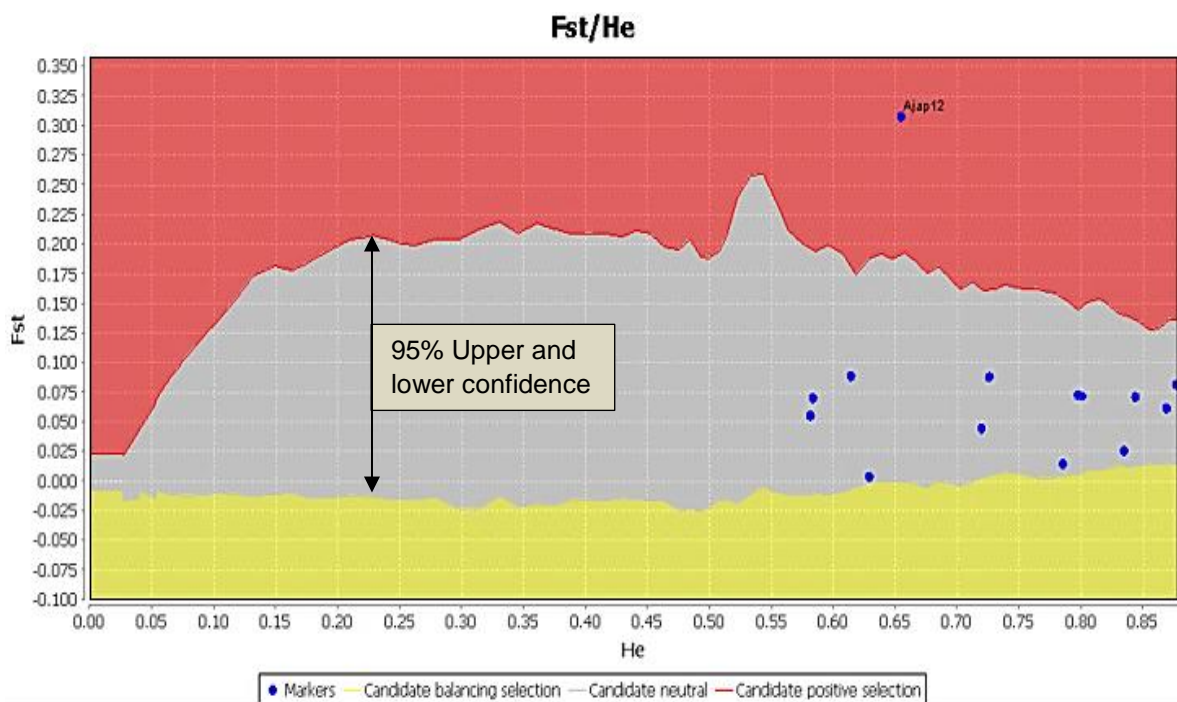


Figure 2.1: Lositan results depicting outlier loci as candidate loci under positive (red) and balancing (yellow) selection. All loci (indicated as blue dots) within the grey region were considered to be neutral.

2.3.2) Genetic diversity and population differentiation

Global exact probability tests revealed that genotypic frequencies for the wild samples confirmed to HW equilibrium expectations, whereas, those for the cultured groups did not ($P < 0.01$), and was subject to a significant reduction in all estimates of genetic diversity, with the exception of H_o (KW: $P < 0.05$; Figure 2.2). Based on all estimates of genetic diversity, Kruskal-Wallis statistical test revealed no statistically significant ($P > 0.05$) differences between the cultured groups.

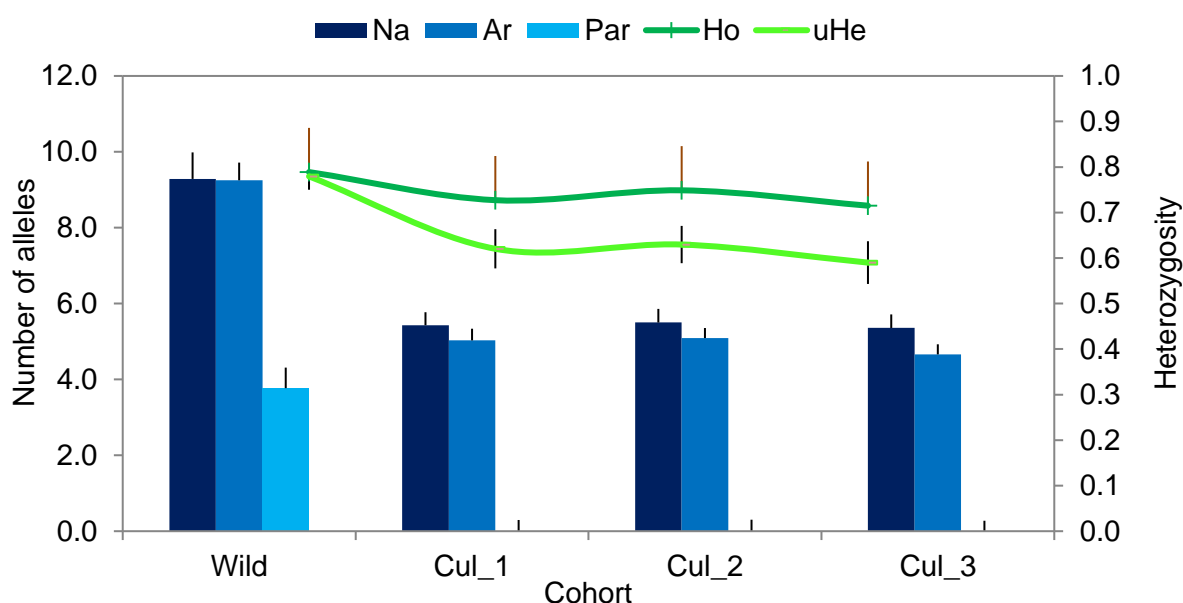


Figure 2.2: Summary of genetic diversity statistics expressed as mean number of alleles (N_a), allelic richness (A_r), private allelic richness (PA_r), observed heterozygosity (H_o) and unbiased expected heterozygosity (uH_e), for all three cultured groups and the wild broodstock cohort. Error bars indicate standard error.

Significant pairwise F_{ST} values were detected for all three wild-cultured group comparisons as well as for the marketable-sized individuals and both cul_1 and cul_2 (Table 2.2). Significant genotypic differentiation between the wild- and cultured animals is further reflected in the PCoA plot showing little overlap between the population cohorts (Fig. 2.3). Separation of the cultured animals can also be seen by the majority of the individuals clustering in the first and third quadrant along with two wild individuals. Across the wild population cohort and all three cultured groups,

AMOVA analyses revealed no within population variance and a significant global F_{ST} of 3% (Table 2.3).

Table 2.2: Population pairwise F_{ST} values inferred from 14 microsatellite loci.

	Wild	Cul_1	Cul_2	Cul_3
Wild	-			
Cul_1	0.0952**	-		
Cul_2	0.0911**	-0.0031	-	
Cul_3	0.1212**	0.0022*	0.0027*	-

*Statistical significance at the 5% nominal level

**Statistical significance at the 1% nominal level

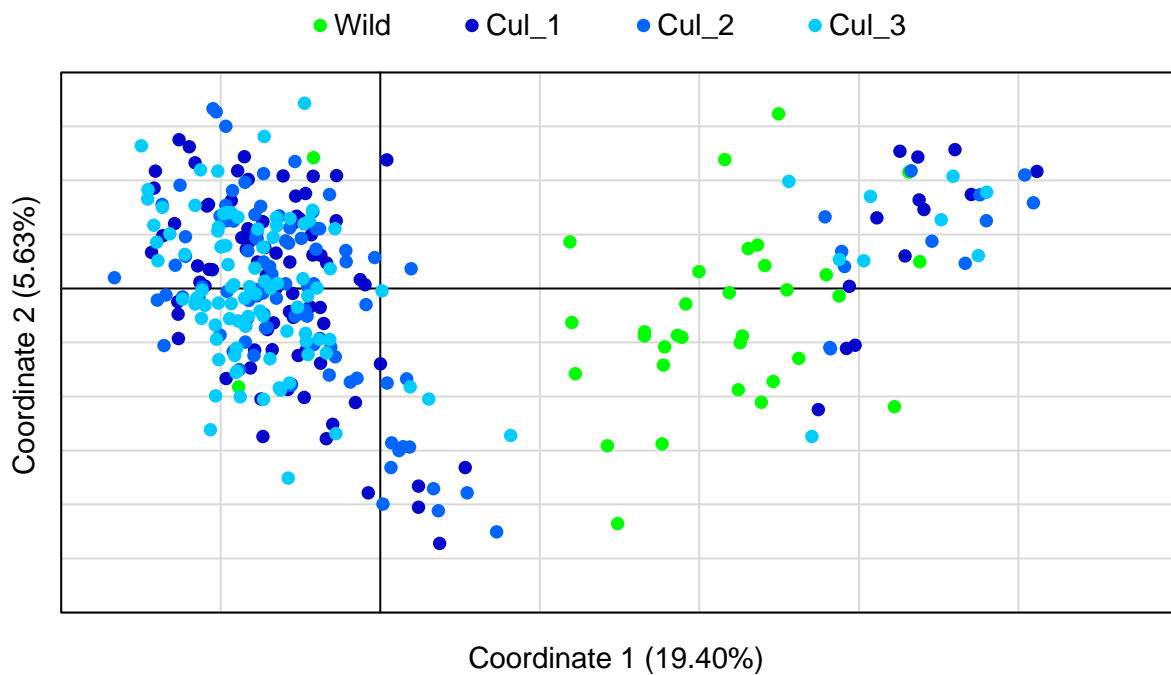


Figure 2.3: Scatterplots of principal coordinate analysis (PCoA) illustrating population distinctness. Plots represent individual genotypes and colours represent populations.

Table 2.3: Locus by locus AMOVA results over all 14 loci, with populations clustered into two main groups, wild and three cultured groups

Source of variation	Sum of squares	Variance components	% of variation
Among populations	78.212	0.1408	3.0859
Among individuals	1207.088	-0.7315	-1416.0270
Within individuals	1706.000	5.1548	112.9411
Total	2991.300	4.5641	
F_{IS} : -0.1654	P : 1		
F_{ST} : 0.0309	P : 0.001**		
F_{IT} : -0.1294	P : 1		

**Statistical significance at the 1% nominal level

2.3.3) Relatedness, effective population size and parental contribution

Mean relatedness for the wild individuals was non-significant, but amongst the cultured groups the relatedness coefficients were significantly larger than zero, with cul_3 showing the highest value that was also significantly different from the other groups (*i.e.* CI fell outside the upper and lower bounds for the null hypothesis of no difference) (Figure 2.4). Effective population size for the wild samples, as estimated by the LD method, was 395.4 (95%CI: 107.6- ∞), whereas the (combined) cultured individuals displayed a value of 6.8 (95%CI: 5.5-8.1), substantially lower than that estimated for the wild individuals. The Wilcoxon signed-rank test, as assessed by the IAM pattern of mutation for recently reduced effective population size, also indicated significant deviation from mutation-drift equilibrium in all three cultured cohorts, suggesting the occurrence of a genetic bottleneck ($P < 0.05$).

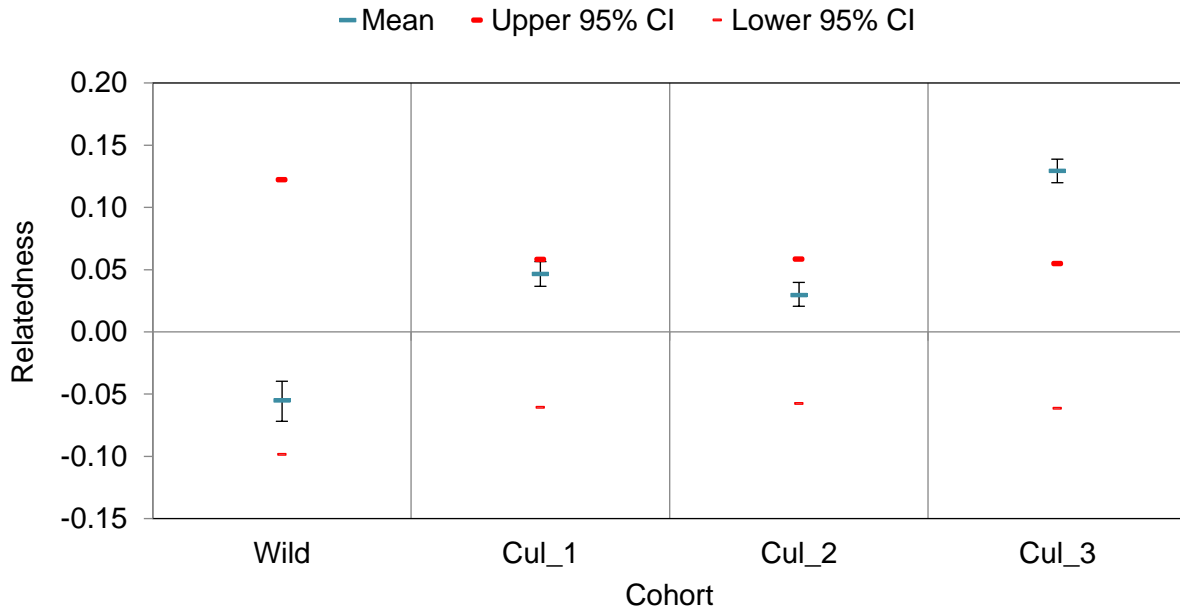


Figure 2.4: Mean within population pairwise relatedness values amongst the wild and cultured groups based on the Queller and Goodnight estimator. Error bars indicate 95% confidence intervals about the respective means. Upper (U) and lower (L) bounds in red indicate 95% confidence intervals for the null hypothesis of no difference between the cohorts.

Exclusion based Vitassign indicated that seven out of twelve parents successfully contributed to the spawn, three of which were males (Sires; S) and four of which were females (Dames; D). Subsequent application of the maximum-likelihood approach (in Colony) confirmed the genotypic exclusion results and revealed five full sib groups, including two half sib groups (Table S2.3). Offspring family numbers and sizes across the four weaning tanks were highly variable: three of the tanks represented only four or fewer families. Levels of contribution across the three sires in the combined weaning group were highly skewed (with S1 siring 87% of cul_1), though proportions did not change significantly throughout the rearing period ($P > 0.05$, Figure S2.1). For the marketable-sized individuals, however, levels of contribution across dames were significantly different from that detected in cul_2 ($P < 0.05$), which caused family sizes after the grow-out period to be in statistically different proportions than those previously found in the hatchery ($P < 0.05$, Figure 2.5). A single full-sib group (assigned to parental pair S1/D2) represented 88% of the

marketable-sized individuals with male S1 siring 91% of all individuals. Nonetheless, none of the families with low starting contributions were eliminated during culture.

