Microsatellite markers as a tool in genetic enhancement and husbandry of *Haliotis midae*: a South African case study.

by
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Abstract

The decline of *Haliotis midae* (perlemoen) populations together with the ensuing collapse of commercial abalone fisheries in South Africa have shifted the responsibility to abalone farms to meet the demand for perlemoen. Attention has recently turned to the genetic enhancement of cultured abalone in order for the farms to remain competitive in the international aquaculture market. To develop a successful breeding programme it is imperative to draw on a good foundation of high levels of genetic diversity and to successfully maintain these levels in order to create an enhanced strain of cultured abalone.

A Performance Recording Scheme (PRS) was established as the first breeding programme for *Haliotis midae* to utilise molecular tools. This programme was aimed at enhancing the growth rate of abalone in order to shorten the production times on farms. The current study made use of 12 species-specific microsatellite markers to assign parentage to a group of faster-growing PRS animals, as selected by the abalone farms, in order to select a diverse on-farm generation of broodstock. Additionally, the influence of standard selection practises on the genetic diversity of a population compared to genotypic selection was investigated. This data was also used to study the differentiation and levels of genetic diversities within and between cultured and wild populations.

Selection based on genotypic traits successfully retained genetic diversity while some diversity was lost in phenotypically selected populations. These phenotypic populations differed significantly from each other and wild populations, while the genotypic populations were similar in genetic composition to each other and wild populations of the West coast.

The broodstock populations used in the PRS spawning event were representative of the wild populations from where they were sourced, with no significant differentiation between the broodstock and West coast population. When these broodstock populations were compared to their corresponding offspring populations, only two populations displayed a significant loss in diversity; although all of the offspring populations showed significant differentiation with their corresponding broodstock populations. This was attributed to the differential contribution of broodstock and the effect of artificial selection. It was established that the cultured

populations of the participating abalone farms should be used with caution in ranching and reseeding programmes. These populations differed significantly from both the East and West coast wild populations.

This study concluded that it is possible to retain genetic diversity by selecting breeding animals based on genotypic traits. The loss of diversity in some cultured populations and significant differentiation from the wild populations indicate that animals are exposed to different selection pressures in the cultured environment. The results found in this study highlight the need for the effective management of hatchery practices and the genetic monitoring of the breeding animals.

Opsomming

Die afname in *Haliotis midae* (perlemoen) populasies en die daaropvolgende ineenstorting van die kommersiële perlemoen bedryf in Suid-Afrika het die verantwoordelikheid om in die aanvraag na perlemoen te voorsien, na perlemoen plase verskuif. Die genetiese verbetering van verboude perlemoen geniet tans aandag in ‘n poging om kompeterend te bly in die internasionale mark. Dit is noodsaaklik vir die sukses van ‘n broeiprogram om gebruik te maak van ‘n goeie genetiese basis met hoë vlakke van genetiese diversiteit en die suksesvolle behoud van die vlakke om so ‘n verbeterde lyn te skep.

‘n Groeiprestasie aanteken stelsel [Performance Recording Scheme (PRS)] is gestig as die eerste broeiprogram vir *Haliotis midae* wat gebruik maak van molekulêre tegnieke. Die doel van hierdie program was om die groeitempo van verboude perlemoen te verbeter om produksie tye te verkort. Die huidige studie het gebruik gemaak van 12 spesie-spesifieke mikrosatelliet merkers om ouerskap toe te ken aan ‘n groep vinnig-groeiende PRS-diere, soos geselekteer deur die perlemoen plase, om ‘n diverse generasie gekultiveerde diere te selekteer wat as broeidiere kan dien. Die invloed van standaard seleksie metodes op die genetiese diversitiet van ‘n populasie in vergelyking met genotipiese seleksie is ook ondersoek. Die ouerskap data is ook gebruik om differensiasie en vlakke van genetiese diversiteit tussen verboude perlemoene en wilde populasies vas te stel.

Seleksie gebasseer op genetiese eienskappe het daarin geslaag om genetiese diversiteit te behou, terwyl diversiteit verlore gegaan het in die fenotipies geselekteerde populasies. Hierdie fenotipiese populasies het ook beduidend met mekaar sowel as met die wilde populasies verskil, terwyl genotipiese populasies soortgelyk was in hul genetiese samestelling en nie van die wilde populasies van die Weskus verskil het nie.

Die broeidiere wat in die PRS broeiprogram gebruik is, was verteenwoordigend van die wilde populasies vanwaar hulle oorspronlik gekom het, met geen beduidende differensiasie tussen die broeidiere en die Wes kus populasies nie. Met die vergelyking van die broeidiere en hul ooreenstemmende nageslag, het dit geblyk dat slegs twee populasies ‘n beduidende verlies aan genetiese diversiteit getoon het, alhoewel al die nageslag beduidende populasie

differensiasie met hul ouers getoon het. Hierdie bevindinge is toegeskryf aan oneweredige bydraes van die broeidiere tydens gameetvrystelling en die invloed van kunsmatige seleksie. Hierdie studie het ook vasgestel dat die verboude perlemoen populasies met sorg gebruik moet word om wilde populasies te herstel, aangesien hierdie populasies beduidend verskil het van wilde populasies van beide die Oos en Wes-kus.

Hierdie studie het gevind dat dit moontlik is om genetiese diversiteit te behou deur diere te selekteer op grond van genotipiese eienskappe. Die verlies van diversiteit in sommige van die verboude perlemoen populasies en die beduidende verskil met die wilde populasies dui daarop dat diere in die gekultiveerde omgewing blootgestel word aan verskillende tipes seleksiedruk. Hierdie bevindinge beklemtoon die belang vir effektiewe bestuur van broeiery praktyke en genetiese monitering van broeidiere.

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List of Abbreviations

ºC	degrees Celsius
µl	microlitres
µM	micromolar
A	allelic richness
AB	Abagold
AD	Anno Domini
AFLP	amplified fragment length polymorphism
AQF	Aquafarm
bp	base pairs
CITES	Convention on International Trade in Endangered Species
CTAB	cetyltrimethylammonium bromide
ddH_2O	double distilled water
DNA	deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra-Acetate
EST	expressed sequence tag
EtBr	Ethidium bromide
f	inbreeding coefficient
FAO	Food and Agricultural Organisation
FCA	multifactorial component analyses
Fig	figure
F_{ST}	fixation index
HCL	hydrochloric acid
H_e	expected heterozygosity
HIK	HIK Abalone farm
H_o	observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
I&J	I&J Abalone
LOD	logarithm of the likelihood-odds ratio
M	molar
mg	milligram

ml	millilitre
mM	millimolar
N_a	number of alleles
NaCl	sodium chloride
ng	nanogram
PCR	polymerase chain reaction
PRS	Performance Recording Scheme
QTL	quantitative trait loci
RAPD	random amplified polymorphic DNA
RB	Roman Bay Sea farm
RFLP	restriction fragment length polymorphism
SNP	single nucleotide polymorphism
SSR	simple sequence repeat
STR	simple tandem repeat
TBE	Tris, boric acid, EDTA
Tris	2-Amino-2-hydroxymethyl-propane-1,3-diol
v/v	volume to volume
w/v	weight to volume

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Chapter 1:
Literature review

1.1 Commercial value of abalone

Abalones (family Haliotidae) are marine gastropods comprising of 56 species, of which approximately 25% are of commercial importance (Geiger, 2000). These animals are highly valued, and some of the earliest references to abalone dates back to Japan around 30 AD, as well as to early North American civilisations (Hahn, 1989). Unfortunately, abalone numbers in the wild are decreasing internationally due to over-exploitation, poaching (Hauck and Sweijd, 1999; Hilborn *et al*., 2003) and disease (Lafferty and Kuris, 1993; Altstatt *et al*., 1996; Hobday *et al*., 2001). Several international commercial fisheries closed down or even collapsed due to the resource becoming unsustainable (Karpov *et al*., 2000; Woodby *et al*., 2000; Hilborn *et al*., 2003; Worm *et al*., 2006; Tarbath *et al*., 2007; Morales-Bojórques *et al*., 2008). In South Africa, abalone (known locally as perlemoen) numbers are also affected by habitat destruction (Mayfield *et al*., 2001). This destruction is a result of an increase in rock lobster (*Jasus lalandii*) numbers in abalone breeding grounds, resulting in an increased consumption of sea urchins, which in turn decreases the amount of natural protection available to juvenile abalone (Mayfield *et al*., 2001). As a result of over-exploitation, poaching and habitat destruction, perlemoen was placed on the CITES list of endangered species in 2007. Because fisheries alone could no longer supply the market, abalone farming emerged as a positive alternative.

Internationally, the demand for abalone is one of the highest for aquaculture species, totalling an amount of approximately 40 000 metric tons in 2008. The major producers of cultured abalone are China, Taiwan and Japan. Several other countries, including South Africa, have well established abalone industries (FAO, 2009). There are currently 18 registered abalone farms in South Africa (Britz *et al*., 2009).

Haliotis midae, the only commercially exploited species along the South African coast, is a slow-growing mollusc, taking several years to reach sexual maturity. In the wild, abalone takes 7.2 years to reach sexual maturity (Tarr, 1995), but this can occur as early as 3 years in the warmer East coast waters or under cultured conditions (Wood, 1993). Despite their slow

growth rate, they are one of the largest abalone species, making them highly sought after. Because of the time it takes abalone to reach sexual maturity and their population numbers being low due to habitat destruction, abalone numbers cannot fully recover after bouts of poaching. This has lead to a collapse in commercial harvesting, which created the perfect opportunity for abalone farms to become the main supplier of perlemoen.

Since the establishment of farms in the early 1990's, the abalone industry experienced rapid growth, producing 1037 metric tons in 2008 (FAO, 2009), making this animal the most lucrative in the South African aquaculture sector. Abalone exports dominates South Africa's aquaculture sector with 24% of all exports in 2008 consisting of abalone, constituting 82% of the net value for aquaculture exports (Britz *et al*., 2009). All exports of this species consist of cultured abalone. With the demand for *H. midae* far exceeding that currently supplied by farms, it is likely that this species will continue to enjoy high priority in the global seafood market.

1.2 A selective breeding programme for *Haliotis midae*

To remain competitive in the international aquaculture market, attention has turned to genetically improve abalone, with the emphasis on increased growth which leads to shorter production times. Breeding programmes to genetically enhance strains have been established for several abalone species including *H. asinina* (Lucas *et al*., 2006), *H. rubra* (Appleyard *et al*., 2007), *H. discus hannai* (Hara and Sekino, 2007a) and *H. laevigata* (Kube *et al*., 2007) with varying degrees of success.

In 2006 a Performance Recording Scheme (PRS) was established as a joint effort between Stellenbosch University, the South African government and five commercial abalone farms, as part of a genetic improvement programme for *H. midae*. The main aim was to enhance the growth rate of the species in order to shorten the production times on farms. Five abalone farms, situated in the Western Cape province of South-Africa (Fig.1), participated by each spawning a group of broodstock animals and submitting 3000 juvenile abalone at age 7 months with each farm distributing these animals evenly between them (Fig. 2). After 43 months, hatchery managers selected the faster growing animals from the animals located on

their respective farms. These animals constitute the base population of this study from which new broodstock can be selected.

Figure 1: The location of the five abalone farms participating in the Performance Recording Scheme programme. HIK, Abagold and Aquafarm are situated in Hermanus, and Roman Bay and I&J are situated in Gansbaai.

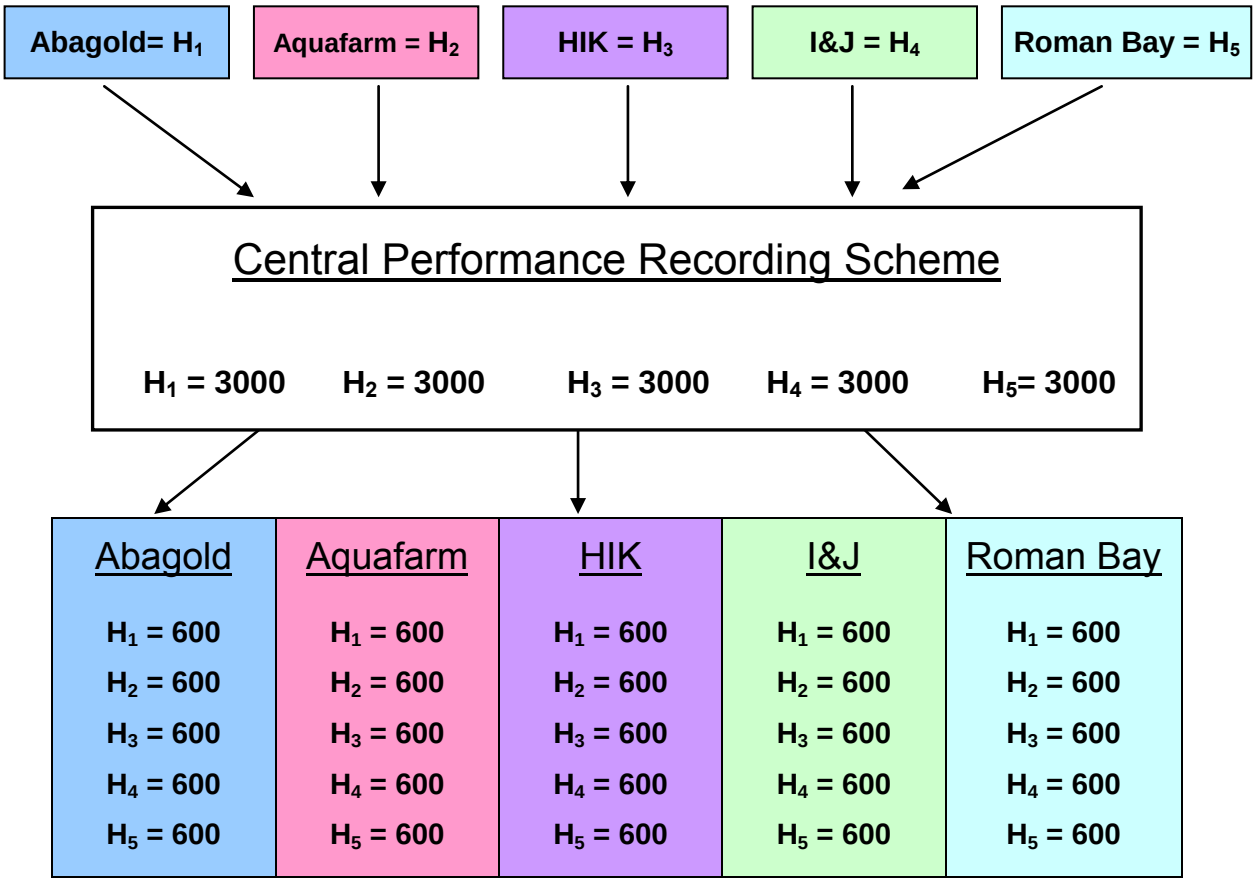


Figure 2: Schematical diagram of the setup of the Performance Recording Scheme (PRS). Each of the five participating farms submitted 3000 animals of the same age to the programme, after which these animals were evenly distributed among the different farms.

1.3 Genetic diversity

When selecting broodstock, it is important to ensure that the genetic diversity of these animals is representative of that of the wild, to ensure high levels of diversity in subsequent generations. This is necessary to form a good genetic base to create an enhanced strain of cultured abalone (Koehn *et al*., 1988; Frankham, 1995a; Hill, 2000; Launey *et al*., 2001; Slabbert *et al*., 2009). A high diversity is not only vital for the enhancement of stocks, but will also increase the ability of the population to resist diseases and allow adaptation to possible environmental changes (Gamfeldt and Kallstrom, 2007). A reduction in variability can have a negative effect on important traits such as growth rate (Koehn *et al*., 1988) and fitness

(Danzmann *et al*., 1989), because of the loss of alleles vital to mechanisms such asgrowth and disease resistance.

1.3.1 Genetic diversity in the cultured environment

Genetic diversity in natural populations is accumulated over a very long period of time, but if broodstock in a cultured environment are not managed appropriately this diversity can be lost in a single generation (Evans *et al*., 2004; Li *et al*., 2004; Frost *et al*., 2006; Lemay and Boulding, 2009; Lind *et al*., 2009). Domestication of a species (such as *H. midae*), is often associated with a loss of genetic diversity after as little as one generation (Horreo *et al*., 2008). Such a loss has been reported for several marine animals including Atlantic salmon (*Salmo salar*) (Horreo *et al*., 2008), white shrimp (*Litopenaeus vannamei*) (Freitas *et al*., 2007), black tiger shrimp (*Penaeus monodon*) (Xu *et al*., 2001), barramundi (*Lates calcarifer*) (Frost *et al*., 2006), Arctic charr (*Salvelinus alpines*) (Lundrigan *et al*., 2005), Atlantic cod (*Gadus morhua)* (Glover *et al*., 2010), Pacific oyster (*Crassostrea gigas*) (Appleyard and Ward, 2006), pearl oyster (*Pinctada fucata*) (Yu and Chu, 2006) and silver-lipped pearl oyster (*Pinctada maxima*) (Lind *et al*., 2009). Similar studies in abalone species (*H. iris*, *H. tuberculata*, *H. rubra*, *H. discus hannai*, *H. discus*, *H. kamtschatkana* and *H. asinina*), have shown a loss of genetic diversity in hatchery stocks when compared to natural populations (Smith and Conroy, 1992; Mgaya *et al*., 1995; Evans *et al*., 2004; Li *et al*., 2004; Hara and Sekino, 2007b; Lemay and Boulding, 2009; Cao and Li, 2010). For *H. midae*, contradicting results have been reported. A loss of genetic variation in hatchery stocks was reported by Evans *et al*. (2004). In 2009, Slabbert *et al*. studied different stocks and found that only one out of three cohorts suffered a significant loss of variation.

Several factors can contribute to a decrease in genetic variability:

1.3.1.1 Inbreeding

Inbreeding is the mating of relatives. According to Hartl and Clark (1989), the determining factor for the rate at which genetic diversity is lost is the rate of inbreeding. It is therefore crucial to minimise inbreeding to restrict a loss of genetic diversity. This is especially

important in commercial breeding programmes, where a high genetic variability is needed for selection.

Inbreeding can have detrimental effects on a population. It can decrease the gene pool and combine recessive, lethal alleles which can lead to a reduction in the fitness of a population, also known as inbreeding depression (Crnokrak and Roff, 1999). This is especially harmful to smaller populations, whereas it takes a longer period of time to detect a loss of heterozygosity in larger populations (Amos and Balmford, 2001). This can result in an increase in mortality rates, poor growth and impaired reproduction (Mustafa *et al*., 2000).

Inbreeding can occur as a result of natural breeding events as well as hatchery management practices. The effects are often more pronounced in cultured populations (Wang *et al*., 2002), because of artificial selection. One of the first aquacultural studies to illustrate the effect of selection on the genetic diversity of a population was that of Wada (1986), on Japanese Pearl oysters (*Pinctada fucata martensii*). The study found that selecting individuals based on commercial traits, in this instance shell width, decreased the genetic diversity of the population. When abalone farms select broodstock, animals are selected based on size, to ensure that abalone with the fastest growth rate are chosen. When only one trait is used to select broodstock, it is possible to choose related individuals that will lower the genetic diversity of the population. When unrelated individuals are used for breeding, the levels of genetic variation can be preserved in subsequent generations (Thorpe *et al*., 2000).

Apart from selection based on commercial traits, inbreeding as a result of hatchery management practices can also occur when there is an insufficient number of breeding animals used (Hansen *et al*., 2001; Wang *et al*., 2002; Evans *et al*., 2004). This is often a problem with abalone breeding, as these animals are highly fecund, and therefore not many animals are used for breeding (Evans *et al*., 2004; Li *et al*., 2004; Hara and Sekino, 2007a). Another cause of inbreeding, one often implemented on abalone farms, is the pooling of gametes after the animals have spawned (Tave, 1986; Withler and Beacham, 1994). This can possibly lead to a reduction in the number of broodstock contributing to the offspring, as competition between sperm cells to fertilise the ova will not result in equal contribution from the males.