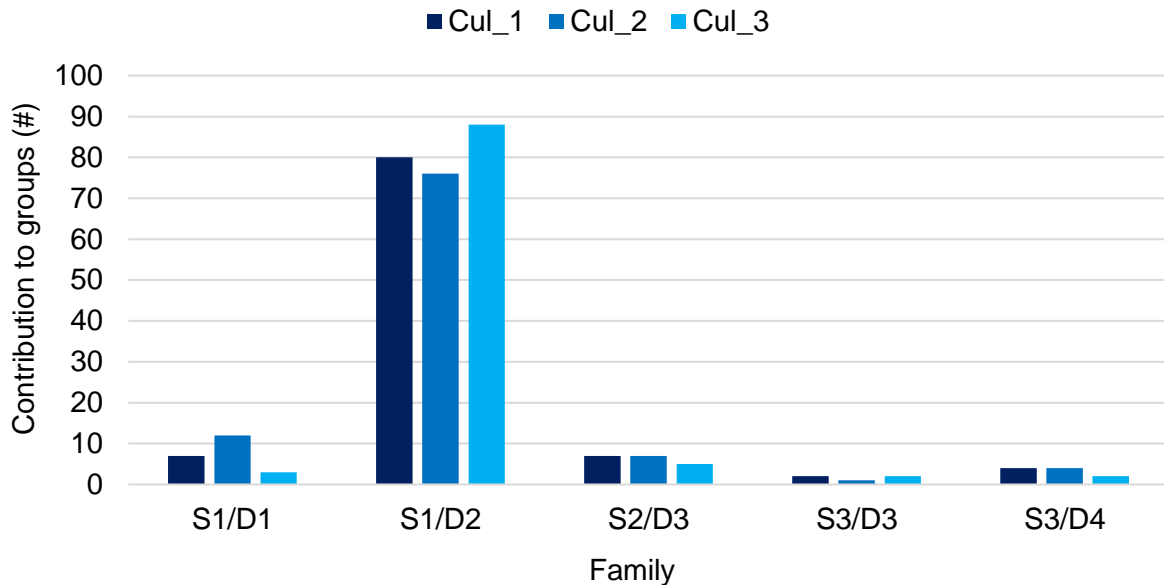


Figure 2.5: Number of offspring individuals from each cultured group, assigned to each of five families.

2.4) Discussion

The results obtained in the current study along with those of Mirimin *et al.*, (2015) indicate that wild individuals of dusky kob harbour moderate levels of genetic diversity, which is comparable to *priori* estimates obtained for other sciaenid species, including European meagre (Haffray *et al.*, 2012) and red drum (Renshaw *et al.*, 2006). Mean observed heterozygosity for the wild individuals was comparable to that detected for the cultured groups yet for the cultured individuals, values were significantly greater than that of expected heterozygosity (Figure 2.2). An inflated estimate of observed heterozygosity can be expected in offspring populations following a recent bottleneck event, as is often the case in first-generations of culture where wild (heterozygote) broodstock are used (Norris *et al.* 1999; Evans *et al.* 2004; Hillen *et al.*, 2017). Consequently, the loss in allelic diversity will occur faster than will loss of heterozygosity due to low impact of low frequency alleles on heterozygosity

estimates (Cornuet and Luikart, 1996; Luikart and Cornuet, 1998). There is evidence that all three F₁ groups suffered a recent genetic bottleneck and this was evident in a loss of allelic diversity. Heterozygosity may therefore, not be particularly useful for evaluating a reduction in genetic variability in first generations of culture (Hedgecock and Sly, 1990). Nevertheless, if individuals sharing similar alleles are used as broodstock in a closed selective breeding programme, the probability of a reduction in levels of heterozygosity is expected to increase as broodstock will inevitably become more related (Waples, 1990; Jorde *et al.*, 1995; Blonk *et al.*, 2009).

The loss in allelic diversity detected in the cultured individuals is an expected result seeing that the breeding population that was used for the spawning event represented only a portion of the wild progenitors analysed. The establishment of such a small founder breeding population could therefore, explain the significant genotypic differentiation of cultured populations from the wild progenitors detected using the pairwise genotypic differentiation tests as well as significant global genetic differentiation that was detected (Figure 2.4, Table 2.2). Differentiation of cultured populations from their progenitor populations have been reported for several other aquaculture species, including finfish species such as salmon (Reilly *et al.*, 1999; Withler *et al.*, 2007), carp (Murakaeva *et al.*, 2003) and Asian seabass (Senanan *et al.*, 2015). An additional important factor that could at least partially account for the levels of differentiation (and loss of allelic diversity) detected in the present study is the low participation of broodstock within the spawning event: parentage analyses indicated an effective broodstock population size of only seven (out of twelve) individuals, which is in line with the linkage disequilibrium estimate of effective population size, and comparable to a similar finding in Japanese flounder where only eight of the fourteen broodstock individuals successfully contributed to the genetic composition of the progeny (Sekino *et al.*, 2003).

In dusky kob, as in many other aquaculture species, when designing mating schemes, usually, the number of female brooders that are used is more than the number of male brooders, because, in practice, a single male should be capable of courting and spawning with more than one female. However, results show that this mating strategy results in high variability in female reproductive success. A similar observation was made for red drum (Gold *et al.*, 2008, 2010). Among many other

factors, the low participation rates amongst female broodstock may be due to differential spawning timings: it is possible that more than one female was ready to spawn at the same time, while available males were not sufficient to fertilise the eggs. On the contrary, for those mass spawning species where hatchery strains have been generated using fewer females than male brooders in the broodstock tank, males have shown to encounter more spawning problems than females when it comes to successful spawning (e.g. Brown *et al.*, 2005; Herlin *et al.*, 2008). Such trends in reproductive success between genders of dusky kob can be further investigated through different mating schemes.

Amongst those brooders that successfully contributed to the spawn, highly variable contributions to offspring numbers were observed. Unequal parental contributions appear to be a common phenomenon in mass spawning species (Sekino *et al.*, 2004; Borrell *et al.*, 2011; Domingos *et al.*, 2014) and, among other factors, could be attributed to broodstock weight and age (Brown *et al.*, 2005), sexual selection and competition - particularly amongst male brooders (Weir *et al.*, 2004; Fessehaye *et al.*, 2006b; 2009). In other mass spawning fish species, such as Japanese flounder and barramundi, it is not an uncommon occurrence for multiple males to follow a single female and race to fertilise her eggs once they are released. Although there are no available records regarding the mating behaviour in the spawning tank in which the cultured animals were founded, parentage analyses did indicate that female D3 was monopolised by both sire S2 and sire S3 (Table S2.3). It is interesting to note that while cohort monopolisation by these two sires might be indicative of male reproductive competition, resulting in unequal contributions between half-sib families S2/D3 and S3/D3 (and, possibly family S3/D4), it is also likely that unequal contributions between family S2/D3 and S3/D3 may be due to sperm competition, resulting from variation in sperm quality. In salmon, sperm competitiveness was shown to skew parental contributions (Campton, 2004; Wedekind *et al.*, 2007). Further investigation into whether sperm competition and mating behaviour play a role in spawning success in dusky kob warrant further study.

In this study, grow-out rearing practices (weaning and culling) did not present a further bottleneck for genetic variability, probably because none of the families were eliminated during culture. Similarly, Domingos *et al.*, (2014) found no significant

changes in any of the indexes used to gauge genetic diversity from 18dph to harvest in three batches of cultured barramundi. Although this was the case in practice, the marketable-sized individuals significantly differentiated from the first two groups (Table 2.2). Because the marketable-sized group did not experience a significant reduction in allelic richness, it would appear that similar alleles, albeit different frequencies, in this particular group led to the significant F_{st} values detected here. Pairwise comparisons of mean relatedness did indicate that levels of relatedness within the marketable-sized group were significantly different from that detected during earlier development stages (Figure 2.4), suggesting an increase in common (*i.e.* high-frequency) alleles within this group. This trend was further reflected in the parentage analyses, indicating a reduction in the relative contribution of some families over time (*e.g.* S1/D1, S2/D2 and S3/D4) whilst, at the same time, a comparatively larger proportion of the marketable-sized group assigned to family S1/D2 (Figure 2.5). Interestingly, however, in the second group analysed here, the highest contributing family represented slightly fewer individuals than that observed for the youngest (weaning) group. Chi-squared analyses also indicated no significant difference in relative family sizes between the weaning group and the market-sized group. Small sample sizes and sampling error may have attributed to the results, given that not all families were sampled from each of the four weaning tanks, probably because each tank represents no more than 30 specimens. The second group, which was collected from only a single tank, may therefore be considered a more suitable representative of relative family sizes (and survival) after mass spawning and prior to culling practices.

If some of the families decreased in size over time, sampled at a time when culling of slow-growing juveniles have been heavily applied, it might be expected that parental pair S1/D2 did not only contribute to the majority of the offspring, but may also have contributed to faster growing animals. It is also interesting to note that, while the relative contribution across sires, and parent S1 for that matter, were not significantly different across the sampling events, the accumulated results showed that relative contribution across the dames were in significantly different proportions than those found prior to culling practices. This was particularly evident for dames D1 and D2. Because dame D1 represented only a small proportion of the market-sized group, it

is possible that parent D2 had the greatest contribution to offspring growth in the population cohort analysed.

2.5) Conclusions

Results demonstrated that the effects of mass spawning hatchery practices on levels of heterozygosity in the cultured animals was significantly less than impacts on allelic richness, as expected as a result of a population bottleneck that was produced by the founder event. Attempts to maximise effective population size through the hormonal induction of wild-caught broodstock still resulted in low participation and highly skewed levels of contribution. Although none of the families with low starting contributions were eliminated following removal of the smallest juveniles by culling, a single full-sib group significantly increased in size, suggesting that culling practices do have the potential to alter the representation of families that will be available for selective breeding. On this account, it is postulated that this particular full-sib group was on average faster growing than others, and that related individuals are likely to be retained as potential broodstock for the next generation of breeders. Further investigation is however needed before final conclusions can be drawn.

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CHAPTER 3

Phenotypic performance and heritability of growth traits in cultured cohorts of dusky kob

Abstract

This chapter investigated, for the first time, the additive genetic variation of growth related traits in dusky kob and how families perform across mass spawning production practices. Pedigree relations were inferred for a total of four experimental cohorts of first-generation (F_1) dusky kob, using 15 microsatellite markers. Two of these cohorts, each yielding a single family, were used to investigate the phenotypic effects of size-grading and culling practices. Collectively, these practices reduced overall variances in juvenile growth, but did not improve growth rate. Parentage analysis of an additional two F_1 cohorts indicated a total of four- and five families, respectively; the latter cohort, representative of three temporal groups F_1 sampled throughout the production cycle (*Chapter 2*), was used to estimate genetic parameters for body weight (W), standard length (L_s) and Fulton's K-index (calculated as $10^6 W/L_s^3$). Direct heritability (h^2) estimates for the traits were inconsistent across the sampling dates. This was attributed primarily to significant changes in relative family frequencies throughout the culture period ($P < 0.05$). Estimates of h^2 for the combined dataset were 0.34 ± 0.25 for W and 0.36 ± 0.27 for L_s , suggesting a significant additive genetic component to juvenile growth rate in dusky kob. Contrary, for K , the h^2 was not significantly different from zero. The pedigree results further indicated an inverse relationship in family growth rate and K in the youngest and oldest (market-sized) groups analysed, indicating that selection for increased growth rate in juvenile dusky kob should have little or no effect on K .

3.1) Introduction

Growth rate is a trait of economic importance that is of major interest to dusky kob aquaculture as production costs in intensive re-circulation systems can be lowered significantly by reducing the duration of the rearing cycle. As with most mass spawning species, production practices for dusky kob commonly involve the communal rearing of offspring from multiple families. Family sizes can be highly variable (Mirimin *et al.*, 2015; *Chapter 2*) and differential family growth, which can promote aggressive behaviour and cannibalism, is frequently observed in the communal tank (O'Sullivan and Ryan, 2001; Timmer and Magellan, 2011). Smaller individuals are commonly preyed upon and the aggressive dominant behaviour of larger animals results in these individuals receiving an increased feed ration size (Baras and Jobling, 2002; Dou *et al.*, 2004). This can further promote juvenile size-disparities and increase cannibalistic behaviour, which can have detrimental effects on overall stock performance (Qin *et al.*, 2004). Consequently, the improvement of juvenile growth performance in commercial mass spawning species is often reliant on the phenotypic variation of growth. Size-grading is a procedure commonly employed in aquaculture to maintain growth disparities, reduce cannibalism, and improve growth rate (Barki *et al.*, 2000; Ahvenharju and Ruohonen, 2007). In South African hatcheries, slower-growing and/or injured juveniles of dusky kob are frequently culled to further assist in maintenance of uniform growth rates. Unlike size-grading, the phenotypic consequences of culling in cultured populations have largely been left unaddressed. For cultured dusky kob, the impact of both these practices on juvenile growth rate has not yet been investigated.

Provided phenotypic measurements on individual growth performance and the genetic relatedness between such individuals within a population is known, the additive genetic (*i.e.* heritable) component underpinning the phenotypic variance for the desired trait(s) can be estimated. Individual relations can be determined using microsatellite markers and genetic parameters (*e.g.* heritabilities and genetic correlations) for the traits can be estimated when offspring from multiple families are raised in a common environment (Wang *et al.*, 2008; Saillant *et al.*, 2007; Ye *et al.*, 2017). The advantage of communal rearing is that potential influences of non-genetic (environmental and physiological) effects on the phenotypic expression of the trait

can be kept to a minimum to ensure accurate estimation of genetic parameters. Genetic parameters for juvenile body weight and length have not yet been estimated for dusky kob in South Africa. In addition, Fulton's conditioning factor (K), a trait derived from weight and length, reflects the body shape of the fish and could be of interest to dusky kob aquaculture if body conformations have an impact on market acceptance. Moderate to high estimates of heritability for fish K are frequently observed (Nilsson, 1994; Kause *et al.*, 2003, 2007) and the strong genetic correlations between K observed at different growth intervals for species such as rainbow trout and salmon (Saillant *et al.*, 2007; Devlin *et al.*, 2010) suggest that body shape differences during early development stages can persist until fish reach market-size.

While the estimation of genetic parameters for growth traits requires data on individual phenotypic performance and family relations, rarely do studies investigate how families perform relative to each other. Culling significantly impacts on family compositions in commercial mass spawning species (Frost *et al.*, 2006) and it is therefore likely to impact on family metrics for growth-related traits at the end of the production cycle. Furthermore, considering that grow-out cohorts (and future potential breeding candidates) of dusky kob are currently being produced from small breeding populations, it will also be of interest to know how male and female broodfish perform relative to each other across mating designs and, consequently, how this will affect the number, sizes and phenotypic performance of the families that are produced. Using phenotypic data and pedigree relations inferred for a total of four experimental cohorts of first-generation (F₁) dusky kob, this study therefore, for the first time estimated genetic parameters for dusky kob growth traits and investigated how families perform across mass spawning practices, involving broodstock populations of variable sizes and sex ratios.

3.2) Materials and methods

3.2.1) Experimental groups, genotyping and phenotyping

Two offspring cohorts collected from each of two aquaculture facilities, coded as Oc and PO, were included for the analyses. Number of broodstock used for each of the

four spawning events, date of spawning, number of offspring genotyped in each sampling period and experiment performed are depicted in [Table 3.1](#). For experiment 1, pedigree relations were inferred for each population cohort across the two facilities. For experiment 2, genetic parameters and phenotypic correlations for growth traits were estimated, whereas, experiment 3 was conducted in an effort to investigate how families perform (in terms of growth and numbers) within and between cohorts across husbandry practices.

Table 3.1: Number of broodstock, offspring genotyped, date of spawning and experiment performed for each spawned cohort.

	Spawn			
	Oc_1	Oc_2	PO_1	PO_2
Broodstock	12	5	4	4
-sires	4	2	1	2
-dams	8	3	3	2
Offspring genotyped	300	100	100	102
Date of spawning	27. Feb. -14	6. Apr. -15	20. Apr. -15	18. Apr. -16
Experiment	1 / 2 / 3	1 / 3	1 / 3	1 / 2 / 3

Spawn Oc_1, as described in *Chapter 2*, represents three F₁ groups, *i.e.* OF₁₁, OF₁₂ and OF₁₃, collected over a two-year duration at approximately one month of age, (during weaning), five months of age (after weaning) and two years of age (during culling practices), respectively. The latter two groups were sampled from a single tank, whilst the weaning group was sampled from four different tanks. Two broodstock individuals that were included for spawn Oc_1 were also included for spawn Oc_2 in the following year. Spawns Oc_2 and PO_1 represent F₁-generation animals that were sampled before harvest at 391 days post hatch (dph) and at 482 dph, respectively. Spawns Oc_2 and PO_1 were collected from four- and three tanks, respectively; for PO_1, 50 individuals were collected from a non-grade (NG)

tank, whilst the remaining two groups each represents 25 individuals that were collected from a small-grade (SG) and large-grade (LG) tank. The latter two (*i.e.* graded) groups were originally in the same tank, where after 30% of the smallest animals was culled at approximately two months of age and subsequently divided into an SG and LG category. The cultured animals representing spawn PO_2 were collected from a single tank.

All F₁ fish were produced through random mating of wild broodstock (with unknown phenotypic performance). Mature broodstock were acclimated to captivity by manipulating temperature and photoperiod to induce oocyte maturation in accordance with industry specific protocols. In addition, all broodstock individuals were hormonally induced prior to spawning, with the exception of one female in spawn PO_1 whose identification is unknown. Methods regarding DNA extraction, PCR amplification and genotyping (using 17 microsatellite markers) of the specimens were previously described in *Chapter 2*. The software program Microchecker v2.2.3 (Van Oosterhout *et al.*, 2004) was used to evaluate the presence of potential genotypic errors and null alleles in the broodstock- and offspring microsatellite data (1000 randomizations, Bonferroni-adjusted 95% confidence intervals). Furthermore, each offspring individual was phenotyped for (wet) weight (W) and standard body length (Ls), and Fulton's conditioning factor (K) was calculated as $K = 10^6 W/Ls^3$, where W is weight in grams and Ls is length in millimetres.

3.2.2) *Experiment 1- Resolving parentage and reproductive success*

Methods regarding DNA parentage analysis for the three F₁ groups of spawn Oc_1 were previously described in *Chapter 2*. The same genetic broodstock database that was used for the parentage analyses of Oc_1 - provided by Mirimin *et al.*, (2013) - was also used to identify potential brooders that contributed to spawn Oc_2, in Vitassign v8.2.1 (Vandeputte *et al.*, 2006). For spawns PO_1 and PO_2, pedigree relations were determined, firstly, by genotypic exclusion using an available genetic broodstock database (Mirimin *et al.*, 2015) in Vitassign and, secondly, using a full-pedigree likelihood method in Colony v2.0.5.0 (Jones and Wang, 2010). In Colony, allele frequencies were calculated for all the offspring individuals and their respective

parents, implementing a genotypic error rate of 0.01 for each marker, and assuming polygamous mating systems in both sexes.

3.2.2) Experiment 2- Estimation of genetic parameters for growth-related traits

Phenotypic data were assessed for normality and homogeneity of variances using a Shapiro-Wilk test and a Leven's test, respectively (with a threshold significance of 5%) in XLStatistics v12.11.22 (Carr, 2012). For most of the cohorts, phenotypic data for W did not approximate a normal distribution and required log-transformation prior to the estimation of (co)variance components. Variance components (for the additive genetic and the residual effects) and standard errors for growth traits W and Ls and the morphometric ratio K were estimated for all three groups of spawn Oc_1 and for the offspring individuals representing spawn PO_2. Estimates were obtained using Average Information Restricted Maximum Likelihood (AI-REML) (Jensen *et al.*, 1997), fitting the single trait mixed model in DMU AI software v6.5.2 (Madsen and Jensen, 2008) as:

$$y = XB + Zu + e \quad (1)$$

where y is the vector of the observed or standardised phenotypes of each trait, B is the vector of fixed effects, u is the vector of the random additive genetic effects $\sim (0, A\sigma_a^2)$ where σ_a^2 is the additive genetic variance and A is the pedigree derived numerator relationship matrix among the animals, e is the vector of residual effects $\sim (0, \sigma_e^2)$ where σ_e^2 is the error variance, and X and Z are incidence matrices relating observations to the fixed effects and the additive genetic effect of the individual animal respectively. To increase statistical confidence in the estimation of variance components for PO_2, families that represented less than 2% of the sampling group were excluded. Poorly represented families of Oc_1 were also excluded when groups were treated separately for the analyses, but included when all three F₁ groups were combined into a single group, fitting age and tank as fixed effects (at three and five levels, respectively). Furthermore, because variances for growth traits W and Ls across the weaning tanks were heterogeneous (Leven's test: $P < 0.01$), estimates of variance components for the combined weaning group were also obtained for standardised traits by scaling the data to a common variance, as

suggested by Hill (1984) (also see Bentsen *et al.*, 2012). For the combined dataset of Oc_1, phenotypes for the weaning group were standardised to variances obtained for the two older F₁ groups (which were sampled from a single tank).

The direct heritability (h^2) for traits W, Ls and K was estimated as $\sigma^2_A/(\sigma^2_A + \sigma^2_e)$, where σ^2_A and σ^2_e are the variances attributed to additive and residual error effects respectively. Phenotypic- (r_p) and genetic correlations (r_g) between traits were calculated for each F₁ group of spawn Oc_1 and for the offspring representatives of spawn PO_2. Bivariate models similar to univariate models (1) were used to obtain covariance components for the traits: the genetic correlation between the traits for each F₁ group, and for the combined dataset of Oc_1, were calculated as $r_g = \sigma_{A1A2}/(\sqrt{(\sigma^2_{A1})} \sqrt{(\sigma^2_{A2})})$, where σ_{A1A2} is the estimated additive genetic covariance component between the traits. For the combined dataset of Oc_1, covariance components were estimated using observed and standardised phenotypes, as suggested by Hill (1984).

3.2.3) Experiment 3- Assessing phenotypic performance within and between families

The impact of size-grading and culling practices on juvenile growth and phenotypic variation of cohort PO_1 was assessed. Differences in mean W and Ls between the different groups (or tanks) were examined using an independent-samples two-tailed t-test (with a threshold significance of 5%), in XLStatistics. The coefficient of variation (CV) of W was also calculated for each group with the formula $CV = SD/\text{mean value}$. Difference in variances for W between the tanks was assessed using Leven's test (with a threshold significance of 5%).

To investigate whether there are expected differences in family growth and levels of contribution over time (throughout the production cycle) of spawn Oc_1, and between families of spawn PO_2, averaged W, Ls and K were determined for each family within each group. To further investigate parental influences on juvenile growth of Oc_1, estimated breeding values (EBVs) for W and Ls were obtained for the seven parents in the combined dataset, using BLUP (Best Linear Unbiased Prediction), from a bivariate model, in DMU AI software v6.5.2 (Madsen and Jensen, 2008).

3.3) Results

3.3.1) Markers, parentage assignment and reproductive success

For spawn Oc_1, all 300 specimens representing three F₁ groups were successfully genotyped using 14 microsatellite markers, as described in *Chapter 2*. For this chapter, a total of 15 microsatellites (*i.e.* including *Ajap24*) were used to genotype an additional 302 offspring individuals. Across all offspring- and broodstock individuals, Micro-checker indicated no major genotypic errors or null alleles at the loci analysed and all individuals were successfully assigned to their parents with the exception of two individuals from Oc_2, which were excluded from further analyses.

In general, male brooders present in the spawning tanks were more likely to contribute as parents than female brooders (*Table 3.2*). Sire S2_{Oc1} and dam D3_{Oc1} (of spawn Oc_1) that successfully contributed to the offspring were the only parents that contributed to spawn Oc_2. A single full-sib group was also detected for the offspring individuals representing spawn PO_1. Contrary, spawn PO_2 generated 100% of all possible crosses between male and female brooders, revealing four full-sib families (*i.e.* two half-sib families); however, levels of contribution across the four parents were highly skewed with 79% of the offspring individuals assigning to sire S2_{PO2}. In addition, family S1/D2_{PO2} represented only a single individual within the sampling cohort, which was excluded prior to the estimation of variance components. A similar observation was made for family S3/D3_{Oc1} of group OF₁₂, and this family was excluded when this group was treated separately for estimating (co)variance components, but included (along with four other animals) when all three F₁ groups were combined for the analysis. Some phenotypic outliers of family S1/D2_{Oc1} were also excluded prior to the estimation of (co)variance components and comprised 2.3% of the total 300 assigned offspring individuals of spawn Oc_1.

Table 3.2: Number of offspring assigned to a parental pair, number of (full-sib) families and broodstock reproductive success across four spawning events. Ns-, Nd-, N tank = number of sires, dams and broodstock present in the spawning tank; Nes, Ned, Ne = number of sires, dames and broodstock that successfully contributed to the spawning event.

	Spawn			
	Oc_1	Oc_2	PO_1	PO_2
Assigned offspring	300	98	100	102
-Families	5	1	1	4
Sires Ns tank	4	2	1	2
-Nes	3	1	1	2
-Nes/Ns tank	0.75	0.50	1.00	1.00
Dames Nd tank	8	3	3	2
-Ned	4	1	1	2
-Ned/Nd tank	0.50	0.33	0.33	1.00
Broodstock N tank	12	5	4	4
-Ne	7	2	2	4
-Ne/N tank	0.58	0.40	0.50	1.00

3.3.2) Phenotypic data and genetic parameters for growth traits

Mean phenotypic data for growth related traits in each mass spawning cohort is provided in [Table 3.3](#). Averaged W and Ls were comparable between cohorts of similar ages, though mean estimates for K varied markedly between the offspring cohorts and were generally lower for PO farm.

Table 3.3: Phenotypic data (observed mean) with standard deviations (sd) for kob growth traits and age (dph = days post hatch) of each offspring group included in this study

Spawn	Age (dph)	Trait					
		W (g)		Ls (mm)		K	
		Mean	sd	Mean	sd	Mean	sd
Oc_1	30	0.51	0.38	27.70	6.65	2.04	0.40
	150	42.17	12.82	139.23	15.32	1.52	0.14
	674	526.90	168.55	326.20	41.59	1.41	0.14
Oc_2	391	301.98	130.69	268.63	38.75	1.47	0.14
PO_1	482	416.31	143.70	330.04	35.75	1.12	0.14
PO_2	129	23.20	10.21	119.30	17.16	1.29	0.12

For spawn PO_1, mean W and L_s for the LG tank were significantly higher than estimates obtained for the SG tank ($P < 0.01$ for W and $P < 0.05$ for L_s), albeit slightly lower than estimates obtained for the NG group ($P > 0.05$ for W and L_s ; Table 3.4). Variances in W and L_s between the LG and SG tank were similar and, collectively, significantly lower than estimates obtained for the NG tank ($P > 0.01$ for W and $P > 0.05$ for L_s).

Table 3.4: Mean body weight (W) and length (L_s) with coefficients of variation (CV, %) for each tank of spawn PO_1. SG = small-grade, LG = large-grade and NG = non-grade.

	W (g)			Ls (mm)		
	SG	LG	NG	SG	LG	NG
Mean	334.5	419.4	445.7	31.5	33.4	33.6
CV %	29	24	36	9	8	12

Heritability estimates for dusky kob growth traits W and L_s for cohort PO_2 were 0.34 ± 0.42 and 0.35 ± 0.41 , respectively, and 0.02 ± 0.10 for the morphometric ratio K . Heritability estimates for growth traits were also imprecise for the three F_1 groups of Oc_1. Estimates for W and L_s , however, were in general higher and more precise than estimates obtained for K , and in the combined dataset, and more accurate (with lower standard errors) than estimates obtained for W and L_s when groups were analysed separately (Table 3.5). The use of standardised phenotypes to correct for heterogeneous variances between the different tanks of the weaning group resulted in higher and slightly more accurate estimates of heritability for growth in this group, and these marginally increased estimates of heritability for both these traits in the combined dataset. The use of standardised traits for estimating the heritability of K and the genetic correlation between W and L_s in the combined dataset provided similar results to those obtained when the data was analysed with observed phenotypes.

Genetic correlations between W and L_s for any given F_1 group, and in the combined dataset, were higher than phenotypic correlations (Table 3.5; Figure S3.1). Because of convergence problems, genetic correlations between K and growth traits W and L_s could not be obtained. Phenotypic correlations between Fulton's conditioning factor K and W were low, in general, with the exception of groups OF_{13} (Figure S3.2) and PO_2 (Figure S3.4) which displayed significant, but contrasting correlations between the two traits. Phenotypic correlations between K and L_s were in general negative and not significant (Figure S3.3; Figure S3.4).

Table 3.5: Genetic parameters (heritability; h^2 and genetic correlations; r_g) \pm standard errors and phenotypic correlations for dusky kob growth traits weight (W) and length (L_s) using observed phenotypes for each group of spawn Oc_1 : standardised estimates of heritability are indicated in brackets. Heritability estimates for Fulton's conditioning factor K (from univariate models) are also indicated.