1.3.1.2 Adaptation to the environment

Artificial selection focuses on specific traits, depending on the species in question. These traits are mostly those with an economic advantage, for example disease resistance, faster growth rates or improved meat quality (Gjøen and Bentsen, 1997). According to a recent survey done by Slabbert (2010), the traits most favoured by the five participating abalone farms were size and growth related traits.

By exposing animals to the artificial selection methods practiced on farms, the genetic composition of a population can be altered within a few generations. This can happen due to shifts in allele frequencies which can ultimately lead to the reduced fitness of the population (Frankham, 2008). Such genetic adaptation to captive environments has been documented in several species including insects (Zouros *et al*., 1982; Frankham and Loebel, 1992), plants (Allard, 1988; Izawa, 2007; Ross-Ibara *et al*., 2007) and fish (Levin *et al*., 2001; Heath *et al*., 2003; Allendorf and Luikhart, 2006; Araki *et al*., 2007).

Natural selection is not restricted to the wild and can take place in a cultured environment where different traits are favoured from that in the wild (Frankham, 2008). In the case of abalone, there are a number of differences between the two environments. Instead of the vast ocean with plenty of rock formations and kelp as shelter, animals are confined to baskets, restricting their domain and movement. Artificial feed replaces natural food sources such as kelp, and spawning of individuals is induced artificially (Pers. Obs.).

Adaptation can result in a decrease in reproductive success and survival rates. This is evident from several studies concerning adaptation in aquaculture species. In a study done by Heath *et al.* (2003), cultured Chinook salmon (*Oncorhynchus tshawytscha*) produced smaller eggs. As these fish were used for restocking, the egg size of the supplemented wild population decreased, resulting in a reduced fitness. Leider *et al*. (1990) found that the reproductive success of steelhead trout (*Oncorhyncus mykiss*) decreased significantly when returned to the wild. With genetic adaptation affecting the survival of animals that are returned to the wild negatively, it is possible that rare alleles that were harmful in the wild are favoured in captivity

and is responsible for most of the adaptation that occurs in a cultured environment (Frankham, 2008).

Adaptation to the cultured environment is essential in the domestication of a species. Although a loss in genetic diversity is expected, it should stillbe limited as far as possible. Failure to do this can reduce the capacity for future selection programmes. Several factors determine the rate at which a population will adapt to its environment and steps can thus be taken to limit this (Frankham, 2008). These factors include the number of generations in captivity (Allard, 1988; Gilligan *et al*., 2003; Allendorf and Luikart, 2006), initial levels of genetic diversity (Ayala, 1965a, b; Reed *et al*., 2003), effective population sizes (Weber and Diggins, 1990) and the intensity of artificial selection (Falconer and Mackay, 1996). Since the current broodstock used on perlemoen farms are wild animals, all subsequent progeny will be first generation offspring. The number of generations in captivity will therefore not affect the rate at which the offspring adapt. The initial levels of genetic diversity is a very important factor and it is one of the aspects taken into consideration in the abalone breeding programme. There are however factors that aren't always feasible in the aquaculture sector. Because commercially important traits are favoured, artificial selection will increase, which will in turn increase the rate of adaptation. Effective population size as a result of differential contribution of breeding animals is also difficult to monitor when molecular techniques such as parentage assignment is not used. Other means to reduce the adaptation rate should therefore be investigated.

1.3.1.3 Differential contribution of broodstock

Abalone are broadcast spawners, releasing numerous gametes directly into the water. As such, it is difficult to manage the contribution of individual broodstock during spawning events. They are also highly fecund, making it common practice on farms to use only a small number of animals as broodstock (Smith and Conroy, 1992; Boudry *et al*., 2002). This results in a population consisting of different families of variable size. Not only is there variation in the number of offspring produced by each individual, but often some animals will not contribute at all, reducing the effective population size. Such variation in broodstock contribution has been studied in several aquaculture species including the Nile tilapia (*Oreochromis niloticus*)

(Fessehay *et al*., 2006), Atlantic cod (*Gadus morhua*) (Bekkevold, 2006; Rowe, 2007) and Gilthead seabream (*Sparus aurata*) (Brown *et al*., 2005). Blonk *et al.* (2009) found a skewed contribution in a spawning event of two broodstock cohorts of Common sole (*Solea solea*). Very few broodstock contributed, with one parent pair being responsible for almost 40% of the offspring. Such differential contribution has also been seen in *Haliotis asinina* (Selvamani *et al*., 2001) and *H. discus hannai* (Hara and Sekino, 2007b). In *H. midae* differential contribution of broodstock was also observed (Van den Berg *et al*., 2010), where the majority of offspring were assigned to a single parent pair. This was also reflected in a study done by Slabbert *et al*. (2009) where several males and females failed to contribute to the offspring. In one of the three cohorts, a mere 24% of the broodstock contributed to the offspring.

Reasons for differential contributions can be physical, with some of the individuals being too old to produce gametes or not producing gametes of a high quality (Slabbert *et al*., 2009). Several genetic factors can also play a role, for instance gamete competition and interaction (Launey and Hedgecock, 2001; Boudry *et al*., 2002). Other reasons include differential larval survival (Lind *et al*., 2010) and hatchery practices (Frost *et al*., 2006; Lind *et al*., 2009).

One way to prevent differential contribution is to make use of a factorial mating design, where the sperm of each male is used to fertilise an equal amount of eggs from different females (Withler and Beacham, 1994; Waples and Do, 1994). In an aquacultural setup however, this is not feasible due to time, space and financial constraints, labour intensity and the practicality thereof.

1.3.2 Importance of genetic diversity in conservation

Apart from playing a pivotal part in the enhancement of an animal for commercial reasons, genetic diversity it is also essential for conservation purposes. The establishment of high levels of initial genetic diversity and the maintenance thereof could allow for future ranching and reseeding opportunities.

As is the case with many endangered species, one of the goals of breeding in captivity is to reintroduce animals into the wild in order to increase natural population numbers. With

abalone population numbers decreasing rapidly, reseeding could be used for this purpose in the future. Ranching can also be used to sustain coastal fisheries. This entails the release of juvenile cultured animals into the sea with the aim of harvesting once they reach market size (Saito, 1984; Mustafa, 2003). This is usually done in a location where there is no wild populations in order to limit possible interbreeding. This is opposed to reseeding (also known as stock enhancement) where the purpose is for cultured animals to interbreed with the wild abalone in order to recover wild populations. Adaptation to captive conditions can hinder this process, since traits selected for in captive conditions (both intentionally and unintentionally) can be unfavourable in the wild (Fleming *et al*., 2000; Chilcote, 2003; McGinnity *et al*., 2003; Araki *et al*., 2007). This can result in a decrease in reproductive success and survival rates. Care should thus be taken to ensure a high diversity is maintained in the cultured environment.

When captive populations are released, they will interbreed with wild populations, creating progeny of mixed origin. These offspring might not be fit for the environment of either parent (Allendorf and Waples, 1996), as an introgression of alleles will result in a different genetic composition. This can either alter the local adaptation of the population, since alleles that might be advantageous in one habitat could be less so in another (Tymchuk *et al*., 2007), or disrupt co-adapted gene complexes (Wallace, 1968). Such gene complexes occur when the interactions between certain loci result in an increased fitness. This disruption of gene complexes and loss of adaptation to the environment could result in outbreeding depression, which is a large concern for conservation biologists (Loeschcke *et al*., 1994), resulting in a reduction in the fitness of a population.

Outbreeding can, however, be positive when it is used to increase the heterozygosity of a population to recover lost alleles for example. This is known as outbreeding enhancement and has shown success in breeding programmes of various animal (Sheridan, 1981), plant (Levin, 1984; Waser and Price, 1989) and fish (Rahman *et al*., 1995; Monson and Sadler, 2010) species. This can facilitate the process of reintroducing captive animals into the wild, by combating processes such as genetic drift. Introgression of cultured animals into a wild population will probably be more successful by crossing the two populations, creating outbred

offspring which can then be introduced to the wild, instead of introducing farm animals directly to the wild (Clifford *et al*., 1998; Fleming *et al*., 2000).

In endangered species, another reason for concern is inbreeding. As confirmed by Frankham (1995b), inbreeding can increase the risk of extinction for endangered species, especially in declining populations. Both past and current inbreeding can accumulate over generations until a threshold is reached for extinction.

If an extinction threshold for wild abalone is reached or population sizes are too small to maintain sufficient genetic diversity, captive animals can be used to aid wild sources. This can be done either by supplementing population numbers, or if there is a large difference in the genetic composition of the wild and captive animals, outbreeding enhancement can be implemented as an alternative.

1.4 The importance of molecular markers in a selective breeding programme

One of the elements of a breeding programme is to select a foundation with high initial genetic diversity in order to ensure that a variety of traits are available for potential selection, both those that are currently valuable as well as those that could be of importance in the future. Failure to do so could result in unsuccessful breeding programmes, as seen in previous fish programmes (Teichert-Coddington and Smitherman, 1988; Huang and Liao, 1990). It is also important that the selected broodstock reflect the genetic composition of that of wild populations to minimise the impact of escaped farm animals on the gene pool of wild abalone as well as for potential reseeding purposes. A popular way to assess and compare genetic diversity is with the use of molecular markers.

Several types of molecular markers have already been used successfully in aquaculture studies (Table 1). One of the earlier studies involving molecular markers as a tool in an aquaculture breeding programme was that of May *et al*. (1980). This study involved the use of allozymes to study linkage associations to determine segregation of biochemical loci in various trout species. More recently microsatellite markers have become a popular marker to

use for population studies since they are very informative due to their high level of polymorphism and the ease of automation of analyses steps. Microsatellite loci consist of tandem repeats of 1 to 6 base pairs (Litt and Luty, 1989). These markers occur in coding and non-coding regions (Liu *et al*., 2001). They are co-dominant, evenly distributed and abundant in most eukaryotic genomes (Liu and Cordes, 2004).

Table 1: Different molecular markers used in aquaculture (Liu and Cordes, 2004).

Marker	Description	Examples of Application
Allozymes	Allelic variations of proteins. Co-dominant, type 1 markers.	Linkage mapping, population studies.
Amplified Fragment Length Polymorphism (AFLP)	PCR-based, multi-locus, dominant markers generated by digestion with restriction enzymes.	Linkage mapping, population studies.
Expressed Sequence Tags (SNP-ESTs, STR-ESTs)	Markers developed from coding DNA. Mostly generates type 1 markers.	Linkage mapping, physical mapping, comparative mapping.
Microsatellites (STRs/SSRs)	Tandemly arranged sequence repeats of 1 to 6 base pairs. High level of polymorphism. Co-dominant markers.	Linkage mapping, parentage assignment, population studies.
Mitochondrial markers	Present on the mitochondria. Maternally inherited instead of Mendelian.	Maternal lineage.
Random Amplified Polymorphic DNA (RAPD)	Bi-allelic locus, dominant marker, generated by PCR.	Fingerprinting for population studies, hybrid identification.
Restriction Fragment Length Polymorphism (RFLP)	Co-dominant markers. Relatively easy to score but low polymorphic levels.	Linkage mapping.
Single Nucleotide Polymorphism (SNP)	Caused by point mutations. Co-dominant, bi-allelic markers.	Linkage mapping, population studies.

Microsatellite markers have been isolated in several species of abalone, including *H. rubra* (Huang and Hanna, 1998; Evans *et al*., 2000), *H. rufescens* (Kirby *et al*., 1998), *H. asinina* (Selvamani *et al*., 2000), *H. discus hannai* (Li *et al*., 2002), *H. discus discus* (Sekino and Hara, 2001) and *H. kamtschatkana* (Miller *et al*., 2001). To date, 264 microsatellites have been isolated in *H. midae* (Bester *et. al.*, 2004; Slabbert *et al*., 2008; Hepple, 2010; Rhode, 2010; Slabbert *et al*. 2010; Jansen, 2011; Slabbert *et al*. 2011). Microsatellite markers have a wide range of applications and have been used successfully in several fields in various species, including population genetics (Nielsen *et al*., 1994; McConnel *et al.,* 1995), linkage mapping (Baranski *et al*., 2006), pedigree analyses (Harris *et al*., 1991; Herbinger *et al*., 1997; Hara and Sekino, 2007a; Lemay and Boulding, 2009), QTL mapping (Guo *et al*., 2011), and strain identification (Glover *et al*., 2010).

Microsatellite loci are excellent for parentage assignment and are often used in aquaculture (Selvamani *et al*., 2001; Li *et al*., 2003; Herlin *et al*., 2008; Slabbert *et al*., 2009). The reason for this is their random and independent Mendelian segregation pattern (Queller *et al*., 1993). Parentage assignment has, amongst others, been successfully used to monitor the contribution of broodstock (Hara and Sekino, 2007a; Horreo *et al*., 2008; Herlin *et al*., 2008) as well as to construct pedigrees to determine relatedness and inbreeding (Bierne *et al*., 1998; Norris *et al*., 1999; Sekino *et al*., 2004).

When using molecular markers as a means to monitor genetic diversity, breeding programmes can be implemented successfully to create an enhanced strain of species for both commercial and conservational use.

1.5 Layout/Aims

This study focuses on the use of microsatellite markers as a tool in broodstock management in an abalone aquaculture setup, with the specific goal of maintaining genetic diversity in subsequent generations. This entails the use of parentage assignment to select unrelated offspring for use as potential broodstock.

Chapter 2:

This chapter will discuss parentage assignment of the faster-growing PRS animals using microsatellite markers to construct pedigrees for use in the selection of first generation broodstock. Recommendations will be made to the farms as to which individuals to subscribe to the breeding programme, to ensure that non-related broodstock are selected to prevent inbreeding. These broodstock, chosen based on their genotype, will also be compared to animals chosen purely on phenotypic traits such as shell-size, to determine the potential effect of conventional selection methods on the diversity and inbreeding of a population.

Chapter 3:

Genetic diversity and differentiation between cultured and wild populations, as well as within and between farms will be determined and discussed to assess whether or not adequate levels of variability are present on the farms. This data will also be used to do an impact assessment to determine the potential of cultured animals currently on the farms to be used for abalone ranching on the West coast of South Africa. Diversity between broodstock and offspring will be compared to study any loss of alleles.

A note on the structure of populations used in this study: Different offspring populations were used for data analyses in chapters 2 and 3. In chapter 2 and section 3.1.4.3 the offspring populations consisted of animals that are currently on the farms, and are therefore of mixed origin (henceforth referred to as mixed offspring) (Fig. 3). Because the PRS offspring were distributed between farms after spawning, parentage data was used to assemble offspring groups from the different farms. For the data analysis of sections 3.1.4.1 and 3.1.4.2, the offspring populations consist of animals corresponding to their original farm location (henceforth referred to as pooled offspring) (Fig. 3).

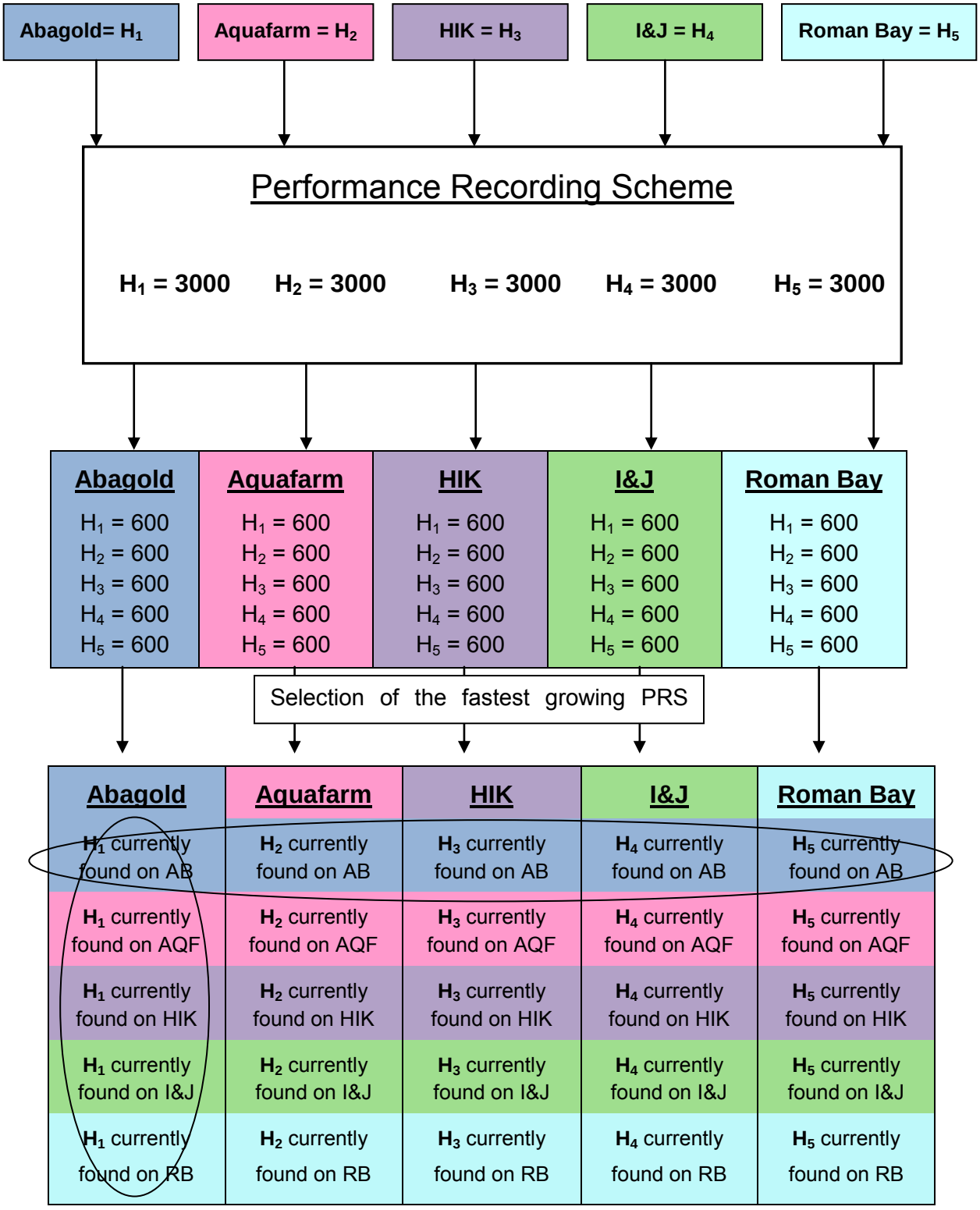


Figure 3: The composition of the offspring populations used in chapters 2 and 3. The mixed offspring populations (horizontal column) consist of animals that are currently on the farms. The pooled offspring populations (vertical column) consist of animals corresponding to their original farm location.

Chapter 2:

Parentage assignment and broodstock selection

In 2006, a Performance Recording Scheme (PRS) was established as the first breeding programme for *Haliotis midae* to make use of molecular tools. This was a joint effort between Stellenbosch University, the South African government and five commercial abalone farms. This programme was aimed at enhancing the growth rate of abalone. Seven months into the programme each farm submitted 3000 juvenile abalones that were evenly distributed between the farms. After 43 months, the hatchery managers selected the faster-growing animals from the animals located on their respective farms. These animals were then subjected to molecular analysis to identify individuals that could be used for breeding purposes to ensure high levels of genetic diversity in subsequent generations.

In order to develop a successful breeding programme, it is recommended that a strong foundation with high levels of initial genetic diversity is used to ensure representation of a variety of traits that could be used for selection. This is necessary not only for traits that are currently valuable, but also for traits that could be of importance in the future (Koehn *et al*., 1988; Frankham, 1995a; Hill, 2000; Launey *et al*., 2001; Slabbert *et al*., 2009). Genetic diversity in natural populations is accumulated over a very long period of time, but if broodstock in a cultured environment are not managed appropriately, this diversity could be lost in a single generation (Evans *et al*., 2004; Li *et al*., 2004; Frost *et al*., 2006; Lemay and Boulding, 2009; Lind *et al.,* 2009).

A limiting factor in establishing a successful breeding programme is the lack of expertise in the field of molecular biology. A significant problem is that conventional selection methods practiced by farms only focus on economically beneficial traits. Because animals are selected based on phenotypic traits, genetic information is not taken into consideration. As such, it is possible to select related individuals, or animals which could lower the genetic diversity of the population. Failure to maintain adequate levels of genetic diversity could result in failure of a breeding programme, as seen in previous fish breeding programmes (Teichert-Coddington and Smitherman, 1988; Huang and Liao, 1990). To date, no information exists to assess

whether or not current selection methods have an effect on inbreeding and genetic diversity of cultured *Haliotis midae* populations.