Analysis	F1 group			
	OF_{11}	OF_{12}	OF_{13}	Combined
$h^2 W$	0.30 \pm 0.32 (0.35 \pm 0.34)	0.54 \pm 0.43	0.08 \pm 0.37	0.28 \pm 0.22 (0.34 \pm 0.25)
$h^2 L_s$	0.27 \pm 0.30 (0.36 \pm 0.35)	0.51 \pm 0.42	0.16 \pm 0.29	0.27 \pm 0.21 (0.36 \pm 0.27)
r_g	0.98 \pm 0.02	0.99 \pm 0.03	0.99 \pm 0.61	0.98 \pm 0.03
r_p	0.97	0.96	0.96	0.95
$h^2 K$	0.16 \pm 0.31	0.27 \pm 0.31	0.16 \pm 0.32	0.02 \pm 0.07

3.3.3) Family contribution and phenotypic performance

Family contribution to average weight at 129 dph of spawn PO₂ was found to be highly variable: among the three families with the highest contribution to the cohort (*i.e.* with at least three offspring assigned within the sampling cohort), the fastest growing family (S2/D3_{PO2}, 37±7.37g) was 73% heavier than the family with the slowest growth (S1/D2_{PO2}, 21±1.09g; Figure 3.1). Interestingly, however, the family with the slowest growth and second lowest averaged K had the greatest contribution to the spawning event (S1/D2_{PO2}, 77%). In addition, this particular family represented all the individuals that were identified below the first quartile for both *W* (<15.25g) and *L_s* (<100.7mm).

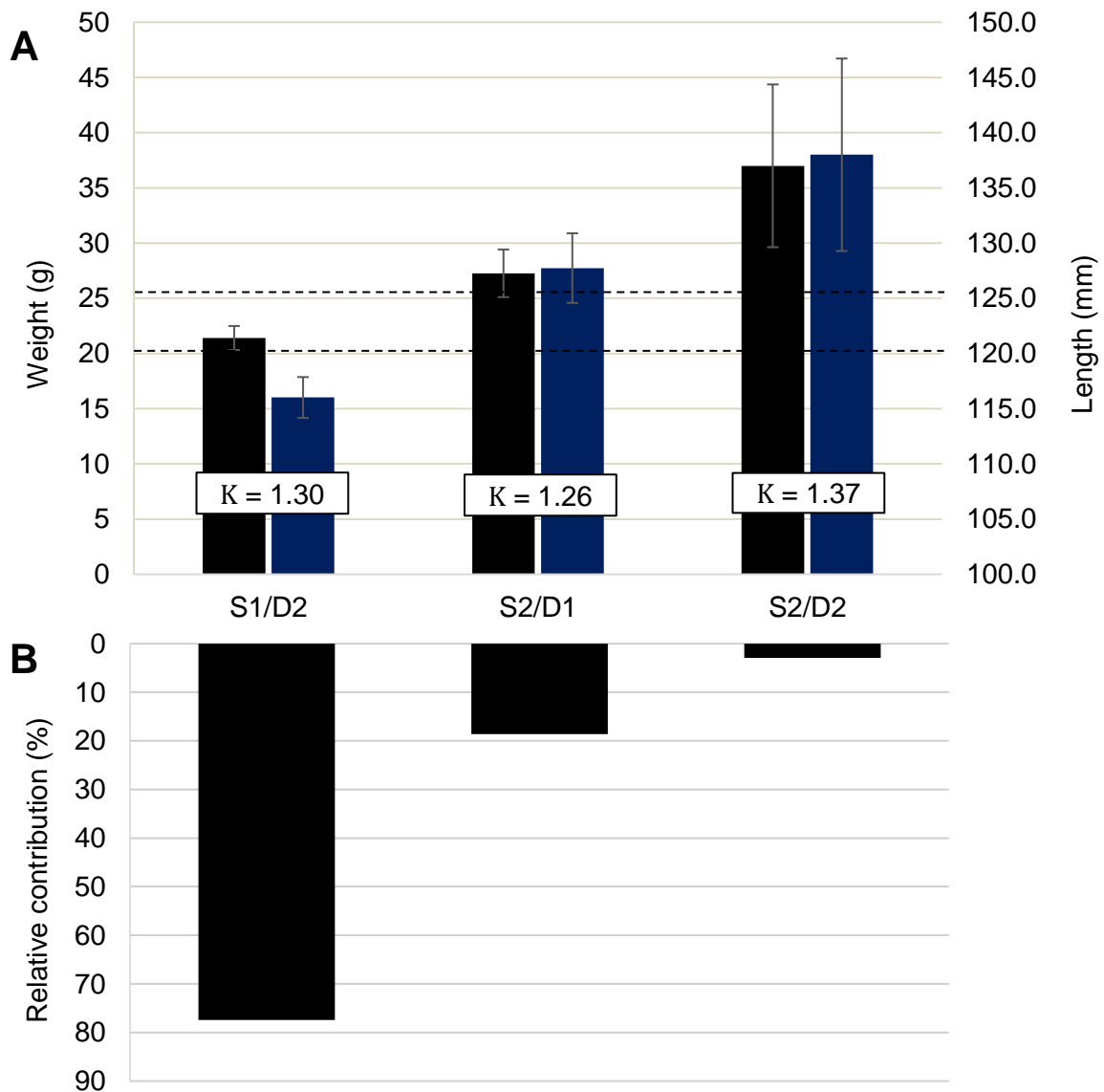


Figure 3.1: Mean (\pm s.e.) (A) weight (black), length (dark blue) and conditioning factor (K), and (B) overall contribution to progeny represented at 129 days-post-hatch (dph) of the three most represented families in cohort PO_2. Dash lines in panel A indicate upper and lower 95% confidence intervals for weight.

On the contrary, for spawn Oc_1, the fastest growing family (S1/D2_{Oc1}) had the greatest contribution to each of the three F₁ groups. For the first group, the remaining four families contributed significantly more to slower-growing animals (Figure S3.5), similar to that observed for the second group (Figure S3.6). For the oldest group however, averaged growth of family S3/D3_{Oc1} was not significantly different from that detected for family S1/D2_{Oc1} (*i.e.* estimates for W did not fall outside the upper and lower bounds for the null hypothesis of no difference). Furthermore, relative family contributions to K were in general inconsistent across the growth intervals; though, for the youngest and oldest groups of Oc_1, the families that had the greatest contribution to K (*i.e.* S3/D3_{Oc1} and S3/D4_{Oc1}) were on average slower-growing. Contrarily, for the second group of the Oc_1, the fastest-growing family had the greatest contribution to K, similar to that observed for PO_2 (which was sampled at a similar age).

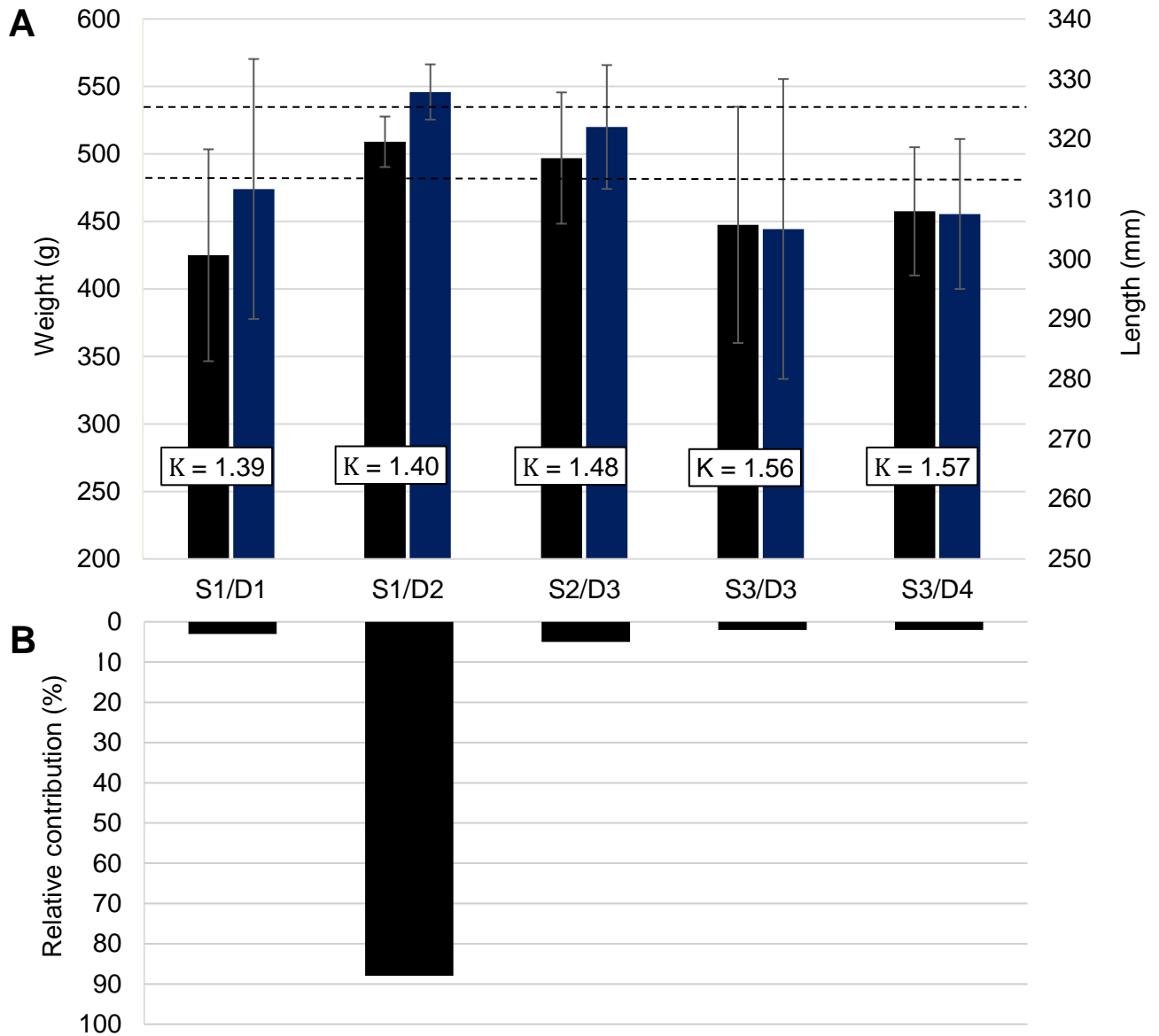


Figure 3.2: Mean (\pm s.e.) (A) family weight (black), length (dark blue) and conditioning factor (K) and (B) relative family contribution to progeny represented in group OF₁₃ (at 679 dph) of spawn Oc₁. Dash lines in panel A indicate upper and lower 95% confidence intervals for weight.

Growth performance also differed markedly with parent of origin in spawn Oc_1. Overall, sire S1 and dame D2 yielded the highest EBVs for traits W and Ls (Table 3.6), although dame D2 showed higher and more accurate estimates, due to more individuals assigning to this parent. Analysis of the combined dataset also revealed positive (but less reliable) EBVs for W and Ls for sire S2 and dame D3, which were the only two parents that contributed to spawn Oc_2.

Table 3.6: Estimated breeding values (EBVs) \pm standard error of the predictions from BLUP, using a bivariate model, for weight (W) and length (Ls) for all parents that successfully contributed to spawn Oc_1. Positive breeding values are indicated in bold.

Parent of Oc_1	EBV for W	EBV for Ls
S1	4.73\pm5.42	6.25\pm7.23
S2	0.17\pm5.42	1.04\pm7.71
S3	-4.89 \pm 6.38	-7.27 \pm 7.65
D1	-3.76 \pm 5.83	-2.76 \pm 6.98
D2	8.49\pm5.51	9.00\pm6.61
D3	0.77\pm6.37	1.48\pm7.64
D4	-5.50 \pm 6.63	-7.71 \pm 6.96

3.4) Discussion

In the present work, two important grow-out rearing practices commonly employed in dusky kob aquaculture, *i.e.* size grading and culling, were tested for their effects on juvenile growth and phenotypic variation. Results demonstrate that variances in juvenile growth can be significantly reduced following a combination of such practices. As may also be expected, grading did contribute to significant differences in averaged growth between the small- and large grade categories analysed here (Table 3.4). However, there is no evidence that suggests that these practices improved juvenile growth; in fact, individuals from both graded categories representing spawn PO_1 were on average slower growing than those individuals that were sampled from the non-graded group. It is unlikely that culling (prior to grading) contributed to a reduction in mean juvenile growth, given that only the smallest animals were discarded. Also, in principle, if individual growth performance does not vary substantially between individuals, as will often be the case if genetically related animals are raised together, elimination of the smallest individuals should have a larger impact on the variation of growth than on the mean (Taris *et al.*, 2006). Parentage analyses did indicate that spawn PO_1 generated only a single full-sib group. With similar variances in growth, it might also be expected that the stress imposed by larger animals over small animals will be less (Ruzzante, 1994; Dou *et al.*, 2004); consequently, the implementation of grading practices should have little effect in disrupting such hierarchies and in improving overall growth performance. Grading has been shown to improve the growth of captive sole, *S. solea* (Blonk *et al.*, 2010), silver perch, *Bidyanus bidyanus* (Barki *et al.*, 2000) and Tilapia, *Oreochromis niloticus*, (Sauod *et al.*, 2005), and the contradictory results from these previous studies were based on the separate rearing of offspring from multiple families. Furthermore, a recent investigation on cannibalism and kin recognition in Asian seabass found that juveniles avoided cannibalising their siblings (Liu *et al.*, 2017) and if this is the case in dusky kob, it might also be expected that cannibalism will be less common if genetically closely related offspring are raised together, regardless of whether grading or culling is applied. Based on personal observations of dusky kob behaviour during sampling of the non-graded group of PO_1, no incidences of cannibalism were observed, despite this sampling group exhibiting a significantly larger variance in growth in comparison to the graded

groups. On the contrary, communal rearing of five families (of Oc_1) did translate into injuries, mostly amongst slower-growing juveniles (personal observations). It therefore postulated that grading may have disrupted social hierarchies between these families, subsequently improving overall growth rate. It is pertinent to note however that environmental factors likely to affect cannibalism, such as light intensity (Timmer and Magellan, 2010), different feeding strategies (Kailasam *et al.*, 2002) and stocking density (Hecht and Pienaar, 1993) were not accounted for in the present study and are likely to have differed amongst the husbandry practices.

The heritability of dusky kob growth traits weight (W) and length (Ls) was estimated at three different time intervals. For the youngest (*i.e.* weaning) group of Oc_1, variances in growth traits between the (non-graded) tanks were heterogeneous, probably due to small sample sizes and in some cases sampling error, leading to unequal sampling of families across the tanks. To correct for heterogeneous variances between the tanks, phenotypes were standardised to a common variance and this resulted in higher and more precise estimates of heritability for growth traits for the combined weaning group. Values obtained for the weaning group are similar to previous estimates at 2 months of age in common carp, *i.e.* 0.33 (Vandeputte *et al.*, 2004), but higher than priori estimates, *i.e.* 0.2-0.3, for barramundi (Domingos *et al.*, 2013) and Japanese flounder (Tian *et al.*, 2011) of similar age. Estimates are likely to differ between samples. However, direct heritability estimates for these earlier studies were better (*i.e.* lower standard errors) due to a more robust experimental design (*i.e.* more families and samples per family). For instance, Domingos *et al.*, (2013) reported estimates of 0.21 ± 0.11 and 0.25 ± 0.14 for W and Ls, respectively, after (29 out of 40) families with four or fewer assigned offspring were excluded. Here, two out of five families each represented no more than 4% of the combined weaning group and with such skewed levels of contributions, the corresponding genetic variance between families can therefore not be accounted for in the overall estimate of genetic variance, resulting in a biased estimate of heritability. Family sizes were less variable within the second group analysed, which may have attributed to more reliable estimates of heritability for growth obtained for this particular group; nonetheless, with only four families included for the analysis likely to produce upward biased estimates, the values obtained here may not be considered with confidence.

It is also possible that estimates of heritability for growth traits W and L_s could have been biased by the occurrence of non-additive (genetic) effects (e.g. dominance and epistasis) and/or maternal effects, given that the animal model(s) implemented for the analyses assumed that all genetic effects were additive. For instance, previous reports on commercially produced rainbow trout, sea bass and salmon, illustrated that maternal effects on offspring growth are more prevalent during early developmental stages and tend to dissipate as fish grow older (Herbinger *et al.*, 1995; Garcia de Leon *et al.*, 1998; Haffray *et al.*, 2012). If this is the case in juvenile dusky kob, maternal effects may have generated upwardly biased estimates of heritability in the first two F_1 groups of Oc_1 analysed (*i.e.* heritability, in the narrow sense, for these groups, may be less than estimated here). As for the oldest (*i.e.* market-sized) group analysed, estimates of heritability for W and L_s were not significantly different from zero and, overall, this group displayed the highest standard errors associated with these estimates. This is most likely due to the fact that three of the five families each represented no more than three individuals within the sampling group, but could also in part be attributed to the fact that family $S1/D2_{Oc1}$ and family $S2/D3_{Oc1}$ shared similar growth rates (*i.e.* estimates for these families did not fall outside the upper and lower bounds for the null hypothesis of no difference; [Figure 3.2](#)). Nonetheless, quite apart from family $S2/D3_{Oc1}$, the pedigree results indicated that the lowest contributing families contributed significantly more to slower-growing juveniles for any given group, indicating that parental influences on growth persisted across the sampling dates. Heritability estimates for the combined dataset are in similar range, *i.e.* 0.3-0.4, (but less precise) to *priori* estimates of heritability for growth reported for barramundi (Domingos *et al.*, 2013), common carp (Vandeputte *et al.*, 2008) and red drum (Saillant *et al.*, 2007), where (co)variance components for juvenile growth traits were also obtained for a population comprising groups that were sampled at different time intervals. Furthermore, genetic correlations between W and L_s were, for any given group, higher than phenotypic correlations, and in the combined dataset ([Figure S3.1](#); [Table 3.5](#)), indicating that the same or closely linked genes are likely involved in expression of both traits.

Considering that Fulton's conditioning factor (K) is a trait derived from both W and L_s , it might be expected that genes influencing the expression of these traits would be similar, and that selection for rapid growth, in particular body weight (as $K = W/L_s$),

will lead to indirect selection for K. However, the low underlying genetic correlations between W and K (Nilsson, 1994; Fishback *et al.*, 2002; Martyniuk *et al.*, 2003; Vandeputte *et al.*, 2004) suggest that this generalised body trait may not be a reliable predictor of fish body weight. In addition, Wang *et al.*, (2008) found that the genetic correlations between K measured at different time intervals during the grow-out phase of cultured barramundi was nonsignificant. The genetic correlations between dusky kob K at different time intervals and between K and growth traits W and Ls for the separate groups could not be estimated, but the fact that estimates of heritability for W and Ls in the combined dataset were moderately high, but not significantly different from zero for K (Table 3.5), suggests that the expression of dusky kob K may differ to a greater extent temporally compared with W and Ls genes. Two major factors may explain why body conformations in cultured kob display such an apparent lack of underlying genetic control. First, it is well known that physiological factors such as lipid stores and protein accretion may alter fish body shape as a reflection of their nutritional intake (Koskela *et al.*, 1998; Kause *et al.*, 2002). Feeding frequency and intensity were likely to have differed not only across the farming practices but also in similar environments over time. Secondly, the data shows that growth in dusky kob, as in most fish species, is non-linear, such that body mass at a given body length can differentially increase or decrease throughout an individual's life time. This may explain why trends in family growth rate and K for the second group of Oc_1 were similar to that detected for cohort PO_2 (which was sampled at a similar age albeit from a different population, Figure 3.1; Figure S3.6), but different from that detected for the weaning and market-sized group of Oc_1 (where the slowest-growing families had the highest averaged K, Figure 3.2, Figure S3.5). Phenotypic correlations among growth-related traits with K differed substantially between the weaning- and market-sized group, however (Figure S3.2, Figure S3.3), and between the second group of Oc_1 and cohort PO_2 (Figure S3.4), which further highlights the influence of environmental noise on expression of K.

Further interesting trends in family growth and sizes were observed. Results demonstrated that the highest contributing family in Oc_1 was, at any given age, on average faster-growing, indicating that culling did lead to contractions in the size of phenotypically inferior families (e.g. S1/D1, S3/D4), which supports the results

obtained from *Chapter 2* (see [Figure 2.4](#)). There is also evidence that dam D2 had the greatest contribution to offspring growth within cohort Oc_1 ([Table 3.6](#)). Interestingly, analysis of PO_2 indicated that the highest contributing family was on average slower-growing. A similar observation was made for barramundi (Domingos *et al.*, 2014) and blunt snout bream (Luo *et al.*, 2017). Such discrepancies in parental contribution to family sizes and growth could be attributed to a combination of physiological factors (such as maturity and weight) and parental genotype (Brown *et al.*, 2005).

Behavioural factors, such as mate selection and competition during the spawning event (Fessehaye *et al.*, 2006b; Weir *et al.*, 2004) and a combination of this and parental genotype, such as the competitiveness of sperm (Wedekind *et al.*, 2007), may also contribute to the production of few families with variable sizes (which may have been the case during spawn Oc_1; *Chapter 2*). Results provide further evidence for mate selection, given that the two parents that successfully contributed to spawn Oc_1 were the only parents to contribute to spawn Oc_2. Furthermore, parentage analysis indicated that all broodstock candidates have the potential to contribute when the adult sex ratio is not biased; however, the breeding population (for spawn PO_2) comprised only four animals: a breeding population of 10 males and 10 females, for instance, may still result in limiting parental contributions (e.g. Liu *et al.* 2012; Domingos *et al.* 2014; Liu *et al.* 2017). Nonetheless, in these earlier studies, family sizes were much more even than that observed in the present study.

3.5) Conclusion

Overall, from this study four major conclusions can be drawn. Firstly, results demonstrate that variances in juvenile dusky kob can be significantly reduced following a combination of size-grading and culling practices; collectively, however, these practices did not improve juvenile growth. Secondly, although estimates of h^2 for juvenile W and Ls were, in general, imprecise (due to small sample sizes and possibly maternal effects), it is concluded that additive genetic effects play a significant role in the expression of these traits. Thirdly, the analysis indicates that the combination of genes influencing the phenotypic expression of juvenile W and Ls

may be different from the combination of genes influencing the expression of K. Lastly, parentage analysis indicated that female broodfish were less likely to contribute than available males when the adult sex ratio was biased, but were equally likely to contribute when the sex ratio was balanced.

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CHAPTER 4

Study conclusions

4.1) Overview

Dusky kob is as an emerging aquaculture species due to its popularity as indigenous line fish and overexploitation in the wild. Cultured kob in South Africa is currently derived from unimproved (wild-caught) broodstock, but considerable efforts were initiated to retain F₁-generation animals with fast growth rate with the specific aim of initiating a selective breeding programme. However, dusky kob's mass spawning behaviour presents a major challenge to the conduct of an efficient selective breeding programme, because single pair mating's can not easily be conducted to record individual family relations and phenotypic performance. Dusky kob also matures at a much larger size than most fish species and therefore, small broodstock populations are employed to generate progeny cohorts from which potential breeding candidates are then selected. Understanding how current production practices impact on the genetic constitution and phenotypic performance of the progeny cohorts is therefore critical for the development of effective genetic management and improvement strategies for dusky kob.

Chapter 2 therefore investigated how genetic diversity is represented and maintained within a commercial hatchery of dusky kob. A wild broodstock genetic database was included along with three cultured groups - collected throughout the production cycle. Although a recent study had assessed genetic diversity in cultured cohorts of dusky kob and compared it to the wild progenitor populations (Mirimin *et al.*, 2015), this study was limited in terms of the size of the cultured- and breeding populations investigated. Additionally, with the exception of a single application to cultured barramundi (Domingos *et al.*, 2014), *Chapter 2* is the first to assess genetic diversity in a mass spawning species across the production cycle, to market size.

In *Chapter 3*, an additional three cultured cohorts - collected across two facilities - were characterised, both in terms of kinship and phenotypic performance, in order to

gain a better understanding of how mass spawning and associated grow-out rearing practices impact on offspring family compositions and phenotypic performance. Lastly, in order to exploit the potential of a selective breeding programme for dusky kob, the use of modern quantitative genetic theory (*via* linear mixed models and Restricted Maximum Likelihood) was employed to estimate, for the first time, heritability and genetic correlations for dusky kob body weight (W), standard length (Ls) and Fulton's condition factor (K).

4.2) Broodstock contribution and genetic diversity in dusky kob

As adults, dusky kob spawn in groups in depths of up to 15m of water (Griffiths, 1997). To overcome inhibition to spawning within the hatchery, hormonal induction is necessary. Results show that, if the adult sex ratio that is used is female-biased, hormonal induction practices will not result in successful seed contribution. It was consistently shown across three mass spawning events that these practices result in high variability in female reproductive success. Moreover, only one female contributed when the size of the breeding population was reduced (*i.e.* from 12 to five or four animals). The low participation amongst broodstock, particularly females, could be attributed to mate selection and/or differential spawning timings. A female-biased sex ratio has been shown to minimize female reproductive success in Japanese flounder (Sekino *et al.*, 2004) and red drum (Gold *et al.*, 2008; 2010), whereas a male-biased sex ratio (which can promote male-male competition) often results in high variability in male reproductive success (Brown *et al.*, 2005; Frost *et al.*, 2006; Herlin *et al.*, 2008; Loughnan *et al.*, 2013), suggesting that differences in gender reproductive performance in commercial mass spawning species are likely dependent on the mating strategy employed. All brooders successfully contributed to the offspring when the breeding population comprised two males and two females, suggesting that greater parental contributions in dusky kob may be expected if a non-biased adult sex ratio is used.

With regards to genetic variability, the largest breeding population analysed (*i.e.* four males and eight females, *Chapter 2*) generated the most desirable outcome. The N_e estimate of the resulting progeny cohort was also higher than *priori* estimates

obtained for cultured populations of the same species (Mirimin *et al.*, 2015); however, its value was nearly 50-fold lower than that obtained for the wild progenitor population. This substantial reduction in N_e could in most part be attributed to the founder event, but also to low participating and highly skewed contributions amongst broodstock within the spawning event. Irrespective of this, the cultured animals did not experience a reduction in heterozygosity, likely as a result of a genetic bottleneck that was produced by the founder effect. Nonetheless, with the implementation of a selective breeding programme it is anticipated that genetic diversity will decrease due to an increase in inbreeding and relatedness (Jorde *et al.*, 1995; Blonk *et al.*, 2009; Knibb *et al.*, 2014).

One family dominated the cohort, both in terms of size and growth, suggesting that selection of broodstock from this cohort, without accounting for individual relatedness, will lead to excessive inbreeding. This family also experienced a significant increase in size following removal of the smallest animals by culling, suggesting that these practices may have the potential to complicate the selection of unrelated broodstock even further. Culling did not lead to the elimination of poorly-represented families; however, such practices may have had a more profound effect in the case of fewer families, as was the case with cultured barramundi where some families had been lost in the short time span 2 dph to 27 dph, following a mass spawn of seven broodfish (Frost *et al.*, 2006). Further, it is pertinent to note that a single parental pair which successfully contributed to the spawn analysed in *Chapter 2*, and had the second greatest contribution to juvenile growth within that spawn (as indicated in *Chapter 3*), was the only to contribute to a different spawn, conducted in the same hatchery in the following year. Thus, if hatchery managers were to establish an F_1 broodstock population comprising animals selected from both spawns, the probability that related animals will interbreed will increase.

4.3) Heritability of growth traits in juvenile dusky kob

Results from *Chapter 3* indicate moderate heritability estimates for W and L_s in juvenile dusky kob, suggesting that these traits could be improved by exploiting additive genetic effects through selective breeding. Strong genetic correlations indicate that similar genes are involved in the expression of both traits, implying that genetic improvement in W could be accomplished merely by selecting on L_s , and *vice versa*. Measurements on L_s , and selection for this trait, in dusky kob may be more feasible as fish grow older, given the large size of sexually matured broodstock. Although genetic correlations between W and L_s at different time intervals could not be estimated, the pedigree results showed that differences in family growth were, at any given age, essentially similar, suggesting that growth rate can be used as early predictor of growth at later stages. Heritability for growth in fish is expected to increase with age (e.g. Su *et al.*, 1996; Saillant *et al.*, 2006; Wang *et al.*, 2008) and may therefore provide a more suitable proxy for evaluating expected genetic gains than during early development stages (where additive genetic variation may be thwarted by maternal effects). However, the oldest group analysed displayed the lowest and most imprecise estimates of heritability due to a reduction in the size of some families after culling, suggesting that these practices may impact on the estimation of genetic parameters in commercial mass spawning species. Similarly, grading of multiple families can lead to an underestimation of heritability estimates (Blonk *et al.*, 2010).

Although heritability estimates obtained for dusky kob condition (K) were low and, in general, not significantly different from zero, it is still encouraging to note that some families maintained shape differences over time, which suggests the presence of some additive genetic variance for this trait. Interestingly, however, there was in general an inverse relationship in K and growth, such that the slowest-growing families displayed on average the highest K . Considering that Fulton's K -index is indicative of the accumulation of body fat, hence nutritional status, of an individual, it would appear, therefore, that slower-growing families of dusky kob are in better physiological condition compared to faster-growing families. However, the low genetic correlations reported among traits such as fillet weight, and visceral and abdominal fat with K (Kause *et al.*, 2002) suggest that K may not be a reliable

predictor of juvenile condition or general “well-being”. Also, in aquaculture, if the objective is to market fillets, a higher K (*i.e.* triangular shape) may have a negative impact on consumer acceptance. Although genetic correlations amongst growth traits W and Ls with K could not be estimated, the inverse relationship detected in the market-sized (and weaning) group suggests that little or no change in K would occur as a correlated response to selective breeding for fast growth.