Several studies have found the minimal kinship selective crossbreeding approach to be the most successful way to limit a loss in the genetic diversity of captive populations (Doyle *et al*., 2001; Sekino *et al*., 2004; Ortego-Villaizan *et al*., 2011). This method entails the selection of individuals showing a lower level of kinship, as determined by the kinship coefficient. This coefficient calculates the probability that alleles of different animals are identical by descent (Falconer and MacKay, 1996), and will therefore give priority to animals with rare genotypes. Ortego-Villaizan *et al*. (2011) found an increase in the number of alleles and expected heterozygosity if a sufficient number of breeding animals were used. These studies also found a decrease in diversity in population groups that were randomly selected. An important drawback of this method however, is that it does not reduce the level of inbreeding (Caballero and Toro, 2000), as selected animals are not necessarily unrelated. To avoid this, parentage assignment was performed in the current study in order to identify unrelated animals. When unrelated individuals are used for breeding, the levels of genetic diversity can be preserved in subsequent generations (Thorpe *et al*., 2000).

Microsatellite markers are often used with great success for parentage assignment in aquaculture (Norris *et al*., 1999; Selvamani *et al*., 2001; Boudry *et al*., 2002; Li *et al*., 2003; Dong *et al*., 2006; Herlin *et al*., 2008; Slabbert *et al*., 2009) and has, amongst others, been successfully used to monitor the contribution of broodstock (Hara and Sekino, 2007a; Herlin *et al*., 2008; Horreo *et al*., 2008), as well as to construct pedigrees to determine relatedness and inbreeding (Bierne *et al*., 1998; Norris *et al*., 1999; Sekino *et al*., 2004).

Microsatellite markers have been isolated in several species of abalone, including *H. rubra* (Huang and Hanna, 1998; Evans *et al*., 2000), *H. rufescens* (Kirby *et al*., 1998), *H. asinina* (Selvamani *et al*., 2000), *H. discus hannai* (Li *et al*., 2002), *H. discus discus* (Sekino and Hara, 2001) and *H. kamtschatkana* (Miller *et al*., 2001). To date, 264 microsatellites have been isolated in *H. midae* (Bester *et al*., 2004; Slabbert *et al*., 2008; Hepple, 2010; Rhode, 2010; Slabbert *et al*., 2010; Jansen, 2011; Slabbert *et al*., 2011).

In this study, microsatellites will be used to assign parents to all the selected PRS offspring. Unrelated animals will be identified and recommendations will be made to the farms of the broodstock animals to be used. These selected broodstock animals, chosen based on their genotype, will also be compared to animals selected by the farms chosen purely on phenotypic traits to determine the potential effect of conventional selection methods on the diversity and inbreeding of a population. The contribution of the current broodstock during spawning events will also be determined to assess whether they are suitable for breeding. Those broodstock animals that are not contributing may be replaced by the newly selected broodstock.

2.1 Materials and Methods

2.1.1 Sample collection and DNA extraction

Forty-three months after the onset of the PRS programme, hatchery managers of five abalone farms selected the fastest-growing abalone on their farm based on shell-size and wet weight. The number of animals selected differed between farms; depending on the number of broodstock individuals they required (Table 1). The participating farms, situated on the West coast of South Africa, are Abagold (Hermanus), Aquafarm (Hermanus), HIK Abalone Farm (Hermanus), I&J Abalone (Gansbaai) and Roman Bay Sea Farm (Gansbaai). Tissue samples of these animals were taken by means of a non-destructive sampling method (Slabbert and Roodt-Wilding, 2006). Three epipodia were clipped from each animal and stored in 99.9% (v/v) ethanol at room temperature until extraction. The wet weight as well as the length and width of the shell were recorded for each animal using a scale and calliper (Fig. 1) (See Appendix A for measurement data).

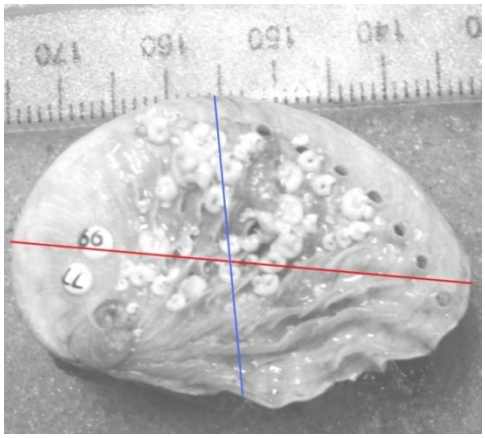


Figure 1: The length (red) and width (blue) of shell sizes were measured for all the sampled animals. (Photo: Slabbert, 2010)

Samples of the broodstock animals were collected and extracted prior to the onset of this study by students of the Molecular Aquatic Research Group (Stellenbosch University). Samples of some of the broodstock animals could not be obtained as these animals were already deceased when this study commenced. Samples of two wild populations, one from the West coast (Saldanha) and one from the East coast (Rietpoint), were also available for comparison with farm populations to assess levels of diversity (Table 1). Wild samples were collected by commercial and government scientific divers.

Table 1: The number of animals selected and sampled from each farm and wild population. A total of 1013 cultured animals and 58 wild animals were sampled.

Cultured populations					**Wild populations**	
Abagold	Aquafarm	HIK	I&J	Roman Bay	Rietpoint	Saldanha
96	199	296	129	293	31	27

DNA extractions were carried out using a cetyltrimethylammonium bromide (CTAB) extraction method as described by Saghai Maroof *et al*. (1984). The three tentacles taken from each animal were placed in 500µl extraction buffer [2% (v/v) CTAB-solution, 1.4M NaCl, 0.2% (v/v) β-mercapto-ethanol, 20mM EDTA, 100mM Tris-HCl; pH8], containing 2µl of a 10mg/ml proteinase K solution (*Sigma Aldrich*) and incubated in a waterbath overnight at 60ºC. DNA was extracted using a 24:1 chloroform:isoamylalcohol mixture and washed with 70% (v/v) ethanol to remove excess salts. After these washing steps, the DNA was precipitated using ice-cold 100% (v/v) isopropanol. Following overnight incubation at -20ºC, pellets were dried at

55ºC in an oven and resuspended in 100μl ddH_2O. Resuspended DNA was stored at -20ºC until further use. Unless otherwise stated, all chemicals used were obtained from Merck (Darmstadt, Germany).

2.1.2 Microsatellite analyses

Twelve microsatellite loci that displayed a high level of polymorphism, had no difficulty in amplifying and had perfect repeats were chosen for genotyping. These loci, divided into two panels, were optimised by the Molecular Aquatic Research Group of the Department of Genetics at Stellenbosch University and will henceforth be referred to as Parent Panel 1 and 2 (Table 2).

Table 2: Details of the parent panels used for genotyping. The size ranges and fluorescent labels of the markers were used as criteria to construct the multiplexes.

Panel	Microsatellite	Fluorescent label	Primer sequence (5'-3')	Size range (bp)	Genbank Accession number	Repeat tract
Parent panel 1	*HmD55*[a]	VIC	F: ATCAAGATAAAACGAGGCG R: ACCACTGTGAAAACGTCCA	183-211	AY303337	$(GTGA)_n$
	HmD59[a]	FAM	F: TATACTGCCATTTCCGTCTG R: TCTGTATTCTGGTCCTGTCG	106-150	AY303338	$(CA)_n$
	HmidPS1.870[b]	NED	F: ACAACAACACACAGCACA R: GTGCCAAAACATATTTCAAAAC	90-120	GU256718	$(CACACG)_n$... $(AC)_n$
	HmidPS1.967[b]	PET	F: ATATGCACACCGAGTGAAATC R: CTAACATGACCAGCGATTGTT	115-150	GU256725	$(TGTC)_n(TG)_n$
	HmRS129[c]	VIC	F: TTGAATCTGACTGAACTGGG R: TATAAGCCACATTCTGAGGAA	251-295	DQ785766	$(GT)_n$
	HmRS27[c]	NED	F: TACCGGTATAAACCGAACAC R: GTTCAGCAAGAAATCAGTCG	224-428	DQ785751	$(TCAC)_n$
	HmRS80[c]	PET	F: AATGGTTCTTTTGATCCCTT R: TCATTATAACATCTGGCCTTG	178-240	DQ785756	$(GAGT)_n(GA)_n$ $(GAGT)_n$
Parent panel 2	*HmNR106*[c]	FAM	F: TCCTTGGCCAGAATAACC R: TATATGGTCTGCATCGCTG	329-389	DQ825709	$(TG)_n$
	HmNR120[c]	PET	F: TTGAGCATGAGTCGTTGAGC R: ACCTGCTCTTTAGCTCAGATGG	235-347	EF121745	$(TGAG)_n$
	HmNR20[c]	FAM	F: CTACAACAAACGCCGATG R: TGCAGTAATAGGGGTACCAG	187-289	EF063097	$(TCC)_n(TAC)_n$
	HmNS19[c]	NED	F: ACAACAACAAAGGTGGTCAA R: CAATGAATAGCTATGGGTCG	178-252	EF033330	$(AAGACCC)_n$
	HmidPS1.818[b]	VIC	F: AATGTAGGGTTGCTTCAAATG R: GAGTGTGTGGGTGTCTCTTTC	85-150	GU256711	$(ATGG)_n$... $(TGGA)_n$...$(AC)_n$

([a]): Bester *et al*. (2004) ([b]): Slabbert *et al*. (in press) ([c]): Slabbert *et al*. (2008)

Polymerase chain reactions (PCR) were performed for all individuals in a GeneAmp® PCR System 2700 (*Applied Biosystems*), using the QIAGEN® Multiplex PCR kit (*Qiagen*). PCR reactions were setup in a final volume of 10μl and contained 2X QIAGEN® Multiplex PCR Master Mix, 40ng DNA and a primer mix containing 0.2μM of each primer. Primer mixes were prepared containing a final concentration of 2μM for all the primers in Parent Panel 1. For Parent Panel 2, 2μM of each primer was added, except for primers of marker *Hm*NR106, of which the final concentration was 4μM. The cycling conditions were as follows: an initial denaturing and activation step of 15 minutes at 95ºC followed by 35 cycles of a denaturing

step at 94ºC for 30 seconds, a 90 second annealing step at 57ºC and a 60 second elongation step at 72ºC, with a final extension step of 30 minutes at 60ºC. After completion of the PCR, amplicons were visualised on an agarose gel [2% (w/v), 1X TBE, EtBr], to assess whether the amplification step was successful. These products were then analysed on an ABI 3730XL DNA Analyzer (*Applied Biosystems*) with the LIZZ600 size standard (*Applied Biosystems*) and scored based on fragment size using GeneMapper® version 4.0 (*Applied Biosystems*). To minimise genotyping errors, the data was verified independently by another member of the Molecular Aquatic Research Group.

The number of alleles, observed and expected heterozygosities and the presence of null alleles were determined for each marker using the software CERVUS version 3.0.3 (Kalinowski *et al*., 2007). Deviation from Hardy-Weinberg equilibrium was calculated with GenePop version 4.0.10 (Raymond and Rousset, 1995).

2.1.3 Parentage assignment

All GeneMapper export files were converted to a GenePop format using Geneticx version 1 [DataMetricx (Pty) Ltd., 2010]. Parents were assigned to offspring using the software Cervus version 3.0.3 (Kalinowski *et al*., 2007). This software is based on likelihood ratios, which are determined by means of allele frequencies for each marker. The software uses simulation to determine the critical values of likelihood ratios used during the assignments (Kalinowski *et al*., 2007). The overall likelihood ratio is expressed as the LOD score. Only individuals typed for at least seven microsatellite markers were included in the assignment. Confidence levels were calculated using the joint LOD scores of both parents. A relaxed confidence level of 80% was used and a strict confidence level of 95%. A proportion of 1% of mistyped loci was allowed. Parent pairs with a LOD score higher than 3 were considered as potential parent pairs, indicating that these pairs are more likely to be the true parents than a pair chosen by random (Cervus help manual).

Parents were assigned to offspring by evaluating the combined LOD score of parent pairs. The parent pair with the highest LOD score was assigned as the parents. In the event where more than one parent pair had the same score and parents could not be assigned by

inspecting the genotypes of the offspring, only one parent was assigned. If this could not be done, animals were left unassigned. Using this data, family trees were visualised with Pedigraph^{TM} version 2.4 (Garbe and Da, 2008). Any unassigned animals were grouped as a population and subjected to multifactorial component analyses (FCA), using the software GENETIX version 4.05.2 (Belkhir *et al*., 2000). Histograms depicting the contribution of the broodstock during the PRS spawning event were constructed using Microsoft® Excel 2007.

2.1.4 Broodstock selection - Phenotypic vs Genotypic

The impact of selection strategies on the genetic diversity and relatedness of breeding animals was determined by comparison of two sets of broodstock. The first selection strategy relied upon favourable phenotypic traits, specifically the size of the animal, colour of the shell and the depth of growth ridges. These phenotypic broodstock were chosen by hatchery managers of each farm from the faster-growing PRS animals. The second selection strategy relied upon the genetic composition of the animals based on genetic diversity and relatedness.

To select genotypic broodstock, the faster-growing PRS offspring were ranked from largest to smallest according to size (based on shell length as determined on the day of sampling). Non-related animals, as determined by parentage assignment, which were the largest, were selected using walk-back selection as described by Doyle and Herbinger (1994). This method entails the selection of the animal with the largest shell-size, and subsequently, selecting the second largest animal that is not related to the animal already selected. This method was repeated until all animals that were not full-sib were included in the broodstock. These animals were recommended to the respective farms to be used for breeding purposes.

Population differentiation for the genotypic and phenotypic broodstock populations was determined using F_{ST} values as calculated in the program FSTAT version 2.9.3.2 (Goudet, 1995). This software makes use of the principles of Weir and Cockerham (1984), and corrects for multiple comparisons. A nominal value for multiple tests of 0.05 was selected.

Because genotypic and phenotypic broodstock for only three farms were available, and varied in sample size, a hypothetical simulation was done. The top 32 genotypic animals and 32 randomly chosen animals were selected for each farm and subjected to the same statistical analyses. The randomly selected animals were chosen arbitrarily without regard to any phenotypic traits.

The broodstocks were also compared by means of multifactorial component analyses, using 1000 permutations, and statistical analyses using the program GENETIX version 4.05.2 (Belkhir *et al.*, 2000). This was done using the default settings of the software and included the average number of alleles across loci, the inbreeding coefficient and observed and expected heterozygosity of the populations as a means to determine the levels of genetic diversity within and between populations.

2.2 Results and Discussion

2.2.1 Microsatellite analysis

The number of alleles for the twelve loci ranged from 7 to 46 in the wild population, 9 to 80 in the broodstock population and 8 to 71 in the offspring populations, with an average number of alleles per locus of 23.9, 37.8 and 30.2 for the respective groups (Table 3). The average expected heterozygosity was 0.904, 0.964 and 0.890 for the wild, broodstock and offspring populations, respectively and the average observed heterozygosity was 0.764, 0.814 and 0.799 for the respective groups. Five of the loci in the wild population, seven in the broodstock population and all 12 of the loci in the offspring population did not conform to Hardy-Weinberg equilibrium (HWE). This could be a result of non-random sampling. Deviation from HWE could also be an indication of selection pressure on these loci. Artificial selection, and adaptation to the cultured environment can cause a shift in the allele frequency of loci (Frankham, 2008), making this, together with inbreeding and a small effective population size, the most plausible explanation for the deviation from HWE seen in the offspring population. Only locus *HmNR106* and *HmRS129* had null allele frequencies higher than that recommended for parentage assignment (r<0.2; Dakin and Avise, 2004). Null alleles can be a result of differential amplification of size-variant alleles (Wattier *et al*., 1998), PCR failure as a result of

poor DNA template (Gagneux *et al*., 1997) or a mutation in the primer annealing site, which prevents allele amplification (Pemberton *et al*., 1995). Null alleles can result in an excess of homozygotes (Jones *et al*., 1998) and may affect parentage assignment by eliminating potential parents. However, a review of literature by Dakin and Avise (2004) found that 90% of the 233 articles studied included loci with null alleles. We therefore did not exclude loci *HmNR106* and *HmRS129* from parentage assignment in this study.

Table 3: Characteristics of the twelve microsatellite loci used for parentage assignment.

	Wild					Broodstock					Offspring				
	N_a	H_o	H_e	HWE	*r*	N_a	H_o	H_e	HWE	*r*	N_a	H_o	H_e	HWE	*r*
HmD55	29	0.828	0.896	0.428	0.033	55	0.793	0.903	0.000***	0.065	45	0.847	0.915	0.000***	0.038
HmD59	20	0.828	0.904	0.144	0.041	38	0.900	0.928	0.000***	0.015	19	0.843	0.896	0.000***	0.032
HmNR106	18	0.397	0.874	0.000***	0.380	26	0.549	0.878	0.000***	0.241	20	0.463	0.828	0.000***	0.302
HmNR120	28	0.776	0.940	0.000***	0.094	35	0.898	0.951	0.228	0.028	30	0.859	0.917	0.000***	0.034
HmNR20	22	0.862	0.921	0.038*	0.027	32	0.871	0.905	0.238	0.020	29	0.912	0.911	0.000***	-0.001
HmNS19	46	0.845	0.967	0.000***	0.066	80	0.961	0.970	0.141	0.004	71	0.866	0.951	0.000***	0.048
HmidPS1.818	13	0.759	0.887	0.349	0.074	22	0.775	0.890	0.000***	0.067	20	0.784	0.843	0.000***	0.035
HmidPS1.870	19	0.948	0.917	0.508	-0.023	28	0.906	0.922	0.067	0.008	24	0.927	0.907	0.000***	-0.015
HmidPS1.967	7	0.707	0.743	0.258	0.024	9	0.780	0.757	0.062	-0.017	8	0.714	0.733	0.023*	0.015
HmRS129	21	0.345	0.899	0.000***	0.450	41	0.566	0.938	0.000***	0.246	25	0.585	0.922	0.000***	0.223
HmRS27	36	0.930	0.967	0.299	0.015	56	0.871	0.969	0.000***	0.053	46	0.885	0.952	0.000***	0.036
HmRS80	28	0.948	0.931	0.462	-0.013	32	0.894	0.921	0.002**	0.012	25	0.905	0.904	0.000***	-0.001
Average	23.92	0.764	0.904			37.83	0.814	0.911			30.17	0.799	0.890		

N_a = number of alleles

H_o = Observed heterozygosity

H_e = Expected heterozygosity

HWE = Hardy-Weinberg equilibrium

r = Null allele frequency

* = p <0.05

** = p <0.01

*** = p <0.001

2.2.2 Parentage assignment

Of the 1000 offspring that had sufficient genotype data, *i.e.* those that had genotypes for at least seven markers, only 431 animals were successfully assigned (See Appendix F for LOD scores of assigned parent pairs). Compared to similar studies done in abalone, this assignment rate is very low. Selvamani *et al*. (2001) assigned 90% - 100% of *Haliotis asinina* larvae to individual families using a combination of three microsatellite markers, while Ruivo (2007) successfully assigned 83.3% and 81.1% of *H*. *midae* offspring from I&J and Roman Bay respectively, using ten microsatellite loci. In a study done by Van den Berg (2008) on the same species, nine microsatellite loci were used to assign 91% and 90% of animals to at least one parent for Abagold and HIK respectively. Slabbert *et al*. (2009) had an assignment rate of 92% in *H. midae* using six microsatellite markers. Only 8% of all the individuals in that particular study were unassigned, in stark contrast to the 56.9% animals that could not be assigned to at least one parent in this study. Failure to assign the remaining 569 animals was ascribed to genotyping errors, mismatches and tagging errors and thus removed from the dataset.