4.4) Considerations for the implementation of a selective breeding programme for dusky kob

The data generated in both experimental chapters highlights that, even if a spawn of 12 breeders was used, a single spawn would not be sufficient to capture and attain adequate numbers of genetically diverse families for an efficient selective breeding programme for dusky kob. Several authors have suggested a base population of a minimum of 50 unrelated broodstock pairs is necessary to limit loss of genetic diversity and avoid excessive inbreeding (Bentsen and Olesen, 2002; Sonesson *et al.*, 2005). Selection of such broodstock is not practical for dusky kob as the species reaches sexual maturity at a very large size (*i.e.* one meter in length). Alternatively, broodstock could be spawned in several isolated small groups (*e.g.* two males and two females) with each generation developed comprising offspring pooled from multiple and diverse spawns (*e.g.* Robinson *et al.*, 2010). Over the years, the South African marine finfish industry have collected four batches of potential F₁ broodstock that were spawned from small breeding populations, similar to that analysed in the present study. The efficiency of conducting multiple small spawns rests on the fact that phenotypic variation, and possibly cannibalism, can be minimised and inbreeding can be limited. To prevent accumulation of inbreeding however, a restricted number of individuals will need to be pooled from each respective spawn. Multiple consecutive spawns will therefore need to be carried out, which will greatly increase the generation time, especially if similar wild-caught broodstock are repeatedly spawned over several years (as was the case in practice). Separate rearing may also introduce environmental and physiological variation: with multiple consecutive spawns, this component will become large, which could mask genetic differences between broodstock candidates and slow genetic improvement.

Although the South African dusky kob industry does not have the capacity to achieve the desired base population size, the industry does have suitable breeding candidates (*i.e.* > 30 animals) and the infrastructure required to greatly increase the size of the breeding populations. Increasing the size of the base population will allow for the greatest number of genetically unrelated families of similar age from which F₁ broodstock candidates can be selected. A large spawn may also stimulate more even contributions amongst parents to the offspring (Loughnan *et al.*, 2013; Domingos *et al.*, 2014; Liu *et al.*, 2017), which will diminish subsequent effects of size grading and culling on genetic variation that will be available for selective breeding. Nevertheless, due to moderate to high heritability of dusky kob growth rate, related animals are still likely to be selected to contribute as parents for the next generation of breeders. While growth rate undoubtedly will remain the major phenotype to be selected for in dusky kob, a selection programme for the species will need to consider both individual growth performance and genetic relatedness, *e.g.* using walk-back selection. Walk-back selection applies DNA profiling as a way to minimize kinship and maximise genetic gains in the absence of pedigree data (Doyle and Herlinger, 1994). Genotyping is an expensive procedure, though it may be argued that for species with extremely long intervals (such as dusky kob, red drum and meagre) the long-term genetic gains and benefits that can be achieved will greatly exceed any initial trepidation. This is particularly evident for dusky kob, given that there are only few commercial hatcheries in operation at present, one of which (where the F₁-broodstock batches are currently being held) commonly distributes seed animals to other hatcheries for grow-out. Whatever achieved in the breeding nucleus may therefore have an extensive impact on the industry.

Another major advantage of conducting a large spawn at the commencement of a selective breeding programme is that genetic parameters can be accurately determined for a variety of different traits. In particular, genetic parameters for growth-related traits could be estimated during early developmental stages to reveal wild-caught broodstock with superior genetic merit for growth. While selective breeding programmes are being considered and/or implemented, such a method of selection will thus allow managers to produce seed animals from only the best performing broodstock, which will result in increased short-term productivity. For this

purpose, farmers should separate the improved line from the unselected populations to prevent genetic deterioration.

4.5) Shortcomings and perspectives on future undertakings

The markers utilised in the present study proved useful for the genetic data analysis and could be used for continued genetic monitoring of cultured populations, as well as for tracing wild from farmed products (Mirimin *et al.*, 2015). The development of more markers or different types of markers may be extremely useful. Expressed sequence tags (ESTs) and single nucleotide polymorphisms (SNPs), especially SNPs, are gaining popularity, and are useful for linkage mapping and marker-assisted selection (MAS). Further, the data generated in *Chapter 2* indicated evidence for directional selection at one of the loci: interestingly, the two parents that had the greatest contribution to the offspring (both in terms of growth and numbers) were the only parents within the broodstock genetic database that was homozygous for this particular locus. Additional analyses such as QTL mapping or gene expression profiling will therefore be useful in confirming that the locus is indeed under selection (e.g. Larsen *et al.*, 2011; Pardo-Diaz *et al.*, 2015).

Heritability estimates for dusky kob weight and length were moderate to high, although the pedigree results also demonstrate the presence of additive genetic variation for these traits, the heritability values obtained can not be considered with true confidence. One clear limitation is the small sample sizes (*i.e.* few families and few individuals for some of the families) that were used. Family sizes also varied considerably across the sampling dates and therefore the true heritability at a given age could not be determined. Specifically, genetic parameters for dusky kob growth traits at harvest should be estimated. Many more samples will also be needed to obtain the true heritability of dusky kob K. Juvenile shape characteristics could impact market acceptance in a developing and more competitive dusky kob industry, apart from K, body depth (BD) or perhaps a body shape index (H), *i.e.* the ratio between BD and Ls could be used as more reliable indicators of body conformation and perhaps juvenile condition. Positive genetic correlations between fish weight and BD or H have been reported (Gjedrem and Thodesen, 2005; Domingos *et al.*, 2013) and the possibility of their simultaneous selection in dusky kob should also be

evaluated. To further exploit the full potential of a selective breeding programme for dusky kob, estimation of genotype by environment (G x E) interactions for growth-related traits should also be conducted (e.g. Dupont-Nivet *et al.*, 2008; Vandeputte *et al.*, 2014; Vlok *et al.*, 2016). As previously mentioned, one of the dusky kob facilities (that was included for the analysis) commonly distributes larvae to other hatcheries for grow-out, which presents an ideal opportunity for investigating whether different genotypes will express the same growth performance and, thus, deliver similar genetic gains across the culture environments.

Future studies should also be directed towards estimating the phenotypic and genetic correlation between aggressive behaviour, such as cannibalism, and growth rate in dusky kob. Genetic correlation between growth and aggressive behaviour may not always be positive, which in turn may confound breeding value predictions for growth (Vollestad and Quinn, 2003; Khaw *et al.*, 2016). Estimating genetic correlations between growth rate and aggressive behaviour, or cannibalism, may be challenging and labour intensive as it requires continuous monitoring of individual aggression and/or tagging, or dissection of stomachs to reveal predators and prey (*via* genotyping). Cannonballed animals could also be DNA profiled, which will give an indication as to whether cannibalism is selective with respect to kin and not just body size. More importantly, the hypothesis that separate rearing of genetically unrelated animals will reduce cannibalistic behaviour and consequently improve growth rate in dusky kob should be tested.

Increasing evidence suggests that adult breeder size is strongly correlated with egg quality (e.g. egg size, number and volume) and larvae size and survival (Su *et al.*, 1997; Vandeputte *et al.*, 2002; Johnson *et al.*, 2010, 2011). However, the results from *Chapter 3* show that there may not always be a positive correlation between offspring family sizes and growth. Adult breeder size was not accounted for in the present study, considering that it is likely to differ markedly between wild-caught broodstock, it will be of interest to know how adult size impacts on the fate of relative family sizes and growth in the hatchery. Multiple spawns of similar broodstock will need to be carried out however, given that physiological factors, mate competition *etc.* may contribute not only to limiting- but also unequal genetic contributions amongst broodstock.

The results obtained in *Chapter 3* demonstrated that early progeny testing (*via* DNA profiling and phenotyping) can be used as a powerful tool to target individual broodstock with superior genetic merit for growth. A major drawback of the technique, however, is that parental breeding values can only be estimated after offspring are large enough to be phenotyped. The RNA/DNA ratio, an indicator of protein synthesis, has been widely used to predict fish condition and growth (review by Buckley *et al.*, 2004; Foley *et al.*, 2016), especially in larvae as metabolic activity and growth of cells (and thus, RNA regulation) during early development stages is at its highest (Johnston, 2006). Faster growing larvae could therefore be detected before the phenotype is expressed (similar to MAS), and subsequently genotyped to reveal faster-growing parents (*e.g.* Marshall and Morgan, 2011; Domingos *et al.*, 2013). The technique has had some success in predicting growth of adult fish, though mostly with fish of similar age (*e.g.* Bulow *et al.*, 1987; Smith and Buckley, 2003). To a larger extent, RNA/DNA ratios could be used for comparisons between groups across space (*e.g.* Vinagre *et al.*, 2008; De Raedemaeker *et al.*, 2012), which will allow farmers to source broodstock from specific geographic locations. For this purpose, estimates of effective population size throughout the species' range, between subpopulations, and ideally individual relatedness, should be considered.

4.6) Concluding remarks

This study is, to date, the most comprehensive survey of genetic variation in farmed populations of dusky kob and South African marine finfish in general. Additionally, this study represents one of first attempt to assess genetic variation throughout the production cycle of a commercial mass spawning species. The obtained results suggest that, even though mass spawning production practices did not impact on genic heterozygosity, most likely as a result of a bottleneck produced by the founder effect, the substantial elevation in genetic relatedness observed in the cultured populations will lead to excessive inbreeding and significant loss of genetic diversity in later generations of selective breeding. This is accentuated if selecting for growth rate as it is a trait with a large genetic component in dusky kob. While growth traits will undoubtedly remain the major phenotype to be selected for in dusky kob culture, and communal spawning is practiced, a selection programme for the species may

also need to consider individual relatedness. Molecular genetics is expected to play a major role in the development of aquaculture breeding programmes and dusky kob is poised to capitalise on this, especially considering its extremely long generation interval.

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APPENDIX A

Supplementary Information for Chapter 2

Table S2.1: Basic marker information for the seventeen microsatellite markers that was used in the present study. Marker name, linkage group, repeat motif, dye colour, size range and source are indicated.

Table S2.2: Summary of diversity statistics detected at 14 microsatellite loci in the wild- and three cultured population cohorts. These include sample size (N), number of alleles per marker (A_n), allelic richness (A_r), observed heterozygosity (H_o), unbiased expected heterozygosity (uH_e), F_{is} values, and HW (Hardy Weinberg) P -values. P -values with a threshold significance of 1% are in bold.

Table S2.3: Number of cultured individuals assigned to each of five full-sib groups. S = sires and D = dams.

Table S2.1: Basic marker information for the seventeen microsatellite markers that was used in the present study. Marker name, linkage group, repeat motif, dye colour, size range and source are indicated.

Multiplex	Marker Name	Repeat Type	Dye	Size Range	Source
I	Ajap06	(GGAT)n	FAM	154 - 206	Mirimin <i>et al.</i> 2013
	UBA05	(CT)n	PET	117 - 170	Archangi <i>et al.</i> 2009
	UBA06	(CA)n	VIC	131 - 183	Archangi <i>et al.</i> 2009
	UBA42	(TGC)n	NED	112 - 200	Archangi <i>et al.</i> 2009
II	Ajap12	(ATCT)n	PET	103 - 188	Mirimin <i>et al.</i> 2013
	Ajap14	(ATCT)n	FAM	80 - 230	Mirimin <i>et al.</i> 2013
	Ajap37	(AGC)n	NED	130 - 248	Mirimin <i>et al.</i> 2013
	UBA40	(CA)n	VIC	133 - 199	Archangi <i>et al.</i> 2009
	UBA851	(AG)n	FAM	203 - 253	Archangi <i>et al.</i> 2009
III	Ajap05	(AGAT)n	PET	110 - 189	Mirimin <i>et al.</i> 2013
	Ajap34	(CAG)n	VIC	167 - 245	Mirimin <i>et al.</i> 2013
	UBA50	(GT)n	FAM	120 - 260	Archangi <i>et al.</i> 2009
	UBA854	(TG)n	NED	120 - 260	Archangi <i>et al.</i> 2009
IV	Ajap24	(AGAT)n	PET	245 - 306	Mirimin <i>et al.</i> 2013
	UBA44	(GT)n	NED	158 - 208	Archangi <i>et al.</i> 2009
	UBA853	(GA)n	FAM	162 - 200	Archangi <i>et al.</i> 2009

UBA53	(CA)n	VIC	187 - 227	Archangi <i>et al.</i> 2009
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Table S2.2: Summary of diversity statistics detected at 14 microsatellite loci in the wild- and three cultured groups. These include sample size (N), number of alleles per marker (A_n), allelic richness (A_r), observed heterozygosity (H_o), unbiased expected heterozygosity (uH_e), F_{is} values, and HW (Hardy Weinberg) P -values: P -values with a threshold significance of 1% are in bold.

Marker	Cohort	N	N_a	A_r	H_o	uH_e	F_{is}	HW P -value
Ajap05	Wild	33	12.000	12.000	0.879	0.887	0,010	0.015
	Cul_1	99	5.000	4.983	0.949	0.712	-0,336	0.000
	Cul_2	100	5.000	4.996	0.940	0.736	-0,280	0.000
	Cul_3	99	5.000	4.835	0.980	0.677	-0,450	0.000
Ajap06	Wild	34	7.000	6.970	0.706	0.722	0,022	0.365
	Cul_1	98	5.000	4.419	0.469	0.475	0,012	0.000
	Cul_2	100	5.000	4.194	0.530	0.480	-0,105	0.000
	Cul_3	100	5.000	3.649	0.410	0.408	-0,005	0.000
Ajap12	Wild	34	12.000	11.911	0.794	0.866	0,084	0.222
	Cul_1	97	6.000	5.240	0.134	0.221	0,396	0.000
	Cul_2	99	6.000	5.142	0.202	0.276	0,268	0.000
	Cul_3	100	6.000	4.859	0.110	0.189	0,418	0.000
Ajap14	Wild	33	14.000	14.000	0.848	0.877	0,033	0.214
	Cul_1	99	7.000	6.234	0.960	0.777	-0,237	0.000
	Cul_2	99	6.000	5.908	0.909	0.777	-0,171	0.000
	Cul_3	99	6.000	5.575	0.990	0.719	-0,380	0.000
Ajap37	Wild	34	6.000	5.999	0.559	0.528	-0,060	0.962
	Cul_1	97	4.000	3.865	0.526	0.574	0,084	0.004
	Cul_2	100	4.000	3.844	0.600	0.567	-0,058	0.338
	Cul_3	100	4.000	3.421	0.530	0.536	0,012	0.176
UBA05	Wild	34	8.000	8.000	0.882	0.860	-0,026	0.540
	Cul_1	98	5.000	4.912	0.714	0.696	-0,026	0.000
	Cul_2	100	5.000	4.800	0.720	0.667	-0,079	0.000
	Cul_3	99	5.000	4.329	0.646	0.639	-0,012	0.000
UBA06	Wild	34	9.000	9.000	0.794	0.869	0,087	0.768

	Cul_1	97	7.000	6.523	0.938	0.795	-0,182	0.000
	Cul_2	100	7.000	6.867	0.960	0.801	-0,199	0.000
	Cul_3	99	7.000	6.641	0.990	0.784	-0,265	0.000
UBA40	Wild	34	8.000	7.970	0.824	0.794	-0,038	0.114
	Cul_1	100	5.000	4.868	0.960	0.759	-0,266	0.000
	Cul_2	100	5.000	4.552	0.900	0.755	-0,194	0.000
	Cul_3	99	4.000	4.000	0.970	0.740	-0,313	0.000
UBA42	Wild	33	8.000	8.000	0.758	0.731	-0,038	0.146
	Cul_1	97	4.000	3.792	0.505	0.445	-0,137	0.000
	Cul_2	100	4.000	3.644	0.490	0.429	-0,142	0.000
	Cul_3	98	4.000	3.629	0.337	0.335	-0,004	0.000
UBA44	Wild	34	12.000	11.941	0.824	0.878	0,063	0.056
	Cul_1	99	8.000	6.380	0.606	0.534	-0,136	0.000
	Cul_2	98	8.000	6.600	0.704	0.565	-0,248	0.000
	Cul_3	100	8.000	5.417	0.530	0.449	-0,182	0.000
UBA50	Wild	34	9.000	8.971	0.765	0.865	0,118	0.063
	Cul_1	97	6.000	5.514	1.000	0.677	-0,480	0.000
	Cul_2	100	7.000	6.030	1.000	0.685	-0,462	0.000
	Cul_3	99	6.000	5.068	1.000	0.670	-0,496	0.000
UBA851	Wild	34	6.000	5.941	0.735	0.622	-0,186	0.665
	Cul_1	100	4.000	3.802	0.730	0.615	-0,189	0.000
	Cul_2	99	4.000	3.872	0.758	0.644	-0,177	0.000
	Cul_3	97	4.000	3.566	0.825	0.651	-0,269	0.000
UBA853	Wild	34	12.000	11.911	0.941	0.857	-0,099	0.958
	Cul_1	98	6.000	5.852	1.000	0.781	-0,283	0.000
	Cul_2	100	7.000	6.743	0.980	0.794	-0,235	0.000
	Cul_3	100	7.000	6.222	1.000	0.777	-0,288	0.000
UBA854	Wild	34	7.000	6.941	0.735	0.721	-0,020	0.586

Cul_1	99	4.000	3.999	0.687	0.667	-0,029	0.000
Cul_2	100	4.000	3.998	0.790	0.682	-0,160	0.000
Cul_3	100	4.000	3.993	0.700	0.658	-0,064	0.000

Table S2.3: Number of cultured individuals assigned to each of five full-sib groups using the combined genotypic dataset. S = sires and D = dams.

Sires	Dams			
	D1	D2	D3	D4
S1	21	245		
S2			19	
S3			5	10

APPENDIX B

Supplementary Information for Chapter 3

Figure S3.1: Scatterplots illustrating correlation analysis for standard length versus body weight for group OF₁1 (**A**), OF₁2 (**B**), and OF₁3 (**C**). Trend line equations, R^2 -values, correlation coefficients (r) and corresponding significance (P) values are also indicated.

Figure S3.2: Scatterplots illustrating correlation analysis for Fulton's conditioning factor versus body weight for group OF₁1 (**A**), OF₁2 (**B**), and OF₁3 (**C**). Trend line equations, R^2 -values, correlation coefficients (r) and corresponding significance (P) values are also indicated.

Figure S3.3: Scatterplots illustrating correlation analysis for standard length versus Fulton's conditioning factor K for group OF₁1 (**A**), OF₁2 (**B**), and OF₁3 (**C**). Trend line equations, R^2 -values, correlation coefficients (r) and corresponding significance (P) values are also indicated.

Figure S3.4: Scatterplots illustrating correlation analysis for standard length versus body weight (**A**), K versus weight (**B**) and – length (**C**) for group PO_2. Trend line equations, R^2 -values, correlation coefficients (r) and corresponding significance (P) values are also indicated.

Figure S3.5: Mean (\pm s.e.) (**A**) weight (black), length (dark blue) and conditioning factor (K), and (**B**) overall contribution to progeny represented at 30 days-post-hatch (dph) of the five families in cohort OF₁1 of spawn Oc_1. Dash lines in panel **A** indicate upper and lower 95% confidence intervals for weight.

Figure S3.6: Mean (\pm s.e.) (**A**) weight (black), length (dark blue) and conditioning factor (K), and (**B**) overall contribution to progeny represented at 150 days-post-hatch (dph) of the four most representative families in group OF₁2 of spawn Oc_1. Dash lines in panel **A** indicate upper and lower 95% confidence intervals for weight.

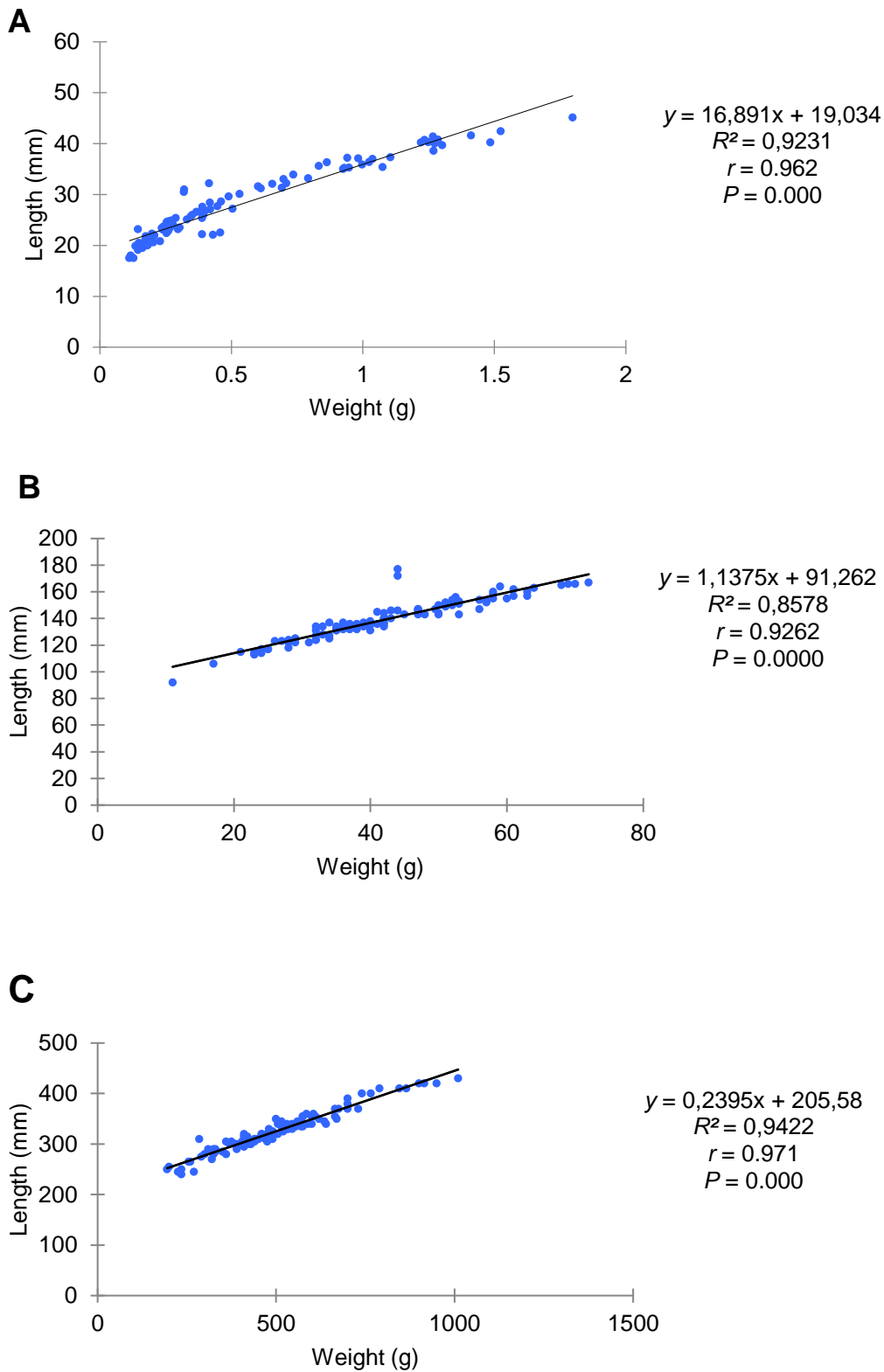


Figure S3.1: Scatterplots illustrating correlation analysis for standard length versus body weight for group OF₁ (A), OF₂ (B), and OF₃ (C). Trend line equations, R^2 -values, correlation coefficients (r) and corresponding significance (P) values are also indicated.

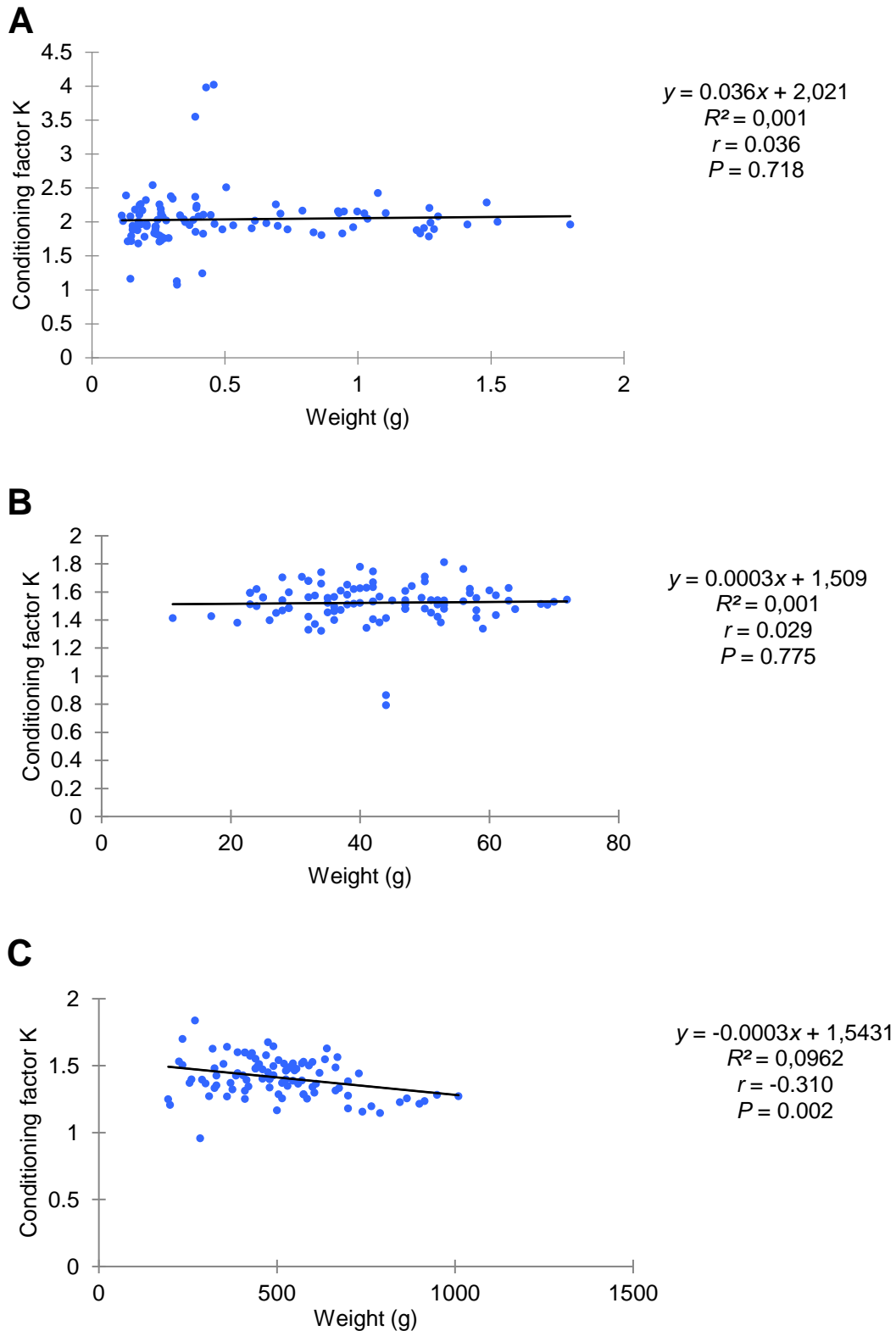


Figure S3.2: Scatterplots illustrating correlation analysis for Fulton's conditioning factor K versus body weight for group OF₁1 (A), OF₁2 (B), and OF₁3 (C). Trend line equations, R^2 -values, correlation coefficients (r) and corresponding significance (P) values are also indicated.