Due to the nature of grow out practices on farms, unassigned animals could be from the unsubscribed broodstock population used for normal (non-experimental animals) commercial purposes. To ensure none of the experimental (PRS) animals had any advantages because of a lower number of animals in some of the baskets, stocking densities were kept the same throughout all the baskets that were monitored. Abalones often rub against each other because of limited space in the baskets as well as to display dominance. This causes the number on the bee tag with which the experimental animals were tagged, to become unclear or for the tag to become dislodged. It is possible that these unassigned animals were mistakenly tagged as PRS animals when animals were retagged.

Since different broodstock groupings were used for the PRS than for commercial spawnings, the genetic composition of the offspring could differ. To test whether or not the unassigned animals were possibly from another broodstock grouping, the unassigned and assigned animals were treated as two separate populations and FCA was done (Fig. 2).

An FCA plot indicates the relationships between different populations using genotypic data. The two populations do not separate on the second axis, which explained 42.76% of the variation, but is separated on the first axis, which accounts for 29.91% (Fig. 2). Taken as a whole, this plot shows distinction between these two populations, which supports the theory that the unassigned animals were not PRS offspring, but possibly non-experimental animals or animals used to increase stocking density.

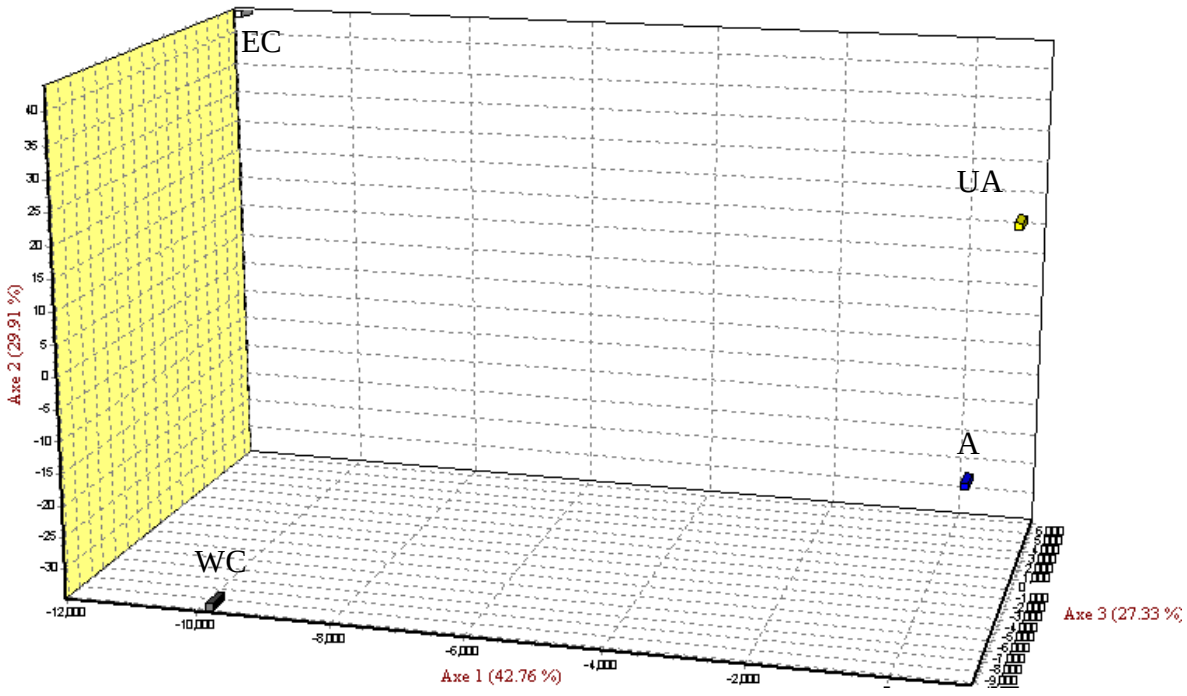


Figure 2: An FCA plot showing the distribution of the assigned animals (A) and unassigned animals (UA). The wild populations were included as reference populations (EC=East coast, WC=West coast).

Family trees of assigned offspring animals were constructed for each farm (Fig. 3).

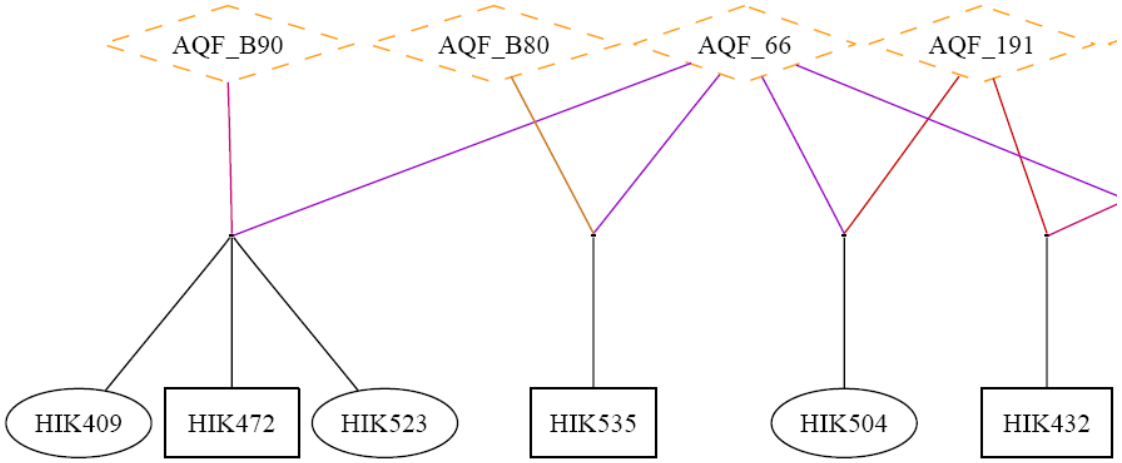


Figure 3: An example of a family tree constructed using assigned animals from HIK. The top row represents the parents (the diamond shape indicates that the sex of the parent is unknown), while the bottom row represents the offspring (a circle indicates female, and a rectangle male). Although the sex of the broodstock was known, Pedigraph did not allow for gender assignment of the parents. AQF=Aquafarm, HIK=HIK.

Histograms were constructed to determine which broodstock animals dominated the spawning event, as well as to identify broodstock that had a low contribution or those that did not contribute at all (Fig. 4 to 8). Contribution data is important since it will add value to various studies and management protocols. The most important of these are the monitoring of spawning events and selection of good quality broodstock in conjunction with other parameters such as sperm and egg quality. Walk-back selection is another area where such data can facilitate research. For example, any disease resistant offspring can be traced back to its progenitors. Since the above data were generated from a single spawning event, it is essential to take data from other spawning events into account before it is used for selection.

The factors influencing the contribution of parents can also be studied in more detail, since potential animals have been identified (Boudry *et al*., 2002; Brown *et al*., 2005; Frost *et al*., 2006; Lind *et al*., 2009). Parameters including animal age, sperm and egg quality, larval quality and other physiological (stress response) and genomic (gene expression) factors can be studied by means of comparative studies.

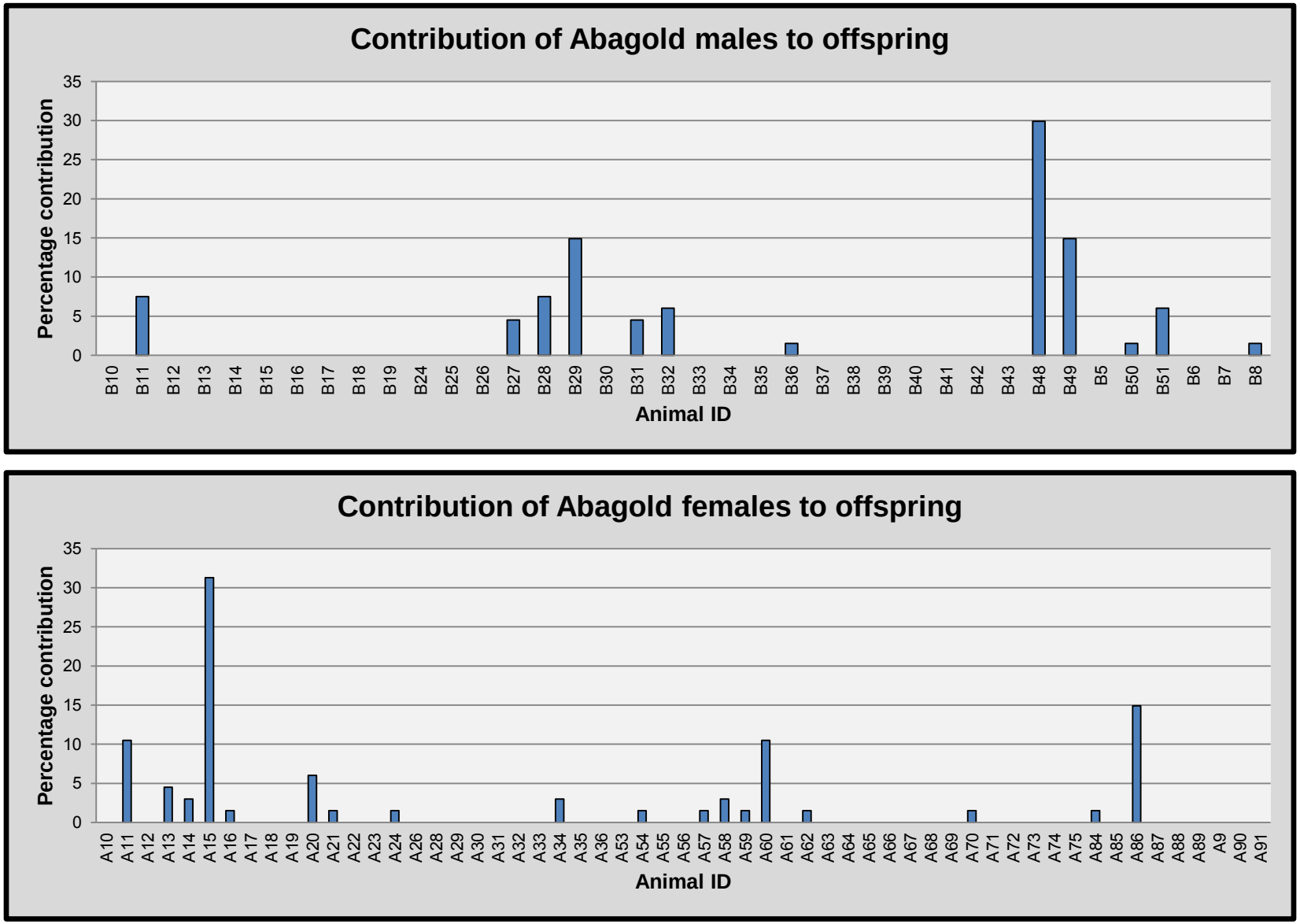


Figure 4: Histograms representing the contribution of each broodstock animal from Abagold to the assigned PRS animals.

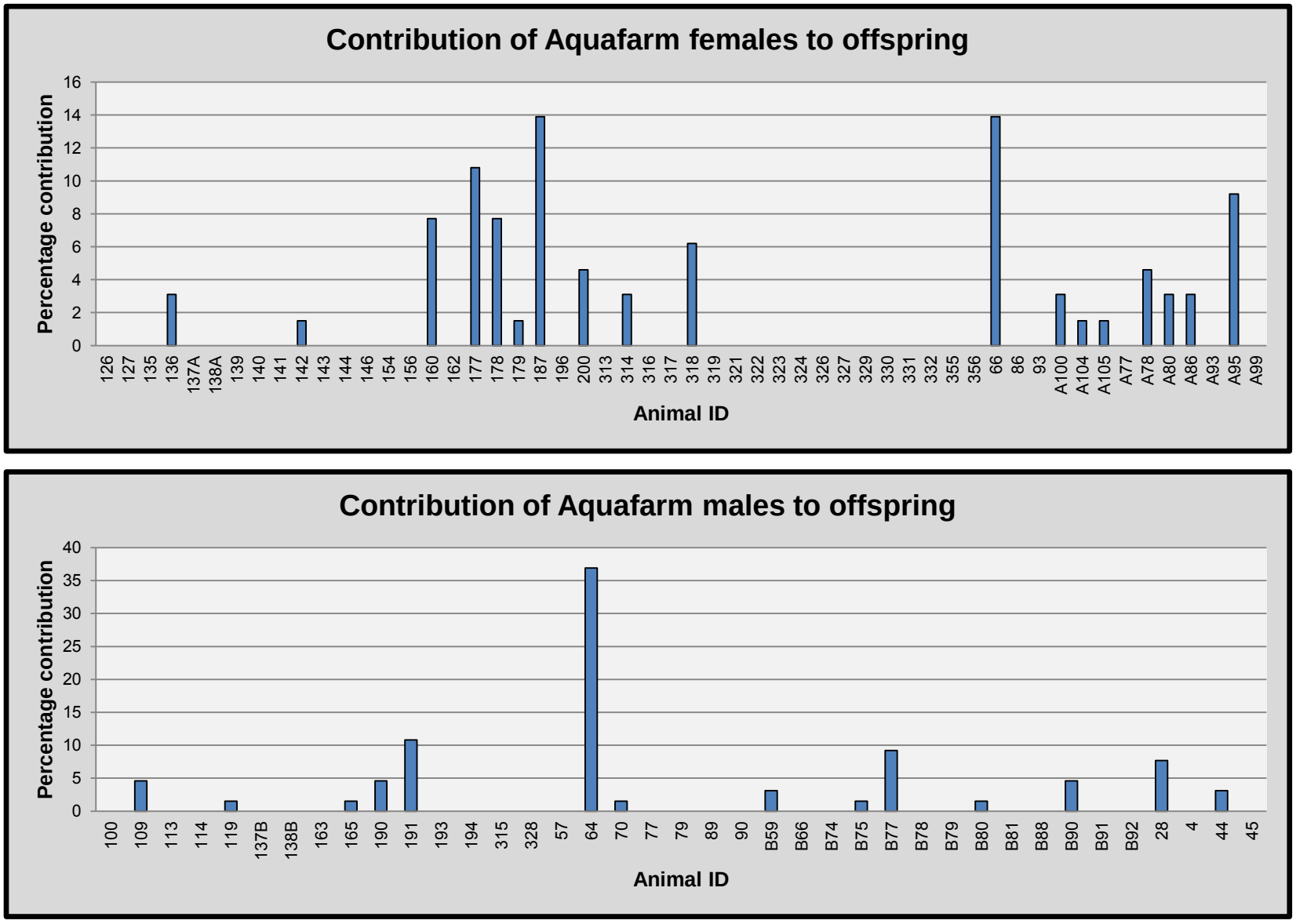


Figure 5: Histograms representing the contribution of each broodstock animal from Aquafarm to the assigned PRS animals.

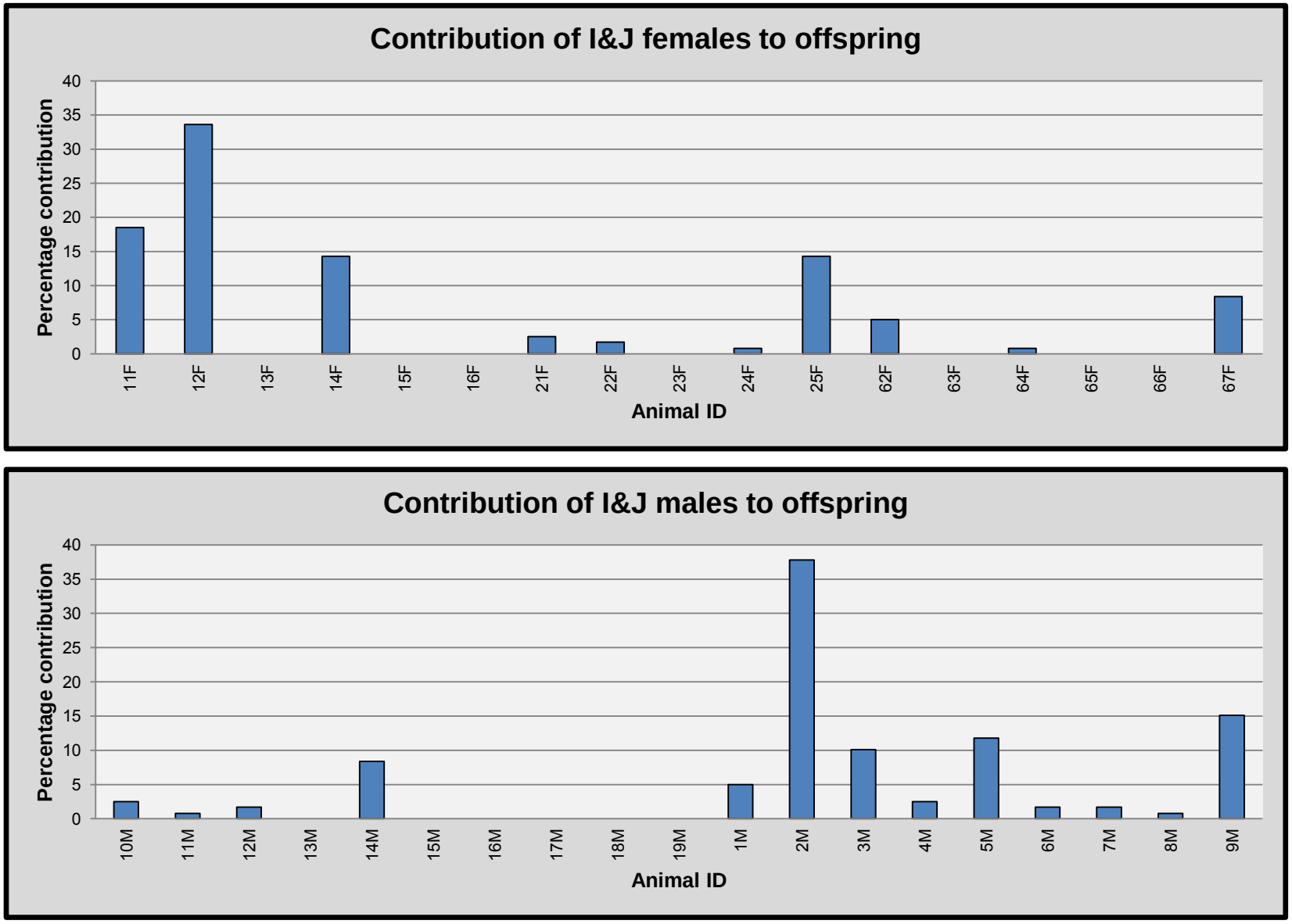


Figure 6: Histograms representing the contribution of each broodstock animal from I&J to the assigned PRS animals.