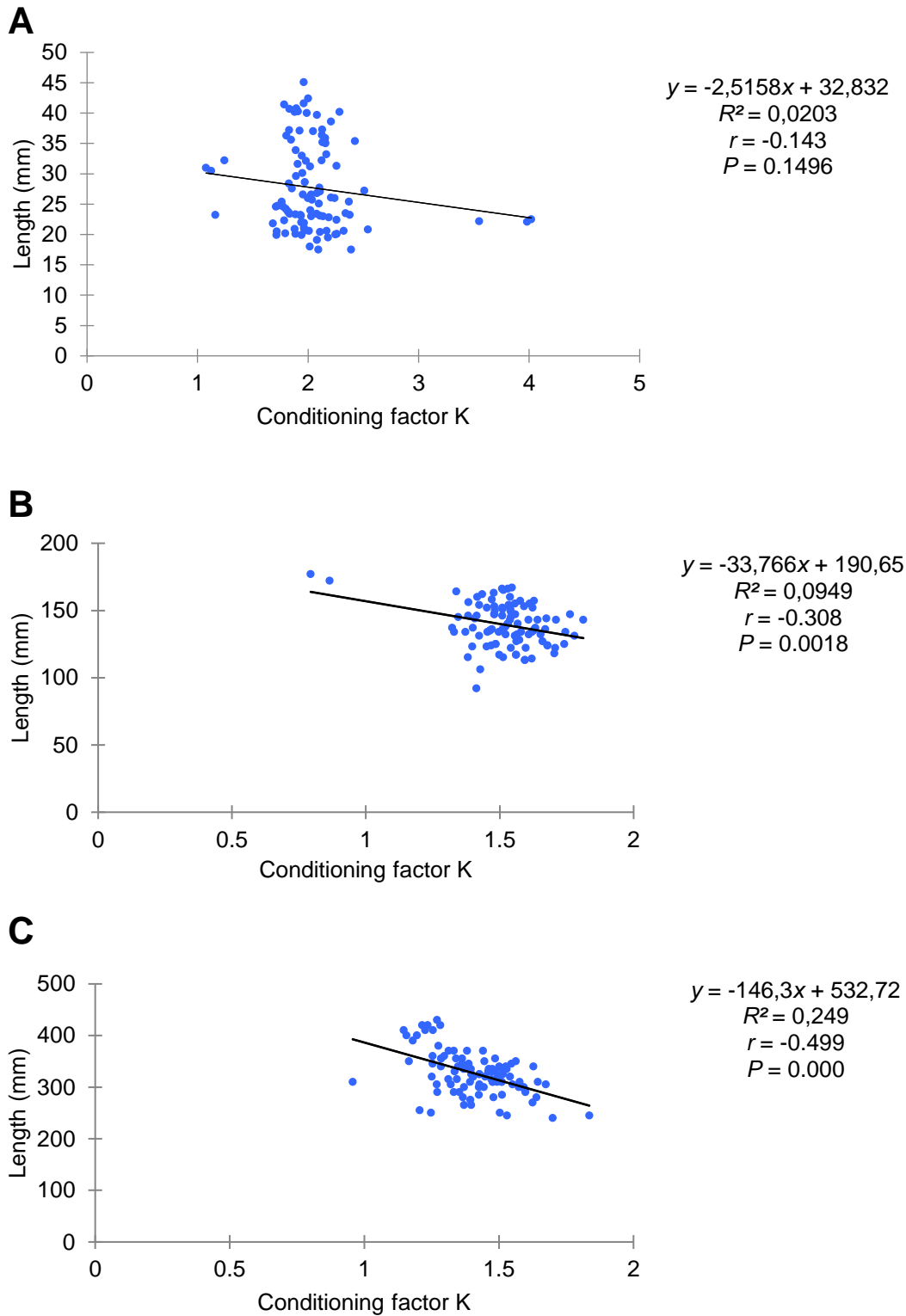


Figure S3.3: Scatterplots illustrating correlation analysis for standard length versus Fulton's conditioning factor K for group OF₁₁ (A), OF₁₂ (B), and OF₁₃ (C). Trend line equations, R^2 -values, correlation coefficients (r) and corresponding significance (P) values are also indicated.

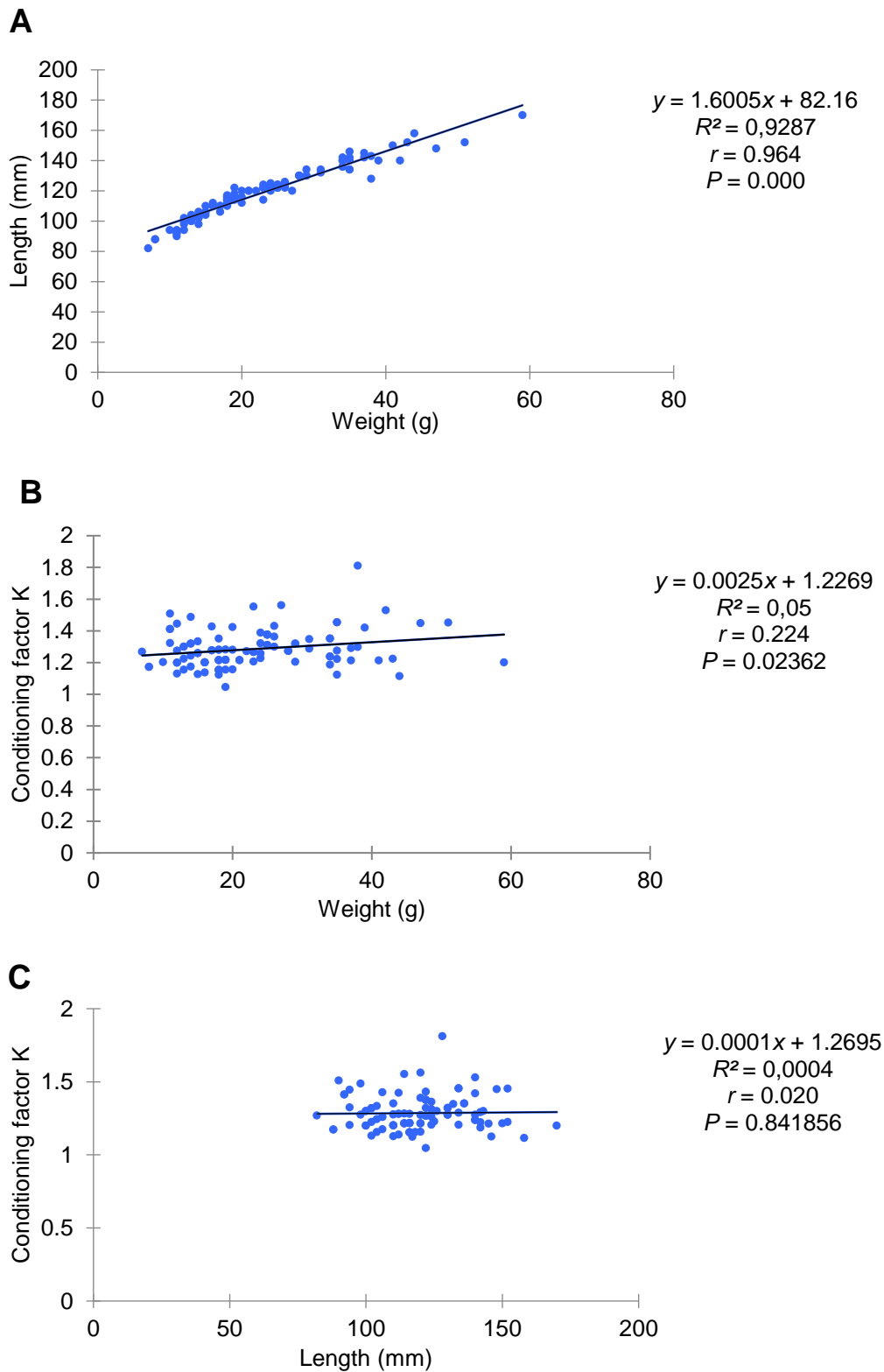


Figure S3.4: Scatterplots illustrating correlation analysis for standard length versus body weight (A), K versus weight (B) and – length (C) for group PO_2. Trend line equations, R^2 -values, correlation coefficients (r) and corresponding significance (P) values are also indicated.

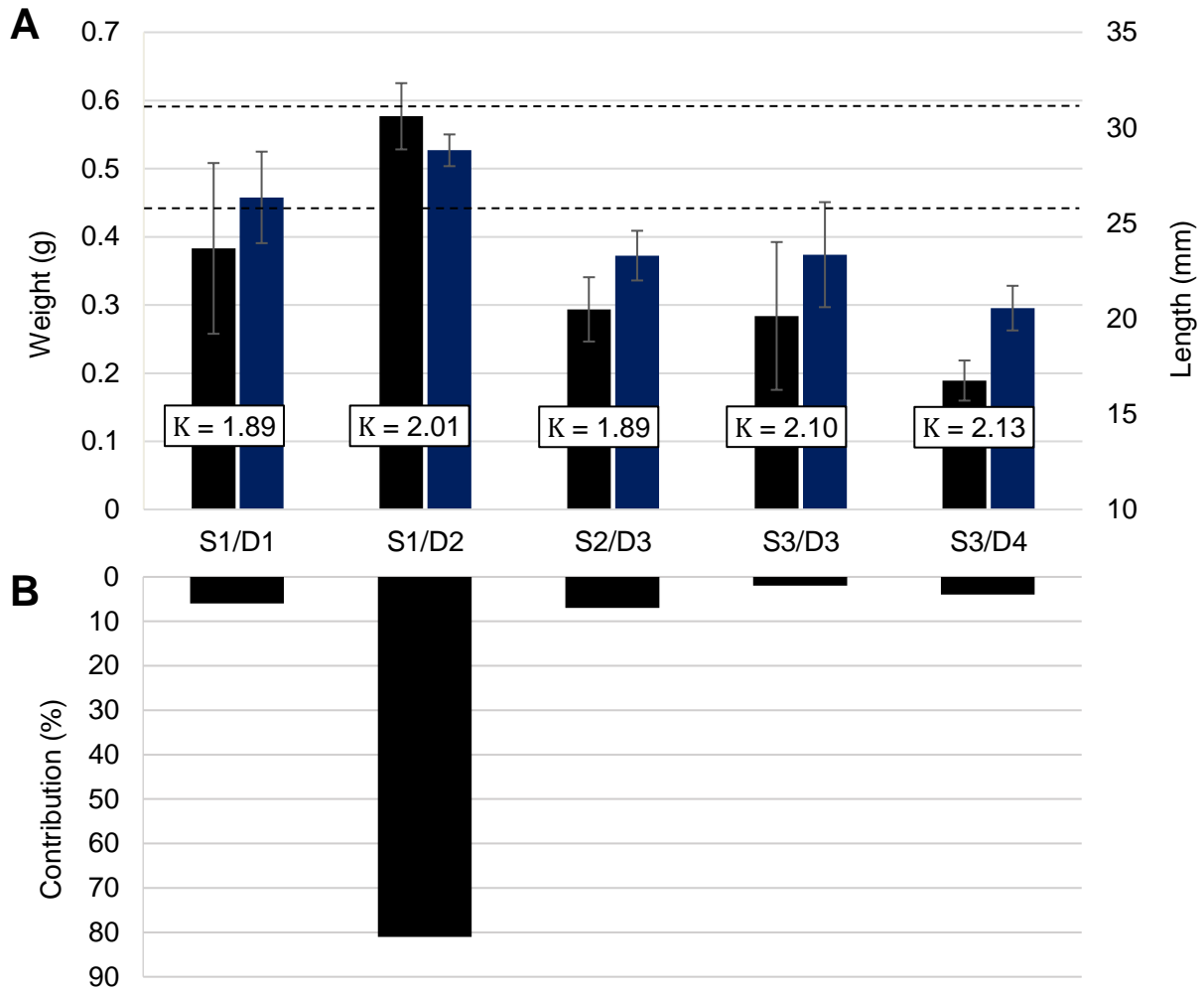


Figure S3.5: Mean (\pm s.e.) (A) weight (black), length (dark blue) and conditioning factor (K), and (B) overall contribution to progeny represented at 30 days-post-hatch (dph) of the five families in cohort OF₁₁ of spawn Oc₁. Dash lines in panel A indicate upper and lower 95% confidence intervals for weight.

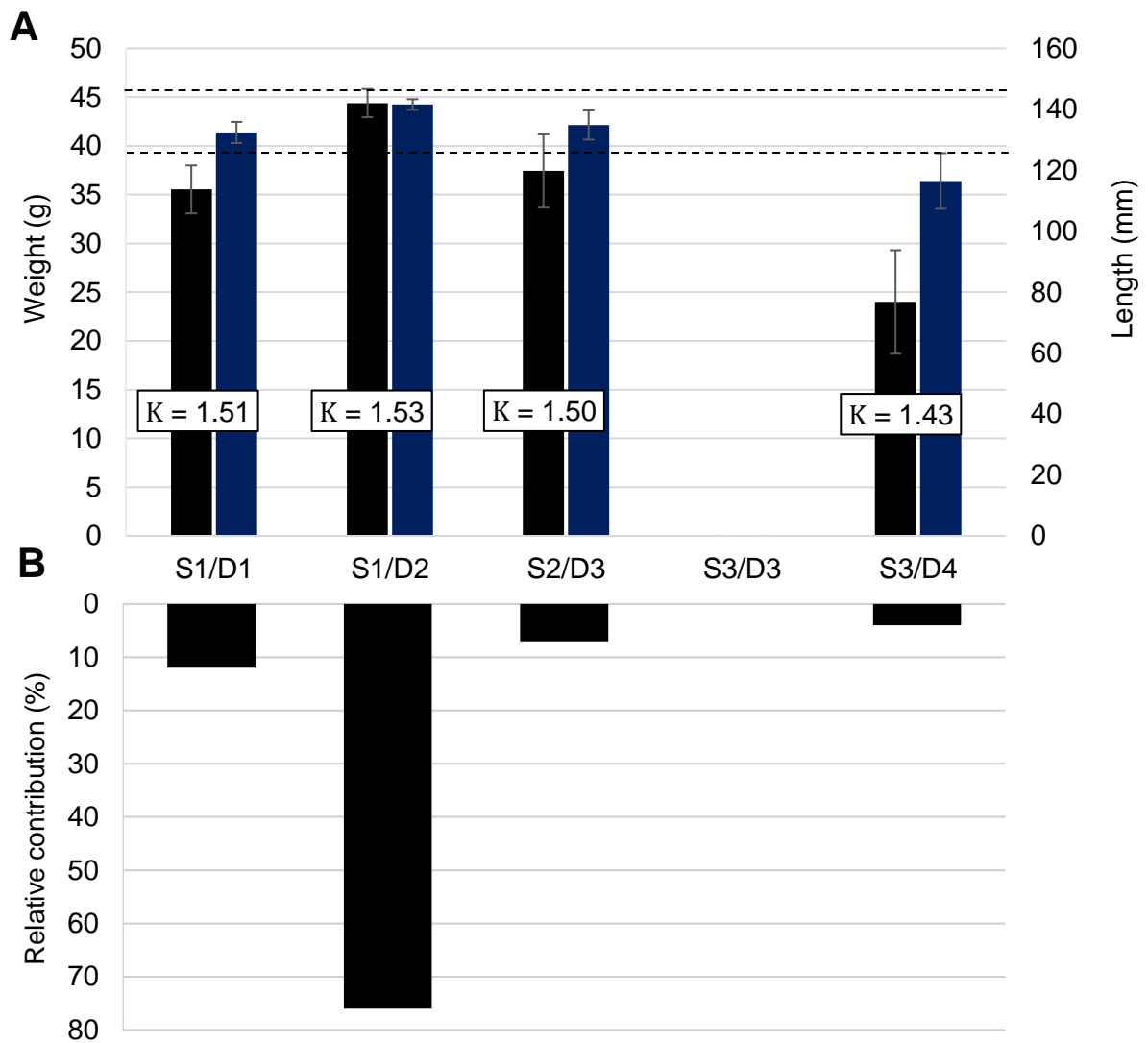


Figure S3.6: Mean (\pm s.e.) (A) weight (black), length (dark blue) and conditioning factor (K), and (B) overall contribution to progeny represented at 150 days-post-hatch (dph) of the four most representative families in group OF₁₂ of spawn Oc₁. Dash lines in panel A indicate upper and lower 95% confidence intervals for weight.