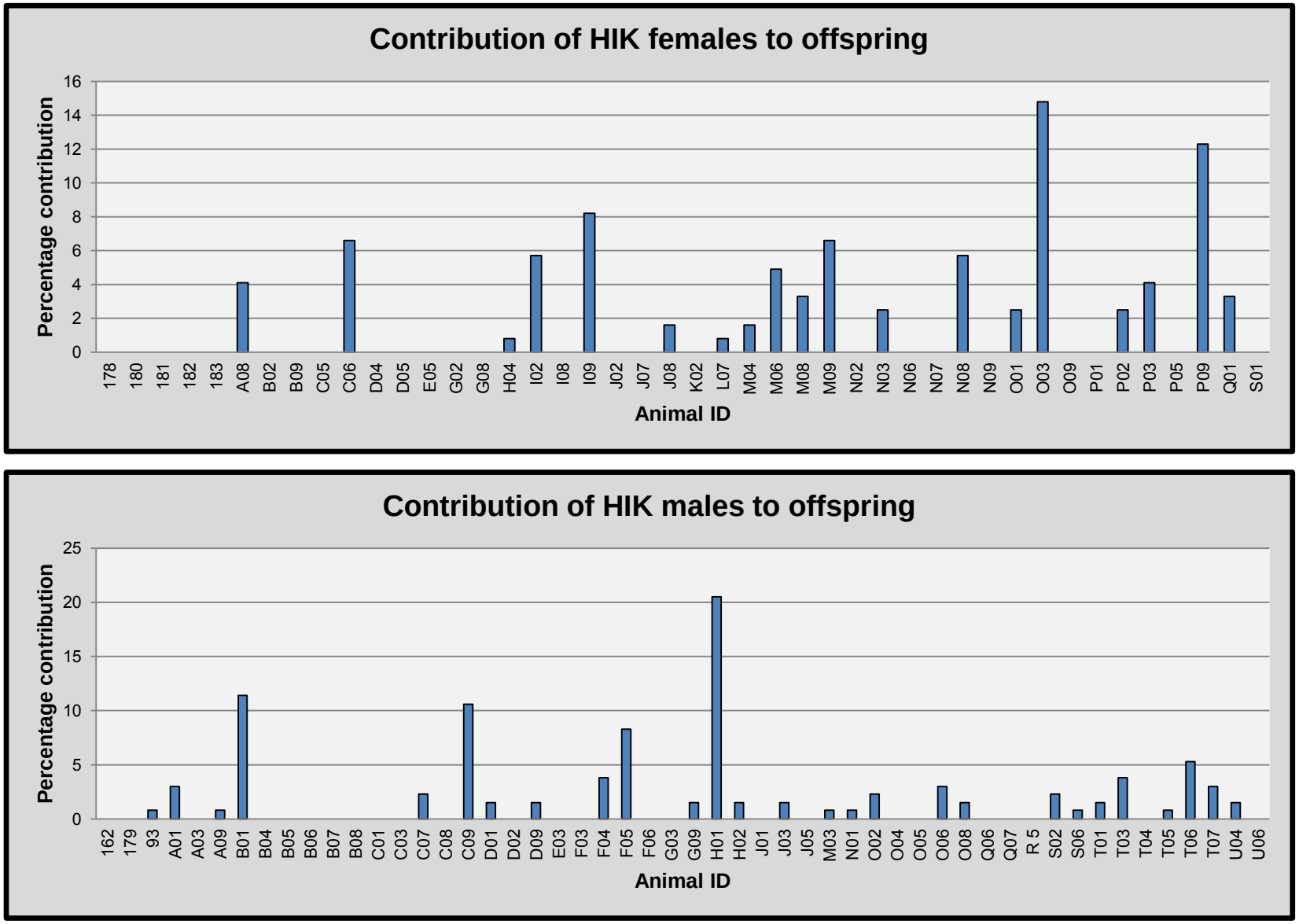


Figure 7: Histograms representing the contribution of each broodstock animal from HIK to the assigned PRS animals.

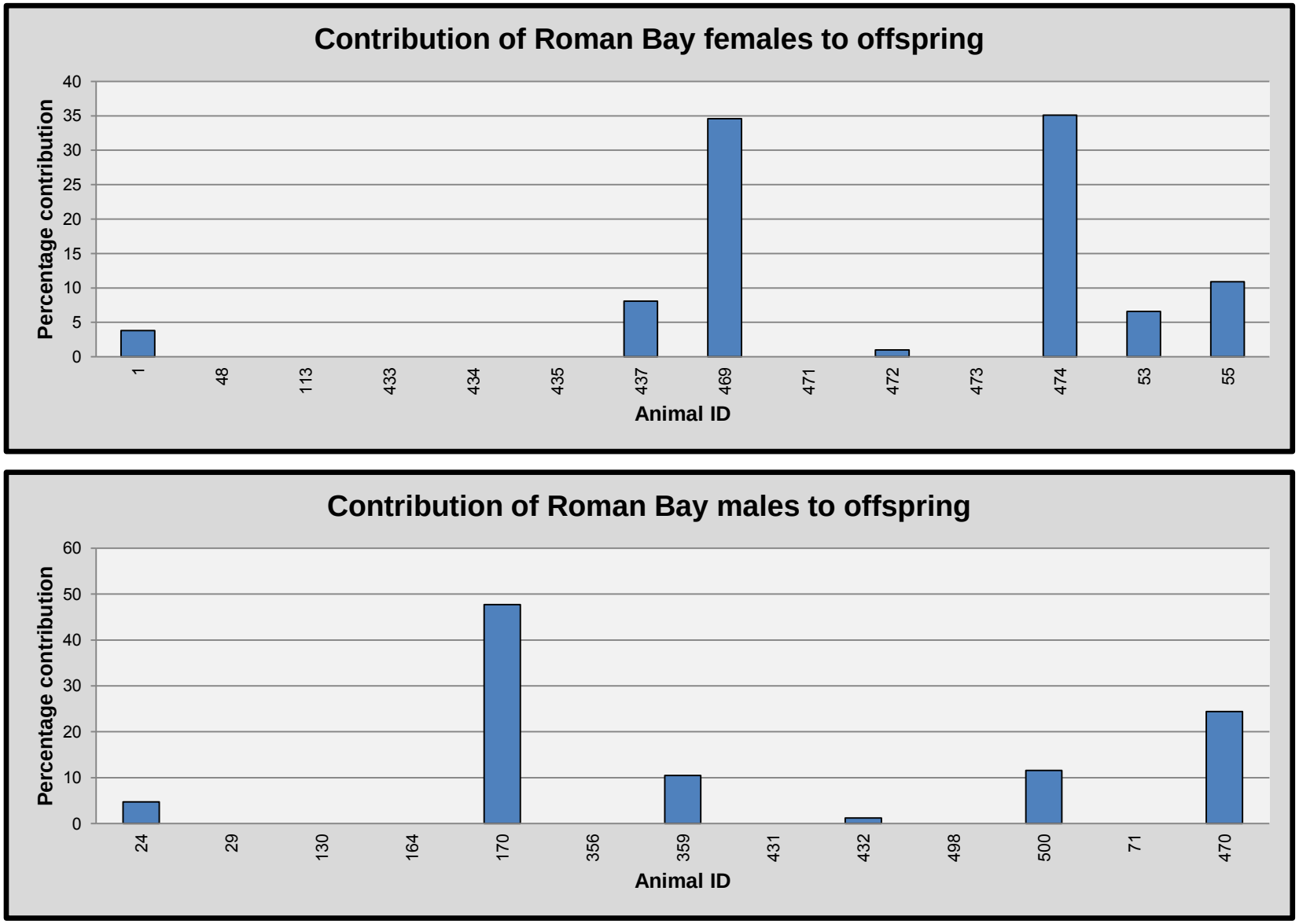


Figure 8: Histograms representing the contribution of each broodstock animal from Roman Bay to the assigned PRS animals.

2.2.3 Comparison of phenotypic and genotypic traits used for selection

To determine the potential effect of different selection methods on the genetic diversity of a population, two selection methods were used to select broodstock for the participating farms. Genotypic broodstock was selected for each farm using the walk-back method as described in Doyle and Herbinger (1994), until no further animals could be selected that were not full-sib to an animal already chosen. Forty-three unrelated animals could be selected as broodstock for Abagold, 71 for Aquafarm, 82 for HIK, and 44 for I&J. No animals could be recommended to Roman Bay because of tag loss early during the study (See Appendix B for the IDs of the recommended genotypic broodstocks).

The farms selected broodstock based on physical traits such as shell-size and colour to obtain a phenotypic broodstock. Due to early tag loss, no phenotypic broodstock were obtained from Roman Bay. I&J did not participate in the phenotypic selection (See Appendix C for the IDs of the phenotypic broodstocks as selected by the farms).

Three dimensional FCA plots were constructed for the three genotypic broodstock groups (Fig. 9) as well as for the three phenotypic broodstock groups (Fig. 10). Pairwise F_{ST} values (Table 4 and 5) were calculated to determine divergence between the respective broodstock groups.

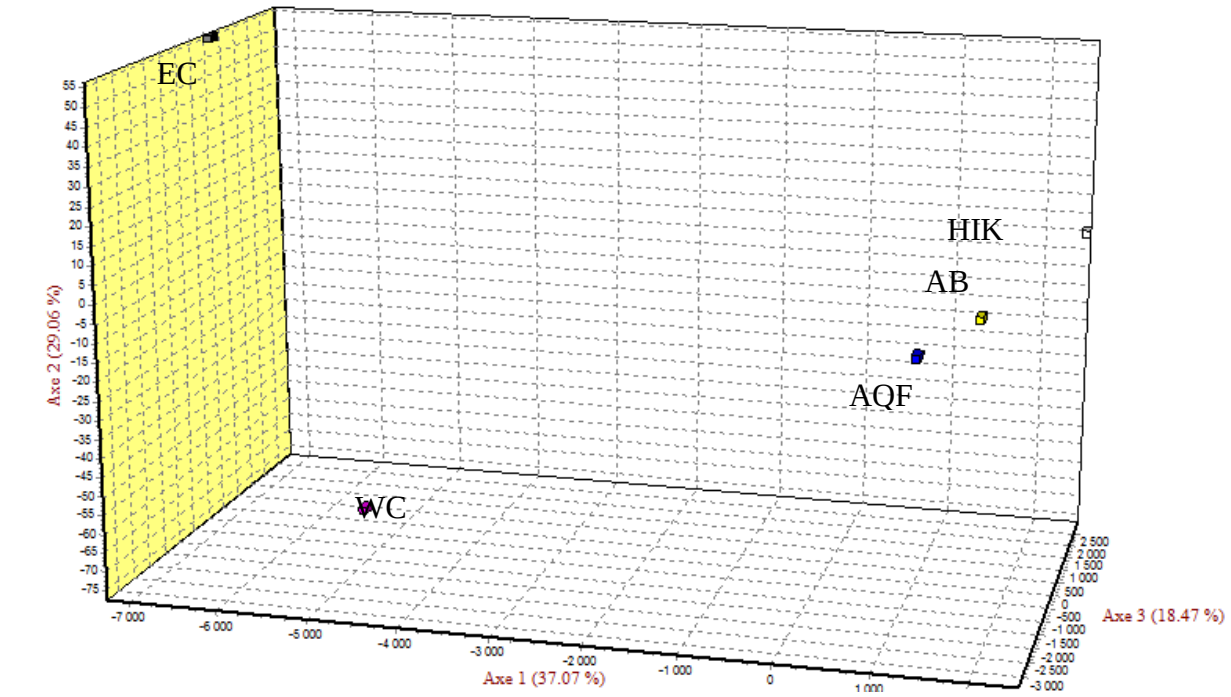


Figure 9: An FCA plot illustrating the genetic divergence between the different genotypic broodstock of each farm (AB=Abagold, AQF=Aquafarm, HIK=HIK). The wild populations were included as reference populations (EC=East coast, WC=West coast).

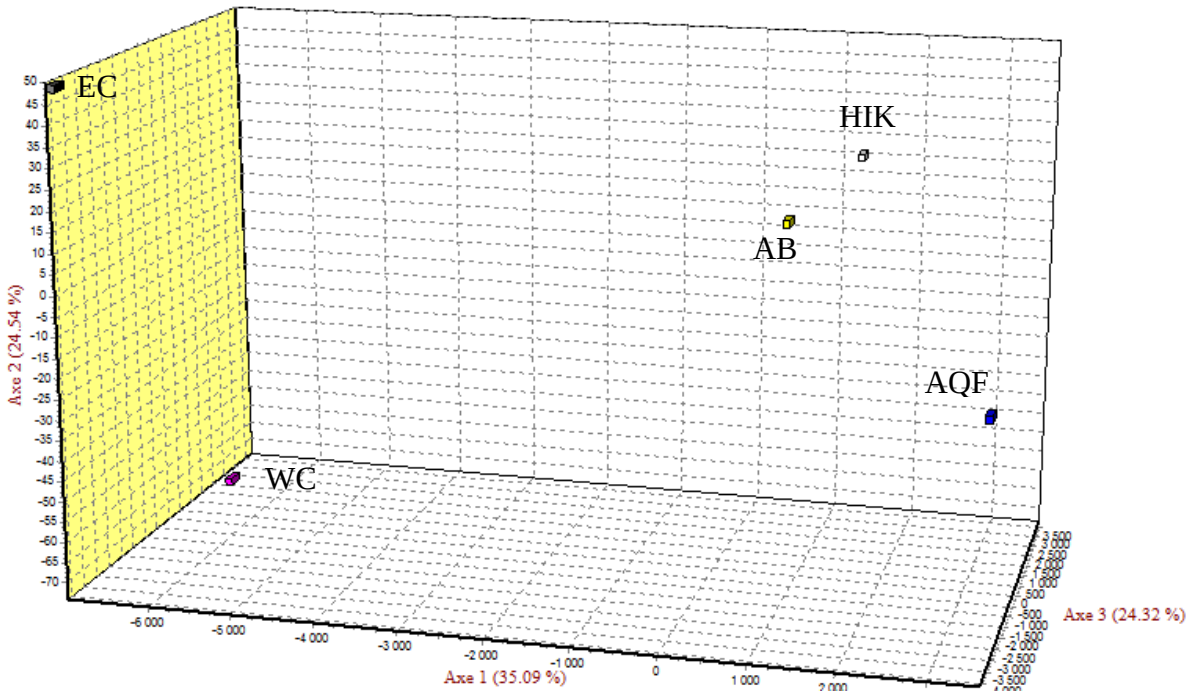


Figure 10: An FCA plot illustrating the genetic divergence between the different phenotypic broodstock of each farm (AB=Abagold, AQF=Aquafarm, HIK=HIK). The wild populations were included as reference populations (EC=East coast, WC=West coast).

Factorial component analysis, visualised as three dimensional plots, depicts the relationships between the different populations. Above plots indicate that there could be a difference between the different selection methods. Not much differentiation is seen between the genotypic broodstock populations, with some separation on the second axis, which describes 29.06% of the variation (Fig. 9), while the phenotypic broodstock groups are more separated, specifically on the first axis, which accounts for 35.09% of the variation (Fig. 10). These plots indicate that there is more variation between the different phenotypic groups, than between the genotypic groups.

This is reflected in the F_{ST} values as well (Table 4 and 5), which indicates the level of genetic divergence between populations. None of the genotypic broodstock populations differed significantly from each other. On the contrary, all of the phenotypic broodstock, excluding the HIK population when compared to Abagold, showed significant divergence from each other. With the exception of the genotypic population from Abagold when compared to the West coast population, and the phenotypic population from HIK when compared to the West coast population, all of the broodstock populations showed significant genetic divergence from the wild populations.

Table 4: Pairwise F_{ST} p-values after adjustment for multiple comparisons ($p<0.05$) of the genotypic broodstock of each farm. Significant values are indicated with an asterisk, (*).

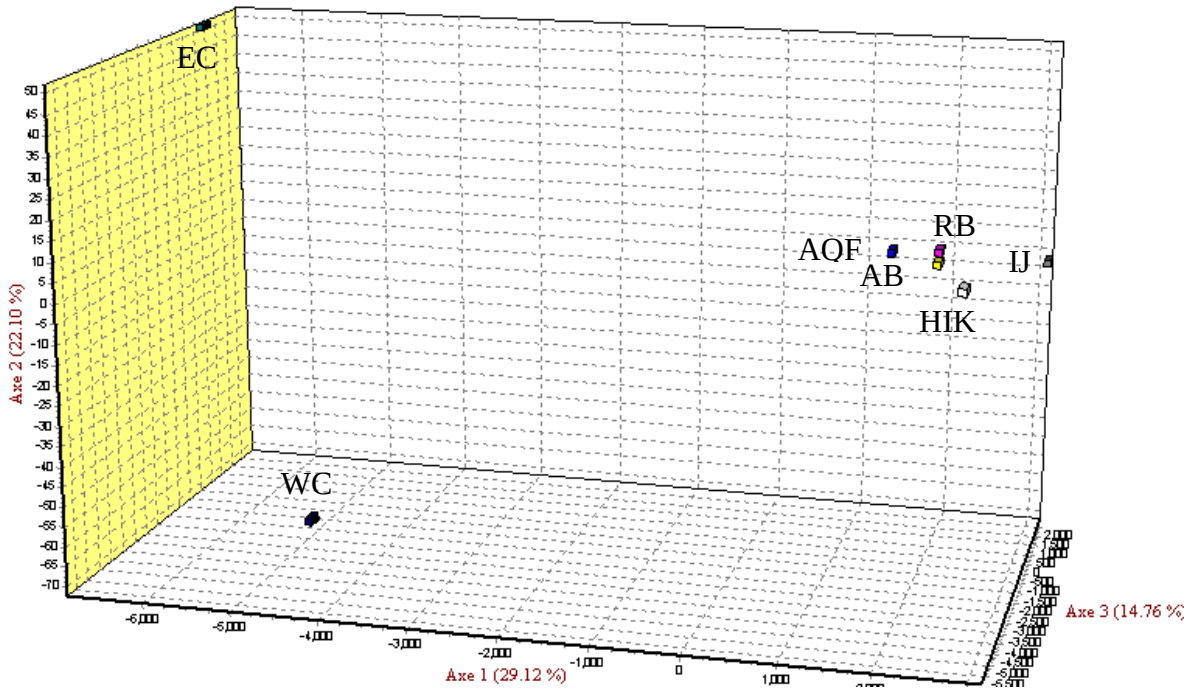
	AQF	HIK	East	West
AB	0.440	0.650	0.005*	0.030
AQF		0.195	0.005*	0.005*
HIK			0.005*	0.005*
East				0.200

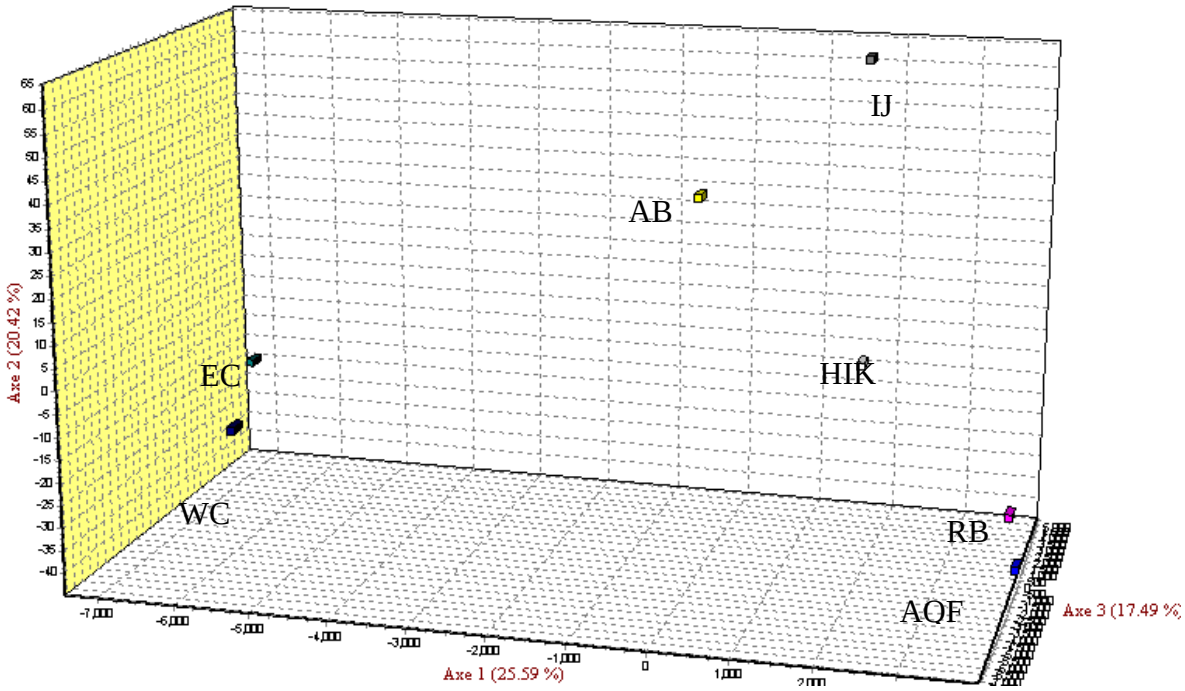
Table 5: Pairwise F_{ST} p-values after adjustment for multiple comparisons (p<0.05) of the phenotypic broodstock of each farm. Significant values are indicated with an asterisk, (*).

	AQF	HIK	East	West
AB	0.005*	0.135	0.005*	0.005*
AQF		0.005*	0.005*	0.005*
HIK			0.005*	0.010
East				0.160

Since only three farms selected phenotypic broodstock of differing sizes, this comparison is not adequate. To confirm that phenotypic selection had an effect on the diversity of the population and to ensure that sample size did not have an effect on the outcome of the comparison between the different broodstock, a hypothetical simulation was done. The top 32 animals from the genotypic broodstock of all five farms as well as 32 samples from the wild populations were compared by means of multifactorial component analyses (Fig. 11a), as well as statistical analyses including pairwise *Fst* values (Table 6), number of alleles, heterozygosity and inbreeding levels (Table 8). This was also done with 32 animals from each farm chosen at random (Table 7, 9 and Fig. 11b). See Appendix D for ID's of the simulated populations.

A]





B]

Figure 11: FCA plots illustrating the genetic divergence between (A): the top 32 genotypic animals of each farm as well as (B): 32 randomly selected animals from each farm. (AB=Abagold, AQF=Aquafarm, HIK=HIK, IJ=I&J, RB=Roman Bay). The wild populations were included as reference populations (EC=East coast, WC=West coast).

The FCA plot of the top 32 genotypic broodstock animals of each population (Fig. 11a), shows that the cultured populations group as a cluster, indicating similarity in genetic composition. This suggests that selection on a genetic level was effective at targeting the alleles that are beneficial for the trait selected for, in this case growth. This similarity is reflected by the pairwise F_{ST} values, which show no significant difference between the genotypic populations (Table 6). In contrast to the genotypic broodstock populations, the FCA plot of the broodstock chosen by random differs greatly (Fig. 11b). These populations are separated on the second axis, which accounts for 20.42% of the population differentiation, with some separation on the first axis, which describes 25.59% of the differentiation. This is corroborated by the F_{ST} values, with five out of the ten pairwise comparisons differing significantly from each other (Table 7). This can be explained by the fact that these animals were chosen by random, which doesn't allow for any selection pattern.

Table 6: Pairwise F_{ST} p-values after adjustment for multiple comparisons ($p<0.05$) of the 32 top animals from the genotypic broodstock of each farm. Significant values are indicated with an asterisk, (*).

	AQF	HIK	IJ	RB	East	West
AB	0.974	0.850	0.991	0.977	0.003	0.100
AQF		0.918	0.999	0.999	0.060	0.389
HIK			0.912	0.961	0.002*	0.002*
IJ				0.977	0.001*	0.018
RB					0.001*	0.055
East						0.162

Table 7: Pairwise F_{ST} p-values after adjustment for multiple comparisons (p<0.05) of the 32 randomly selected animals from each farm. Significant values are indicated with an asterisk, (*).

	AQF	HIK	IJ	RB	East	West
AB	0.002*	0.002*	0.005	0.002*	0.002*	0.007
AQF		0.036	0.002*	0.279	0.002*	0.002
HIK			0.005	0.007	0.002*	0.002*
IJ				0.002*	0.002*	0.002
RB					0.002*	0.002
East						0.179

To determine the impact of the selection methods on genetic diversity, the number of alleles, observed and expected heterozygosity and inbreeding levels were calculated (Table 8 and 9).

The average observed (H_o) and expected heterozygosity (H_e) was 0.803 and 0.890 respectively for the genotypic populations and 0.811 and 0.872 for the populations chosen at random (Table 8 and 9). These values are comparable to similar parentage studies done on *H. midae*. In a study using ten microsatellite loci by Ruivo (2007), the observed and expected heterozygosity values ranged from 0.737 to 0.833 and 0.847 to 0.886, respectively. Van den Berg (2008) reported an average observed and expected heterozygosity of 0.816 and 0.872 using nine microsatellite loci, while Slabbert *et al*. (2009) found the observed and expected heterozygosities to range from 0.511 to 0.618 and 0.651 to 0.784 using six microsatellite loci. Because high heterozygosity levels are observed for several species of abalone including *H. midae*, *H. rubra*, *H. discus hannai* and *H. kamtschatkana* (Evans *et al*., 2004; Li *et al*., 2004; Lemay and Boulding, 2009), it cannot be used on its own to determine whether there is any loss in genetic diversity.

The average number of alleles across loci (N_a) ranged from 17.4 to 18.7 for the genotypic populations, and 15.6 to 17.3 for the populations chosen at random (Table 8 and 9). In all the populations, the genotypically selected animals had a higher number of alleles, comparable to that of the wild populations, than the broodstock chosen at random. All of the random population groups had a lower average number of alleles than the wild populations. This

confirms results found by similar studies where a decrease in number of alleles was seen in populations chosen at random (Doyle *et al*., 2001; Sekino *et al*., 2004; Ortego-Villaizan *et al*., 2011). However, where minimal kinship groups were selected a similar (Doyle *et al*., 2001; Sekino *et al*., 2004) or increased (Ortego-Villaizan *et al*., 2011) number of alleles was observed.

Even though unrelated animals were chosen as genotypic broodstock, the inbreeding levels of the genotypic populations were not lower than that of the populations chosen at random (as indicated by the inbreeding coefficient, *f*, in Table 8 and 9). The inbreeding coefficient is calculated based on the heterozygosity levels of a population, which is very high in *H*. *midae*, [as illustrated in previous studies (Ruivo, 2007; Van den Berg, 2008; Slabbert *et al*., 2009), as well as the current study], explaining why no inbreeding is observed for any of the populations.

Table 8: The levels of genetic diversity for the top 32 animals from the genotypic broodstock of each farm in terms of average number of alleles across loci (N_a), mean expected (H_e) and observed heterosygosity (H_o) and inbreeding (*f*).

Population	N_a	H_e	H_o	*f*
East	17.8	0.879	0.778	0.132
West	18.1	0.893	0.800	0.124
AB	17.4	0.884	0.798	0.114
AQF	18.3	0.890	0.798	0.120
HIK	18.7	0.897	0.814	0.109
IJ	17.9	0.890	0.824	0.091
RB	17.9	0.890	0.779	0.142

Table 9: The levels of genetic diversity for the 32 randomly selected animals of each farm in terms of average number of alleles across loci (N_a), mean expected (H_e) and observed heterosygosity (H_o) and inbreeding (f).

Population	N_a	H_e	H_o	f
East	17.8	0.879	0.778	0.132
West	18.1	0.893	0.800	0.124
AB	15.8	0.874	0.850	0.043
AQF	15.8	0.856	0.756	0.134
HIK	17.3	0.885	0.813	0.097
IJ	15.8	0.875	0.820	0.079
RB	15.6	0.870	0.817	0.078

These results were consistent with the comparison between the genotypic and phenotypic populations. For both these experiments, the genotypic populations had a similar genetic composition with almost no differentiation between the populations. In contrast, both the phenotypic and randomly selected populations showed significant differentiation from each other and the wild populations. In addition to this, the genotypic populations had similar levels of genetic diversity than the wild, while the randomly selected populations had a lower number of alleles than the genotypic and wild populations.

This study suggests that there could be a difference between selection strategies, as indicated by the F_{ST} values and number of alleles of the different populations. It should be noted that these findings reflect the genetic diversity of populations after only one generation of selective breeding and that a cumulative effect could occur after a few generations. Although differences are found between the strategies, selection is still necessary for the enhancement of a species. By selecting for a specific trait, be it phenotypic or genotypic, selection will focus on targeting the allele beneficial for the trait selected for. Selection will therefore be more successful in fixing the desired allele in the populations than selecting animals at random. This study found that it is possible to retain genetic diversity with genotypic selection whilst still selecting for a phenotypic trait, in this case shell-size. It is

therefore advisable to combine phenotypic and genotypic selection strategies to select animals with desirable traits, while still managing the genetic diversity of the population.

2.3 Conclusion

This study demonstrated the value and effectiveness of microsatellite markers in breeding programmes. Twelve loci were used to assign parentage to a selected group of offspring animals. In contrast to the high success rate found in previous *H*. *midae* studies, a substantial amount of animals could not be successfully assigned to parents in this study. This was attributed to a possible tagging error, where non-experimental animals could have been mistaken as PRS offspring, or genotyping errors. Although only 43.1% of the animals could be assigned to parents, this data was successfully used to establish the contribution of broodstock animals during the PRS spawning event. Using the parentage data, unrelated animals were selected and recommended to the farms as potential future broodstock.

This study indicates that it is indeed possible to retain genetic diversity when using unrelated animals for breeding purposes. The average number of alleles of the top 32 genotypic animals of each farm compared well to the wild populations, with both having an average of 18.0. These findings also suggest that although artificial selection did not result in an increase in inbreeding levels, it can lower the genetic diversity of a population, since the populations selected at random had an average number of alleles of 16.1.

Selection based on phenotypic traits can also affect population structure as is evident from the significant population differentiation between the artificially selected populations found in this study. The differentiation between phenotypic populations as opposed to the genetic similarity of the genotypic populations is also an indication that phenotypic selection was not effective at capturing target alleles. Failure to capture alleles that will be beneficial to growth rate will not shorten production times. This will restrain the progress of the breeding programme rendering it economically invaluable. It is thus of critical importance to employ genetic tools to ensure the success of a breeding programme.

Chapter 3:

Comparison of genetic diversity between wild and cultured populations

The genetic enhancement of cultured abalone has become the focus for aquaculture farms in order to stay competitive in the international market. Breeding programmes to genetically enhance strains have been established for several abalone species including *H. asinina* (Lucas *et al*., 2006), *H. rubra* (Appleyard *et al*., 2007), *H. discus hannai* (Hara and Sekino 2007a) and *H. laevigata* (Kube *et al*., 2007). Until 2006, no such programme existed for *H. midae*. In collaboration with the South African government and Stellenbosch University, five commercial farms along the West coast of South Africa established a Performance Recording Scheme (PRS), as the first breeding programme for *H. midae* to make use of molecular tools. This was aimed at increasing the growth rate of farmed animals to shorten production times. After seven months, each farm submitted an equal amount of animals to be evenly distributed among them. Forty-three months later, the participating farms selected the faster-growing animals on their farms. These animals were then subjected to molecular analysis to establish the levels of genetic diversity of the hatchery-reared offspring and current broodstock.

Maintaining genetic diversity is a critical part of breeding programmes and is necessary for the improvement of species for commercial purposes. Not only will a high diversity increase the general fitness of a population (Gamfeldt and Kallstrom, 2007); it also serves as a platform for further selection with the aim of creating an enhanced strain (Koehn *et al*., 1988; Frankham, 1995a; Hill, 2000; Launey *et al*., 2001; Slabbert *et al*., 2009). Apart from playing a pivotal part in the enhancement of species for commercial reasons, a high genetic diversity is also essential for conservation purposes. Establishing and maintaining a cultured population with high levels of genetic diversity may allow for future ranching and reseeding opportunities. For stocking enhancement, the levels of genetic diversity between cultured and wild populations should be similar in order to prevent outbreeding depression. This phenomenon can result in a decrease in the fitness of a population (Loeschke *et al*., 1994) due to both the disruption of co-adapted gene complexes (Wallace, 1968) and changes in the local adaptation of a population (Tymchuk *et al*., 2007).

Several studies in aquaculture species including the Pacific abalone (*Haliotis discus hannai*) (Li *et al*., 2004); pearl oyster (*Pinctada fucata*) (Yu and Chu, 2006) and the Chinese freshwater pearl mussel (*Hyriopsis cumingii*) (Li *et al*., 2009) have shown that the levels of genetic diversity of cultured animals may differ from that of the wild. This is mainly due to artificial selection, where animals are selected on traits that are not always favourable in the wild. This can lead to adaptation to the cultured environment, and can alter the genetic composition of a population within a few generations (Frankham, 2008). Even though adaptation is necessary for the domestication of a species such as *Haliotis midae*, it should be closely monitored to limit the loss of genetic diversity.

Domestication of a species (such as *H. midae*), is often associated with a significant loss in genetic diversity (Horreo *et al*., 2008). Several studies done on abalone species (*H. iris*, *H. tuberculata*, *H. rubra*, *H. discus hannai*, *H. discus*, *H. kamtschatkana* and *H. asinina*), have shown a decrease in the diversity levels of hatchery stocks (Smith and Conroy, 1992; Mgaya *et al*., 1995; Evans *et al*., 2004; Li *et al*., 2004; Hara and Sekino, 2007b; Lemay and Boulding, 2009; Cao and Li, 2010).

To compare the levels of diversity of the offspring originating from a specific farm and because the PRS offspring were distributed between the different farms, parents had to be assigned (see Chapter 2). Microsatellite markers were utilised for this purpose. Microsatellite markers have also been used to establish the levels of genetic diversity in several organisms, including plants (Dirlewanger *et al*., 2002; Martin *et al*., 2010), mammals (Saitbekova *et al*., 1999; Boersen *et al*., 2003), fish (Sekino *et al*., 2004; Ortego-Villaizan *et al*., 2011) and abalone (Hara and Sekino, 2007b; Lemay and Boulding, 2009; Miller *et al*., 2009).

In this study, genetic diversity levels between wild and cultured populations from each farm were compared by means of 12 microsatellite markers. This was done to determine the levels of genetic diversity of the broodstock populations at the start of the PRS programme and to establish whether the broodstock was representative of the wild populations. These levels were then compared to that of the offspring to investigate a possible loss of diversity from

parent to offspring. This data was also used to establish the potential of participating abalone farms to supply animals for abalone ranching in the Western Cape area.

3.1 Materials and Methods

3.1.1 Sample collection and DNA extraction

Forty-three months after the onset of the PRS programme, hatchery managers of five abalone farms selected the fastest-growing abalone on their farm based on shell-size and wet weight. The number of animals selected differed between farms; depending on the number of broodstock they required (Table 1). The participating farms, situated on the West coast of South Africa, are Abagold (Hermanus), Aquafarm (Hermanus), HIK Abalone Farm (Hermanus), I&J Abalone (Gansbaai) and Roman Bay Sea Farm (Gansbaai). Tissue samples of these animals were taken by means of a non-destructive sampling method (Slabbert and Roodt-Wilding, 2006). Three epipodia were clipped from each animal and stored in 99.9% v/v ethanol at room temperature until extraction. The wet weight as well as the length and width of the shell were recorded for each animal using a scale and calliper (Fig. 1).

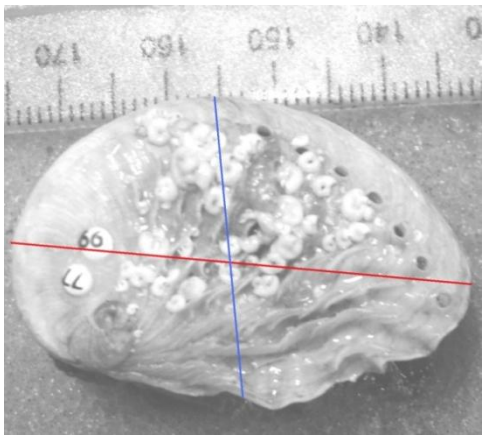


Figure 1: The length (red) and width (blue) of shell sizes were measured for all the sampled animals. (Photo: Slabbert, 2010)

Samples of the broodstock animals were collected and extracted prior to the onset of this study by students of the Molecular Aquatic Research Group (Stellenbosch University).

Samples of some of the broodstock animals could not be obtained as these animals were already deceased whenthis study commenced. Samples of two wild populations, one from the West coast (Saldanha) and one from the East coast (Rietpoint), were also available for comparison with farm populations to assess levels of diversity (Table 1). Wild samples were collected by commercial and government scientific divers.

Table 1: The number of animals selected and sampled from each farm population. A total of 1013 cultured animals and 58 wild animals were sampled.

Cultured populations					**Wild populations**	
Abagold	Aquafarm	HIK	I&J	Roman Bay	Rietpoint	Saldanha
96	199	296	129	293	31	27

DNA extractions were carried out using a cetyltrimethylammonium bromide (CTAB) extraction method as described by Saghai Maroof *et al*. (1984). The three tentacles taken from each animal were placed in 500μl extraction buffer [2% (v/v) CTAB solution, 1.4M NaCl, 0.2% (v/v) β-mercapto-ethanol, 20mM EDTA, 100mM Tris-HCl; pH8], containing 2μl of a 10mg/ml proteinase K solution (*Sigma Aldrich*) and incubated in a waterbath overnight at 60ºC. DNA was extracted using a 24:1 chloroform:isoamylalcohol mixture and washed with 70% (v/v) ethanol to remove excess salts. After these washing steps, the DNA was precipitated using ice-cold 100% (v/v) isopropanol. Following overnight incubation at -20ºC, pellets were dried at 55ºC in an oven and resuspended in 100μl ddH_2O. Resuspended DNA was stored at -20ºC until further use. Unless otherwise stated, all chemicals used were obtained from Merck (Darmstadt, Germany).

3.1.2 Microsatellite analyses

Twelve previously optimised microsatellite loci were chosen for genotyping and divided into two multiplex panels, Parent Panel 1 and 2. Polymerase chain reactions were performed for all of the sampled individuals with both these panels and scored based in fragment size using the software Genemapper® version 4.0 (*Applied Biosystems*). The number of alleles observed and expected heterozygosities and the presence of null alleles were determined for each marker using the software CERVUS version 3.0.3 (Kalinowski *et al*., 2007). Deviation from

Hardy-Weinberg equilibrium was calculated with GenePop version 4.0.10 (Raymond and Rousset, 1995). See section 2.1.2 for details.

3.1.3 Parentage assignment

Parents were assigned to offspring using the software Cervus version 3.0.3 (Kalinowski *et al*., 2007). Only individuals typed for at least seven microsatellite markers were included in the assignment. Parent pairs with a LOD score higher than 3 were considered as potential parent pairs and the pair with the highest LOD score was assigned as the parents. In the event where more than one parent pair had the same score and parents could not be assigned by inspecting the genotypes of the offspring, only one parent was assigned. If this could not be done, animals were left unassigned. See section for 2.1.3 for details.

3.1.4 Data analyses

3.1.4.1 Broodstock versus pooled offspring

Allele frequencies of the 12 microsatellite loci used in this study were determined for each population with the software Genepop version 4.1.10 (Raymond and Rousset, 1995). This data was used to determine the presence of private alleles, in order to determine which alleles, if any, were not present in the offspring populations.

The levels of genetic diversity were calculated for each farm population (broodstock and corresponding pooled offspring groups). Genetic diversity was considered as the average number of alleles across all loci (N_a), allelic richness as well as expected (H_e) and observed (H_o) heterozygosity, calculated by the software GENETIX version 4.05.2 (Belkhir *et al*., 2000), using the default settings. Because the number of individuals differed between populations, allelic richness (A) was calculated in addition to the number of alleles (N_a), since this parameter is unbiased for sample size. This was done using the default settings of the software FSTAT version 2.9.3.2 (Goudet, 1995). Genetic diversity was compared between the broodstock and corresponding offspring populations to determine a possible loss of alleles

and heterozygosity. The significance of any loss was determined by means of a non-parametric Mann-Whitney U test, using the software XLSTAT Version 2011.4.02 (*Addinsoft*).

To determine the population differentiation between the broodstock population and its corresponding pooled offspring population, pairwise F_{ST} values were calculated using the software FSTAT version 2.9.3.2 (Goudet, 1995). This software makes use of the principles of Weir and Cockerham (1984), and corrects for multiple comparisons. A nominal value for multiple tests of 0.05 was selected.

3.1.4.2 Broodstock versus wild

To evaluate the levels of genetic diversity available to the PRS programme, broodstock populations were compared to the wild populations. The levels of genetic diversity (allelic richness, number of alleles as well as observed and expected heterozygosity) for these populations were calculated as in section 3.1.4.1. Population differentiation (pairwise F_{ST}) between the individual broodstock and wild populations were calculated as in section 3.1.4.1.

3.1.4.3 Mixed offspring versus wild

For this section, the offspring populations consisted of the selected PRS animals that are currently found on the farms. The levels of genetic diversity (allelic richness, number of alleles as well as observed and expected heterozygosity) and genetic divergence (pairwise F_{ST}) of the mixed offspring populations were compared to the wild as described in section 3.1.4.1. This was done to investigate the possibility of using the cultured animals currently on the farms for abalone ranching in the Western Cape of South Africa.

3.2 Results and Discussion

3.2.1 Microsatellite analyses

An average number of alleles per locus of 23.9 for the wild, 37.8 for the broodstock and 30.2 for the offspring groups were found. The average expected heterozygosity was 0.904, 0.964

and 0.890 respectively and the average observed heterozygosity was 0.764, 0.814 and 0.799 for the respective groups. Five of the loci in the wild population, seven in the broodstock population and all 12 of the loci in the offspring population did not conform to Hardy-Weinberg equilibrium (HWE). This was attributed to artificial selection, adaptation to the environment and a small effective population size. See section 2.2.1 for details.

3.2.2 Parentage assignment

Of the 1000 offspring that had sufficient genotype data, only 431 animals were assigned. Compared to similar studies done in *H. midae*, this assignment rate is very low (Ruivo, 2007; Van den Berg, 2008; Slabbert *et al*., 2009). Failure to assign the remaining 569 animals was ascribed to mismatches and tagging errors and these animals were removed from the dataset. Due to the nature of grow out practices on farms, it is possible that the unassigned animals could be non-experimental animals used to increase stocking densities. This was supported by FCA analyses, which indicated that the unassigned and assigned animals grouped as two distinct populations. See section 2.2.2 for full details.

3.2.3 Data analyses

3.2.3.1 Broodstock versus pooled offspring

Allele frequencies for each locus of the broodstock and pooled offspring populations were determined (Appendix E, see Table 2 for allele frequencies of locus *HmD55* as example).

Private alleles were present for all 12 loci in the broodstock populations, and seven loci in the offspring populations. Private alleles can occur as a result of genotyping errors or mutation. Microsatellites have a very high mutation rate of 10^{-2} to 10^{-5} (Weber and Wong, 1993; Vigouroux *et al*., 2002). Replication slippage is also quite common for these markers (10^{-3} to 10^{-5}) (Schlotterer and Tautz, 1992; Ellegren, 2002), and will result in new alleles due to changes in the number of repeats. Some of the private alleles may also result from a population bottleneck caused by the differential contribution of broodstock (Norris *et al*., 1999; Sekino *et al*., 2002). This will result in some alleles being lost in the offspring population, for

example allele 166 of locus *HmD55*, which is present in the broodstock population of Abagold, but not in the Abagold offspring population (Table 2).

Table 2: The allele frequencies of locus *HmD55* for each broodstock and pooled offspring population.

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
166	-	-	0.012	-	-	0.008	0.015	-	-	-
170	0.017	0.037	0.018	0.046	-	-	0.029	0.004	-	-
174	0.011	-	0.018	0.008	-	-	0.015	0.030	-	-
178	0.044	0.060	0.024	0.146	0.030	0.074	0.029	0.129	0.022	-
182	0.117	0.015	0.124	0.131	0.010	0.152	0.147	0.060	0.130	0.015
186	0.156	0.142	0.188	0.269	0.177	0.123	0.147	0.160	0.217	0.296
188	-	-	0.012	-	-	-	-	-	-	-
190	0.167	0.045	0.141	0.062	0.152	0.062	0.250	0.043	0.196	0.156
192	0.039	0.052	0.018	-	0.020	-	-	-	-	-
194	0.128	0.134	0.135	0.146	0.172	0.205	0.118	0.103	0.087	-
196	0.011	-	-	-	0.025	0.037	-	-	-	-
198	0.061	0.112	0.053	0.062	0.029	0.057	0.029	0.039	0.044	-
200	0.017	0.008	0.029	0.008	0.039	0.070	-	0.004	0.044	0.024
202	0.022	0.052	0.012	0.015	0.039	0.004	0.059	0.026	0.044	0.009
204	-	-	0.006	-	-	-	-	0.013	-	-
206	0.017	-	0.059	0.023	0.010	-	0.015	0.043	0.065	0.101
208	-	-	-	-	-	-	-	0.073	-	-
232	0.006	-	0.006	-	0.005	-	-	-	-	-
234	0.006	0.052	0.012	0.008	0.015	0.004	-	-	0.022	0.105
236	-	-	0.006	-	-	-	-	-	-	-
238	-	-	-	-	-	-	0.015	-	-	-
240	0.006	-	-	-	0.010	-	-	-	-	-
242	-	-	-	-	-	-	0.029	0.030	-	-
246	-	-	-	-	0.005	0.016	0.015	0.009	-	-
248	0.017	0.082	-	-	0.005	-	-	-	-	-
252	0.006	-	-	-	0.005	0.043	-	-	0.022	0.013
256	-	0.008	0.012	-	-	-	-	-	-	-
258	-	-	-	0.031	-	-	-	0.004	-	-
260	0.006	0.015	0.006	-	0.020	0.004	-	-	-	-
262	-	-	-	-	0.005	-	-	-	0.022	0.004
268	-	-	0.006	-	0.005	-	-	-	-	-
272	-	-	-	-	0.005	0.004	-	-	-	-

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
274	-	-	-	0.008	-	-	0.015	-	-	-
276	-	-	-	-	-	-	-	0.065	-	-
280	-	-	-	-	0.005	-	-	0.004	-	0.007
284	-	-	-	-	0.010	0.004	0.015	-	-	0.007
286	-	-	-	-	0.005	-	-	-	-	-
288	0.006	0.008	0.012	-	0.005	-	-	-	-	0.002
290	-	-	-	-	-	-	0.015	0.004	0.022	0.002
292	-	-	0.006	-	-	-	-	0.004	-	-
294	0.006	-	0.012	-	0.010	0.008	-	-	-	-
298	0.006	0.060	-	-	-	-	-	-	-	-
300	-	0.015	-	0.008	-	-	-	-	-	-
302	-	0.008	0.006	-	-	-	-	-	-	-
306	0.006	-	-	-	0.005	-	-	-	-	-
308	0.006	-	0.006	-	-	-	-	-	-	0.004
312	-	-	-	-	0.005	-	-	-	-	-
316	-	-	-	-	0.005	0.008	-	-	-	-
322	0.006	-	-	-	-	-	-	-	-	-
324	0.006	0.015	-	-	-	-	-	-	-	-

The levels of genetic diversity of each population are reported in Table 3. Genetic diversity was measured in terms of allelic richness, the average number of alleles across loci and expected and observed heterozygosity.

Table 3: The levels of genetic diversity of the individual broodstock and pooled offspring populations, measured in terms of allelic richness (*A*), average number of alleles across loci (N_a), and expected (H_e) and observed (H_o) heterozygosity.

		A	N_a	H_e	H_o
Broodstock	Abagold	27.2	27.9	0.905	0.812
	Aquafarm	25.2	25.8	0.901	0.799
	HIK	25.7	26.2	0.908	0.817
	I&J	20.0	20.6	0.898	0.834
	RB	15.5	15.9	0.882	0.827
	Average	**22.7**	**21.3**	**0.894**	**0.807**
Offspring	Abagold	16.1	16.3	0.868	0.829
	Aquafarm	18.0	18.2	0.881	0.839
	HIK	19.3	19.8	0.885	0.820
	I&J	16.5	16.8	0.867	0.799
	RB	16.2	16.3	0.802	0.762
	Average	**17.2**	**17.5**	**0.882**	**0.808**

The average observed and expected heterozygosity values of 0.894 for the broodstock populations and 0.882 for the offspring populations (Table 3), are comparable to similar population studies on *H. midae*. In a study using ten microsatellite loci by Ruivo (2007), the observed and expected heterozygosity values ranged from 0.737 to 0.833 and 0.847 to 0.886 respectively. Van den Berg (2008) reported an average observed and expected heterozygosity of 0.816 and 0.872 using nine microsatellite loci, while Slabbert *et al*. (2009) found the observed and expected heterozygosities to range from 0.511 to 0.618 and 0.651 to 0.784 using six microsatellite loci. As mentioned previously, due to high heterozygosity levels in *H. midae* additional parameters are needed to determine whether there is any loss in genetic diversity.

No difference was seen between allelic richness (*A*) and the average number of alleles (N_a) for the broodstock and offspring populations (Table 3). The Mann-Whitney U test to determine the significance of the loss in genetic diversity was consequently only performed for the average number of alleles (Table 4).

The average number of alleles of 21.3 for the broodstock populations and 17.5 for the offspring populations does not show a very large loss of alleles from broodstock to offspring

(Table 3). Upon closer inspection, a large loss of alleles from the broodstock to offspring populations was seen for each farm, with the exception of Roman Bay. Four broodstock animals from Roman Bay could not be genotyped which could explain the higher value of 16.3 for the offspring population compared to 15.9 of the broodstock population.

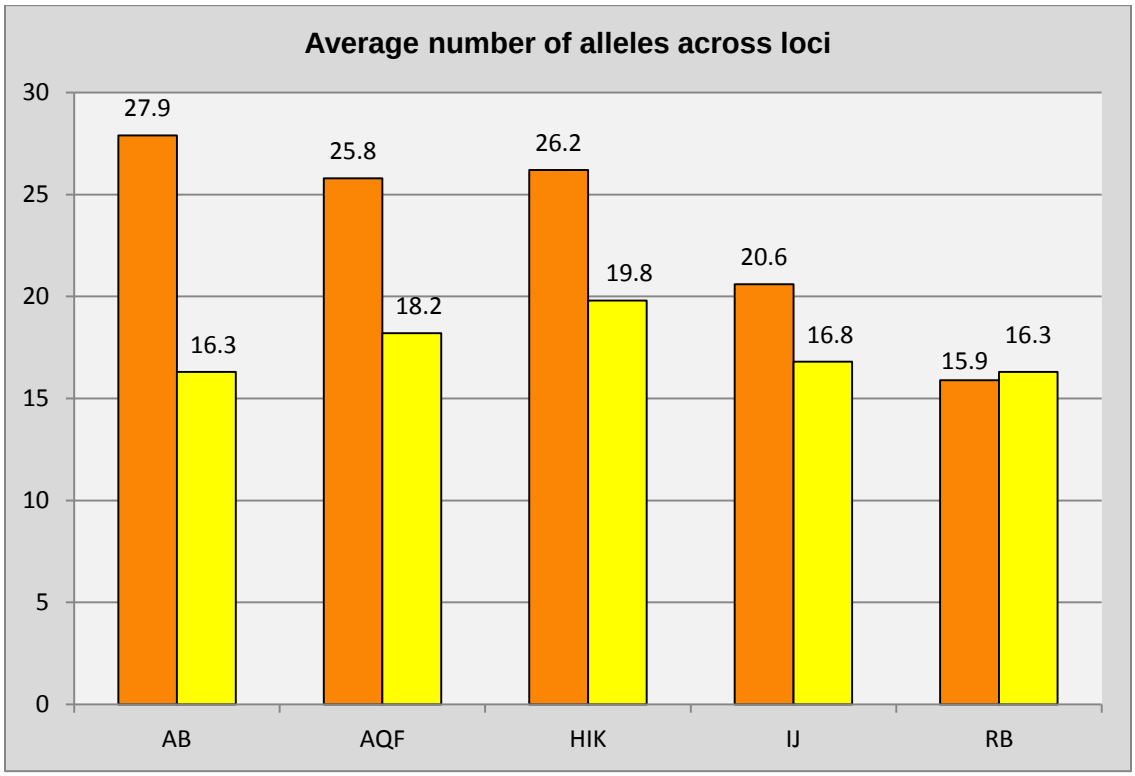


Figure 2: The average number of alleles per farm (Broodstock populations = Orange, Offspring populations = Yellow)

The loss of alleles from broodstock to offspring is visualised by means of a histogram in Figure 2. Although it seems as if all of the farms, with the exception of Roman Bay, show a loss of alleles from broodstock to offspring, only Abagold and Aquafarm had a significant loss ($p<0.05$), as determined by the Mann-Whitney test (Table 4). This loss corresponds to similar studies done in aquaculture species such as the Japanese flounder (*Paralichtys olivaceus*), Atlantic salmon (*Salmo salar*) and silver-lipped pearl oyster (*Pinctada maxima*) (Sekino *et al*., 2002; Horreo *et al*., 2008; Lind *et al*., 2009). A loss has also been seen in several abalone species including *H. discus hannai* and *H. kamtschatkana* (Li *et al*., 2004; Lemay and Boulding, 2009), and specifically *H. midae* (Evans *et al*., 2004). In another study done on *H. midae*, Slabbert *et al*. (2009) reported a significant loss for only one of the three studied

cohorts. This was ascribed to the differential contribution of that particular cohort's broodstock, with only 24% of the animals contributing during spawning.

Table 4: A comparison of the average number of alleles between the broodstock and pooled offspring populations of each farm by means of a Mann-Whitney test.

Population	*U*	p-value
AB Broodstock vs AB Offspring	119	0.004**
AQF Broodstock vs AQF Offspring	109	0.031*
HIK Broodstock vs HIK Offspring	99.5	0.116
I&J Broodstock vs I&J Offspring	98	0.138
RB Broodstock vs RB Offspring	66	0.755

* = $p<0.05$

** = $p<0.01$

Differential contribution reduces the effective number of breeding animals, which results in only a few animals founding the new population. This is known as a population bottleneck and can result in a loss of genetic diversity. Several studies have shown the differential contribution of broodstock animals to be the cause of a loss of genetic diversity in cultured populations (Boudry *et al*., 2002; Li *et al*., 2004; Horreo *et al*., 2008; Lemay and Boulding, 2009). In the current study, the broodstock populations of the farms that displayed a significant loss of alleles had a contribution of only 31.6% for Abagold and 37.1% for Aquafarm (Table 5). These values were less than those of the other farms, with I&J having the highest contribution of 63.9%. The low values of contribution could explain the loss of alleles seen in these populations. The loss of alleles without an accompanying loss of heterozygosity further supports the idea of a short-term population bottleneck (Nei *et al*., 1975).

Table 5: The number of broodstock animals of each farm that participated and contributed in the PRS spawning event.

	Abagold	Aquafarm	HIK	I&J	Roman Bay	Average
Number of broodstock animals participating in spawning event	95	89	104	36	23	69.40
Number of broodstock contributing in spawning event	30	33	48	23	13	29.40
Percentage of broodstock contributing in spawning event	31.6	37.1	46.2	63.9	56.5	47.0

Another explanation for this loss could be the effect of artificial selection. Because animals are selected based on one or more specific traits, in this instance growth, genotypes or alleles providing such an advantage will be preferentially selected. This can change the genetic composition of the cultured population, and may cause a shift in the allele frequency (see Table 2). Similarly, alleles that are not favourable in the cultured environment or have a negative effect on the particular trait will be selected against, causing a possible loss of alleles in the cultured population (see Table 2).

Population differentiation between the broodstock population and its corresponding pooled offspring population was determined with pairwise F_{ST}. Even though only the Abagold and Aquafarm offspring differed significantly from their parents in terms of number of alleles, all of the offspring populations showed significant population differentiation ($p<0.05$) with their corresponding broodstock populations. This indicates that although the genetic diversity levels of the broodstock and offspring populations of farms HIK, I&J and Roman Bay are similar or not significantly different, these farms do have genetically different compositions. This could be a result of artificial selection. By exposing animals to the artificial selection methods practiced on farms, the genetic composition of a population can be altered within a few generations. This can happen due to shifts in allele frequencies (Frankham, 2008), and is evident in the allele frequency data of this study (Table 2 and Appendix E). Another explanation could be adaptation to the local environment. Since different traits are favoured on the farms than in the wild, animals better suited for the cultured environment will have the best survival rate (Frankham 2008).

A loss of alleles and/or shifts in allele frequencies as a result of the differential contribution of the broodstock could also result in population differentiation between broodstock and offspring populations. This is especially true for populations in which some broodstock dominated the spawning event, since the genetic composition of the offspring population will more closely represent those broodstock animals than the entire broodstock population.

High levels of genetic diversity are vital for the success of enhancement programmes. This is necessary to ensure that all the desired traits are present to create an enhanced strain of cultured abalone (Koehn *et al*., 1988; a, 1995; Hill, 2000; Launey *et al*., 2001; Slabbert *et al*., 2009). A high level of diversity can also increase the ability of the population to resist diseases and allow easier adaptation to possible environmental changes (Gamfeldt and Kallstrom, 2007). A reduction in variability can have a negative effect on important traits such as fitness (Danzmann *et al*., 1989), because of the possible loss of alleles vital to mechanisms including larval survival and disease resistance. Two of the abalone farms in this study showed a significant reduction in genetic variability of the offspring while the remaining farms, although not significant, also displayed lower levels of diversity than the broodstock populations. This should be closely monitored to limit any further losses.

3.2.3.2 Broodstock versus wild

To determine if the broodstock animals used for the PRS were representative of wild populations, the genetic diversity levels and genetic differentiation of these populations were determined. The levels of genetic diversity, measured as allelic richness (A), the average number of alleles (N_a) as well as the observed (H_o) and expected (H_e) heterozygosities are indicated in Table 6. Similar values are seen for allelic richness and the average number of alleles for each population, with an average allelic richness of 16.3 and average number of alleles of 17.9 for the wild and an average allelic richness of 20.3 and average number of alleles of 21.3 for the broodstock populations. These values are much higher in the broodstock than that of the wild, and this is most likely due to the small sample sizes of the wild populations included in this study. The heterozygosity levels for the wild and broodstock populations are comparable to each other as well as to other studies done on *H. midae* (Ruivo, 2007; Van den Berg, 2008; Slabbert *et al*., 2009).

Table 6: The levels of genetic diversity of the individual wild and broodstock populations, measured in terms of allelic richness (*A*), average number of alleles across loci (N_a), and expected (H_e) and observed (H_o) heterozygosity.

		A	N_a	H_e	H_o
Wild	West	16.6	18.1	0.893	0.800
	East	16.0	17.8	0.879	0.778
	Average	**16.3**	**17.9**	**0.886**	**0.789**
Broodstock	Abagold	27.2	27.9	0.905	0.812
	Aquafarm	25.2	25.8	0.901	0.799
	HIK	25.7	26.2	0.908	0.817
	I&J	20.0	20.6	0.898	0.834
	RB	15.5	15.9	0.882	0.827
	Average	**20.3**	**21.3**	**0.894**	**0.807**

Population differentiation between the broodstock and wild populations was determined with pairwise F_{ST} (Table 7). Three of the five broodstock populations showed significant genetic differentiation with the wild population of the East coast, while no significant differentiation between the broodstock populations and the wild population of the West coast was seen. This was expected when considering that all of the broodstock animals were sourced from fishing zones B and C from the West coast as determined by the Department of Agriculture, Forestry and Fisheries of South Africa. This, together with the levels of genetic diversity, indicates that all of the broodstock populations used by the farms were representative of the wild population from where they were sourced. This supports the findings of Slabbert *et al*. (2009), who found no significant difference between the broodstock populations of Roman Bay and wild populations of *H*. *midae*.

Table 7: Pairwise F_{ST} p-values after adjustment for multiple comparisons (p<0.05) of the broodstock populations of each farm, compared to wild populations of the West and East coast. Significant values are indicated with an asterisk, (*).

	RB	AB	AQF	HIK	East	West
IJ	0.141	0.145	0.021	0.005	0.010	0.250
RB		0.179	0.505	0.276	0.012	0.376
AB			0.002*	0.002*	0.002*	0.074
AQF				0.002*	0.002*	0.850
HIK					0.002*	0.183
East						0.145

3.2.3.3 Mixed offspring versus wild

To assess whether it is possible to use the cultured animals currently on the farms as broodstock to establish ranching programmes in the Western Cape of South Africa; the genetic diversity and population differentiation of the mixed offspring and wild populations were determined. The levels of genetic diversity, measured as allelic richness (*A*), the average number of alleles (N_a) as well as the observed (H_o) and expected (H_e) heterozygosities are shown in Table 8. Similar values are seen for allelic richness and the average number of alleles for each population, with an average allelic richness of 16.3 and average number of alleles of 17.9 for the wild and average allelic richness of 19.2 and average number of alleles of 19.9 for the offspring populations, respectively. The values for the mixed offspring groups are higher than that of the wild populations, and this is most likely due to the small sample sizes of the wild populations included in this study. The heterozygosity levels for the wild and broodstock populations are comparable to each other as well as to previous studies on *H. midae* (Ruivo, 2007; Van den Berg, 2008; Slabbert *et al*., 2009).

Table 8: The levels of genetic diversity of the individual wild and mixed offspring populations, measured in terms of allelic richness (*A*), average number of alleles across loci (N_a), and expected (H_e) and observed (H_o) heterozygosity.

		A	N_a	H_e	H_o
Wild	West	16.6	18.1	0.893	0.800
	East	16.0	17.8	0.879	0.778
	Average	**16.3**	**17.9**	**0.886**	**0.789**
Offspring	Abagold	16.1	16.3	0.868	0.829
	Aquafarm	18.0	18.2	0.881	0.839
	HIK	19.3	19.8	0.885	0.820
	I&J	16.5	16.8	0.867	0.799
	RB	16.2	16.3	0.802	0.762
	Average	**19.2**	**19.9**	**0.882**	**0.808**

Population differentiation between the mixed offspring and wild populations was determined with pairwise F_{ST}. All five offspring populations differed significantly ($p<0.05$) from the East and West coast populations. This agrees with similar studies done on abalone. Hara and Sekino (2007b) observed significant population differentiation between hatchery and wild populations along with a substantial decrease in allelic diversity in *H. discus*. Similar results were seen in the study Lemay and Boulding (2009) on *H. kamtschatkana*. Evans *et al*. (2004) found cultured populations of *H. rubra* to be significantly differentiated from the wild populations, along with a decrease in genetic diversity. Although a similar decrease was seen for *H. midae* in that particular study, the cultured populations did not differ from the wild. This supports the observations of Slabbert *et al*. (2009) who found only one of the three cohorts studied to be significantly differentiated from the wild *H. midae* populations. This was attributed to a small number of contributing breeders in that particular cohort.

Because all of the broodstock populations used for the PRS were representative of the wild (see section 3.4.2.2), we expected the offspring populations to be similar to the wild. This is not the case, indicating that artificial selection or adaptation to the cultured environment could have resulted in the divergence of the cultured animals due to shifts in allele frequencies (Frankham, 2008). What is interesting is that most of the offspring populations differ from each other, with eight of the ten pairwise comparisons showing a significant difference. This suggests that the selection method and even the local environment of the farms could differ from each other (Adkison, 1995; Oetjen *et al*., 2010) but this will have to be verified by

comparing the distributed offspring of a particular farm. This study was not designed to investigate differences between farms, but could be an important aspect to look at in future.

Another phenomenon often associated with the cultured environment is the differential contribution of the broodstock animals during mass spawning events. A skewed contribution is often identified as the main cause for genetic differences between cultured and wild populations (Horreo *et al*., 2008; Lind *et al*., 2009; Slabbert *et al*., 2009) and is also a reason for concern in the current study. On average, only 47% of broodstock animals contributed to the offspring (Table 5).

The genetic differentiation between the cultured and wild populations suggests that if these farm animals were to be used for ranching or reseeding in the Western Cape environment, it could have a negative impact on the wild populations. The shifts in allele frequencies could alter the adaptation of the populations, since alleles that might be advantageous in one habitat could be less so in another (Tymchuk *et al*., 2007). It could also disrupt co-adapted gene complexes (Wallace, 1968). Such gene complexes occur when the interactions between certain loci result in an increased fitness. This disruption of gene complexes and the lack of adaptation to the wild environment could result in outbreeding depression, which is a concern for conservation biologists (Loeschke *et al*., 1994), resulting in a reduction in the fitness of a population. This is evident from several studies concerning adaptation in aquaculture species. Leider *et al*. (1990) found that the reproductive success of steelhead trout (*Oncorhynchus mykiss*) decreased significantly when returned to the wild. Other studies also show similar results of reduced fitness and reproductive success in fish (Fleming *et al*., 2000; McGinnity *et al*., 2003), as well as abalone (Schiel and Welden, 1987; Tegner and Butler, 1989), where a low survival rate is seen for hatchery-reared larvae that are released into the ocean.

3.3 Conclusion

It is important to distinguish between the requirements for a selective breeding programme and for conservation. For conservation purposes it is necessary that animals that are released into the ocean are representative of the wild populations. This is to prevent possible shifts in allele frequencies which could disrupt local adaptation of the wild populations (Tymchuck *et al*., 2007). It is therefore imperative that there should be no differentiation between the ranching and reseeding populations and wild populations. In this study, all of the broodstock populations were representative of the wild populations of the West coast. This can be expected, since this was the area where the broodstock animals were obtained from. We expected the offspring populations to have a similar genetic composition as the wild populations, since there was no significant differentiation between the broodstock and the wild. This was not the case, with all five mixed offspring populations showing significant differentiation from the wild populations. This was attributed to differential contribution of the broodstock, artificial selection and adaptation to the environment. Based on these results, the offspring populations currently on the farms are not suited for ranching or reseeding purposes in the Western Cape region.

To enhance a strain of animals by selecting for desired traits however, it is expected that allele frequencies will shift, since the target allele/s will become more prevalent in the selected populations. It is therefore expected that populations will differentiate when selection is employed. For both selective breeding and conservation purposes, it is however crucial to limit the loss of genetic diversity. This will ensure a variety of commercially important traits are available for selection (Koehn *et al*., 1988; Frankham, 1995a; Hill, 2000; Launey *et al*., 2001; Slabbert *et al*., 2009) as well as to ensure that wild populations can cope with possible environmental changes (Frankham, 1995a). When the levels of genetic diversity between broodstock populations and their corresponding offspring were investigated in this study, we found two offspring populations, Abagold and Aquafarm, displaying a significant loss in number of alleles. This was once again attributed to artificial selection, adaptation to the cultured environment and differential contribution of the broodstock. Although only two offspring populations showed a loss in genetic diversity, all five populations differed significantly from their corresponding broodstock population, as well as from each other.

The possible reasons for the loss of genetic diversity in this study, namely artificial selection, adaptation to the environment and the differential contribution of breeding animals are a result of management practices in the cultured environment. The results found in this study highlight the need for effective management of hatchery practices and the genetic monitoring of the breeding animals in order to limit the loss of genetic diversity.

Chapter 4:
Conclusions and future prospects

In 2006 a breeding programme for *H. midae* was established with the aim of utilising traditional selection as well as molecular tools for the enhancement of this species. This was a joint effort between Stellenbosch University, the South African government and five commercial abalone farms. This programme, known as the Performance Recording Scheme (PRS), was aimed at shortening the production times on abalone farms, by selecting for superior genotypes. This study utilised molecular tools to reconstruct families in order to identify unrelated animals that could be used for breeding purposes.

For this study PRS broodstock and offspring were typed using 12 microsatellite loci. The primary goal was to reconstruct pedigrees within the superior PRS stock and use this molecular and phenotypic data to select a diverse on-farm generation of broodstock, which could serve as a foundation for further domestication of this species. Additionally, we investigated whether standard selection practises based on phenotype alone influenced the genetic diversity of a population compared to genotypic selection. This data was also used to study the genetic diversity and differentiation within and between commercial and wild populations.

4.1 Parentage assignment and broodstock selection

The results of the pedigree reconstruction were unexpected. Only 43% of the animals could be assigned successfully, in stark contrast to the high success rates (82.2%; 90.5%; 92%) from previous studies in *H. midae* (Ruivo, 2007; Van den Berg, 2008; Slabbert *et al*., 2009). We theorised that non-experimental animals used to keep stocking densities consistent were selected by mistake. The PRS was running for four years and the chances of mixing groups were not unfounded, (results not given; part of an internal auditing study). FCA analyses supported this observation, but genotyping errors and mismatches during assignment could not be discounted.

The parentage data indicated that there was an uneven contribution during the PRS spawning event. Breeding animals that contributed poorly, or not at all, were identified and recommended to the farms as broodstock to be replaced. New breeding animals were selected to replace these broodstock and were selected based on genotypic traits. This genotypic selection was compared to the selection strategy currently used on the farms, which relies on phenotypic traits that are of commercial importance.

No significant population differentiation existed between most of the genotypic populations as determined by FCA analyses and pairwise F_{ST} tests. On the contrary, significant differentiation was seen for all of the phenotypic populations. The hypothetic simulation confirmed these results, since no differentiation was observed between the top 32 genotypic animals of each farm, whilst the populations selected at random differed significantly from each other. It was also established that it is possible to maintain the levels of genetic diversity in genotypically selected populations. The same could not be said for the random populations, since a lower number of alleles were observed for these populations when compared to the genotypic groups.

4.2 Comparison of genetic diversity between wild and cultured populations

All five broodstock populations were representative of the wild populations from where they were sourced, with no significant differentiation between the broodstock and West coast populations. The levels of genetic diversity present at the start of the PRS were comparable to the wild with a higher number of alleles for the cultured populations.

When the offspring populations were compared with their corresponding broodstock, only two farms, Abagold and Aquafarm, displayed a significant loss in genetic diversity. This was attributed to poor broodstock contribution during the spawning event, since the broodstock of these farms had the lowest contribution values of 32% and 37% respectively. It should be noted that, although not significant, all of the farms, with the exception of Roman Bay, showed a loss in number of alleles. The similar values for the broodstock and offspring populations of Roman Bay were ascribed to missing broodstock animals that could not be genotyped. The

loss seen in the HIK and I&J populations might not be significant in this study, but warns of the possible danger if the number of alleles were to continue to decrease for a few generations. Apart from the differential contribution of the broodstock, artificial selection was also proposed as a possible reason for this loss. Even though Abagold and Aquafarm were the only farms where the offspring populations differed significantly from their parents in terms of number of alleles, all of the offspring populations showed significant population differentiation with their corresponding broodstock populations. The differential contribution of broodstock animals and the effect of artificial selection were once again suggested as reasons for this differentiation between populations. Local adaptation was also suggested as a possible cause for this differentiation.

All of the mixed offspring populations differed significantly from both the West and East coast wild populations. This differentiation between cultured and wild populations suggests that if these farm animals were to be used for ranching or reseeding in the Western Cape environment, it could have a negative impact on the wild populations.

4.3 Future prospects

This study showed that genetic technologies can be very useful for any breeding programme or aquaculture industry in a pre-domestication phase. Genotypic selection is a more focussed means of selection as seen in the greater structure of these selected animals compared to the random and phenotypic selection. Beneficial alleles can therefore be fixed much earlier and effectively. The genetic diversity of early stage, single trait breeding schemes can be monitored and managed until a domesticated line has been successfully established. When ranching and reseeding become practical realities, genetics will play a major role to monitor and identify stocks for such undertakings, both on conservation level as well as commercial level.

The results found in this study highlight the need for the effective management of hatchery practices and the genetic monitoring of the breeding animals. The academic sector is in the perfect position to act as a support structure to assist with this. This study successfully employed microsatellite markers to evaluate and support a breeding programme for *H. midae*.

This served as an indication of how molecular tools can be applied for the benefit of the aquaculture industry. Based on this study there are several directions and areas of research that can be explored in future.

One of these is the comparison of broodstock that dominated the spawning event and those that contributed poorly. These animals should be monitored over several spawnings to determine if their contributions are consistent. Once the relevant animals are identified they can be compared in terms of parameters possibly influencing contribution (Boudry *et al*., 2002; Brown *et* al., 2005; Frost *et* al., 2006; Lind *et al*., 2009). These include animal age, sperm and egg quality, larval quality and other physiological (stress response) and genomic (gene expression) factors. Breeding animals that consistently outperform the other abalones can then be crossed preferentially to produce offspring with a probable growth advantage.

Another aspect that can be investigated is the difference in management practices between farms and what the possible impact on the genetic composition of a population would be. Although being spawned from the same broodstock group, the mixed offspring populations in this study differed significantly from each other, suggesting that the selection method and even the local environment of the farms could differ from each other. A possible link between the environment and population differentiation can therefore be investigated. This use of genotypic data to investigate how cultured populations differ from the wild can be supported by other analytical disciplines, such as transcriptomics (Vandersteen *et al*., 2010) and gene expression studies (Larsen *et al*., 2007).

Apart from assisting the aquaculture industry, molecular tools are also of great importance in conservation. Although this study indicates that the populations studied were not suitable for ranching or reseeding purposes, the idea of using cultured animals to replenish wild stocks should not be disregarded. This study only evaluated one population per farm. It is possible that other cultured populations will have similar allele frequencies to that of the wild and will consequently not pose a threat to the wild populations. The genetic profiles of these animals will therefore have to be evaluated before reintroducing them to the wild. The feasibility of using genotypic data for this should be explored, both in terms of cost and practicality.

This study has demonstrated the value of microsatellite markers in breeding programmes and serves as an example of how genetic tools can assist the aquaculture industry.

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APPENDIX A

MEASUREMENT DATA

Abagold

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	PRS 9F	10.4	7.2	196	Female
-	PRS 78	10.1	6.9	177	Female
-	PRS 6F	10.1	7.1	203	Female
-	PRS 87	10.1	6.5	162	Female
-	PRS 4F	10.0	7.0	195	Female
-	PRS 69	10.0	7.0	194	Female
-	PRS 72	10.0	7.1	195	Female
-	PRS 14	9.9	7.2	185	Male
-	PRS 82	9.7	6.8	180	Male
-	PRS 3	9.7	7.1	184	Female
-	PRS 5F	9.7	7.4	202	Female
-	PRS 68	9.5	6.8	172	Female
-	PRS 80	9.5	6.6	154	Female
-	PRS 30	9.4	6.6	169	Male
-	PRS 96	9.4	6.6	145	Female
-	PRS 64	9.4	7.1	173	Female
-	PRS 63	9.4	6.7	148	Female
-	PRS 70	9.4	6.3	149	Female
-	PRS 85	9.4	6.7	132	Female
-	PRS 42	9.3	6.6	145	Male
-	PRS 111	9.3	6.7	139	Male
-	PRS 33	9.3	6.8	177	Male
-	PRS 59	9.3	6.9	160	Female
-	PRS 1	9.3	6.1	95	Female
-	PRS 10F	9.3	7.0	142	Female
-	PRS 93	9.3	6.6	159	Female
-	PRS 25	9.2	6.2	144	Male
-	PRS 44	9.2	6.6	149	Male
-	PRS 106	9.2	6.2	133	Female
-	PRS 60	9.2	6.6	163	Female
-	PRS 71	9.2	6.5	146	Female
-	PRS 90	9.2	6.4	154	Female
-	PRS 39	9.1	6.4	153	Male
-	PRS 41	9.1	6.4	132	Male
-	PRS 29	9.1	6.2	146	Male
-	PRS 7F	9.1	6.6	142	Female
-	PRS 100	9.1	6.2	130	Female
-	PRS 95	9.1	6.3	151	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	PRS 109	9.1	6.3	142	Female
-	PRS 79	9.1	6.6	133	Female
-	PRS 40	9.0	6.5	143	Male
-	PRS 84	9.0	6.5	143	Male
-	PRS 105	9.0	6.7	146	Female
-	PRS 10	9.0	6.5	149	Female
-	PRS 8F	9.0	6.5	151	Female
-	PRS 97	9.0	6.3	125	Female
-	PRS 89	9.0	6.2	152	Female
-	PRS 62	9.0	6.8	145	Female
-	PRS 43	8.9	6.3	151	Male
-	PRS 45	8.9	6.7	141	Male
-	PRS 68	8.9	6.6	138	Female
-	PRS 83	8.9	6.6	134	Female
-	PRS 99	8.9	6.3	133	Female
-	PRS 67	8.9	6.3	129	Female
-	PRS 81	8.9	6.2	132	Female
-	PRS 26	8.8	6.3	134	Male
-	PRS 52	8.8	6.2	136	Male
-	PRS 36	8.8	6.2	137	Male
-	PRS 51	8.8	6.4	139	Male
-	PRS 61	8.8	5.7	130	Female
-	PRS 91	8.8	6.2	141	Female
-	PRS 50	8.7	6.1	133	Male
-	PRS 47	8.7	6.3	122	Male
-	PRS 32	8.7	6.4	146	Male
-	PRS 75	8.7	6.5	138	Female
-	PRS 88	8.7	6.3	146	Female
-	PRS 110	8.7	6.2	120	Female
-	PRS 65	8.7	6.2	112	Female
-	PRS 98	8.7	6.1	131	Female
-	PRS 15	8.6	6.0	118	Male
-	PRS 102	8.6	5.9	122	Female
-	PRS 66	8.6	6.2	115	Female
-	PRS 30	8.5	5.6	110	Male
-	PRS 31	8.5	6.4	115	Male
-	PRS 37	8.3	6.0	121	Male
-	PRS 103	8.3	5.6	110	Female
-	PRS 55	8.2	5.6	115	Male
-	PRS 54	8.2	5.3	102	Male
-	PRS 53	8.2	5.7	110	Male

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	PRS 76	8.2	5.8	110	Female
-	PRS 104	8.2	6.1	117	Female
-	PRS 57	8.1	5.8	102	Male
-	PRS 21	8.1	5.5	117	Male
-	PRS 38	8.1	5.8	115	Male
-	PRS 92	8.1	5.3	105	Female
-	PRS 107	8.0	6.0	117	Female
-	PRS 48	7.8	5.1	90	Male
-	PRS 46	7.6	5.5	98	Male
-	PRS 49	7.4	5.0	87	Male
-	PRS 28	7.4	5.3	89	Male
-	PRS 16	7.4	5.1	77	Male
-	PRS 22	7.2	5.6	99	Male
-	PRS 56	6.6	4.8	87	Male

Aquafarm

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	A328	11.5	8.4	234	Female
-	B209	11.4	8.2	255	Male
-	A259	11.3	8.3	239	Female
-	A241	11.2	7.9	222	Female
-	A336	11.2	8.1	204	Female
-	B259	11.2	8.4	240	Male
-	B212	11.1	7.8	252	Male
-	A213	11.0	7.7	233	Female
-	A277	11.0	8.0	202	Female
-	A322	11.0	7.9	212	Female
-	A23	10.9	7.5	212	Female
-	B201	10.9	7.9	250	Male
-	B257	10.9	80	208	Male
-	B210	10.9	7.7	210	Male
-	B240	10.9	8.0	256	Male
-	B208	10.8	8.3	231	Male
-	A221	10.7	7.9	206	Female
-	B218	10.7	7.4	218	Male
-	B226	10.7	7.7	237	Male
-	A260	10.7	7.9	229	Female
-	A252	10.7	7.4	218	Female
-	B245	10.7	8.1	236	Male
-	A220	10.6	7.2	223	Female
-	A280	10.6	7.5	215	Female
-	A315	10.6	7.6	186	Female
-	B219	10.6	7.8	219	Male
-	B256	10.6	8.0	215	Male
-	A235	10.5	7.5	195	Female
-	B207	10.5	7.9	235	Male
-	A316	10.5	7.5	186	Female
-	B238	10.5	7.8	238	Male
-	B252	10.5	7.7	230	Male
-	A234	10.4	6.9	195	Female
-	A285	10.4	7.5	200	Female
-	A301	10.4	7.7	190	Female
-	A304	10.4	7.6	230	Female
-	A302	10.4	7.4	206	Female
-	B220	10.4	7.4	188	Male

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	B227	10.4	7.4	191	Male
-	A240	10.4	7.5	212	Female
-	A320	10.4	8.0	192	Female
-	A332	10.4	7.2	187	Female
-	A317	10.4	7.3	171	Female
-	B265	10.4	7.9	228	Male
-	B233	10.3	7.2	194	Male
-	B234	10.3	7.3	219	Male
-	B232	10.3	7.5	172	Male
-	B241	10.3	7.6	207	Male
-	B244	10.3	7.5	194	Male
-	B236	10.3	7.5	204	Male
-	B258	10.3	7.4	194	Male
-	A209	10.2	7.3	188	Female
-	B222	10.2	7.2	183	Male
-	A243	10.2	7.2	184	Female
-	A298	10.2	6.9	190	Female
-	B263	10.2	7.4	192	Male
-	B251	10.2	7.5	202	Male
-	B266	10.2	7.4	185	Male
-	A232	10.1	7.4	196	Female
-	A303	10.1	7.2	188	Female
-	B231	10.1	7.7	212	Male
-	A268	10.1	7.3	180	Female
-	A253	10.1	7.3	183	Female
-	A314	10.1	7.3	155	Female
-	A342	10.1	7.7	190	Female
-	B255	10.1	7.4	198	Male
-	A230	10.00	7.6	214	Female
-	A211	10	7.6	201	Female
-	A257	10.0	7.1	167	Female
-	A247	10.0	7.5	202	Female
-	A275	10.0	7.4	198	Female
-	B223	10.0	7.6	179	Male
-	A311	10.0	6.9	166	Female
-	B267	10.0	7.1	168	Male
-	A323	10.0	7.0	167	Female
-	A339	10.0	7.0	167	Female
-	A341	10.0	7.2	207	Female
-	B202	10.0	7.0	183	Male
-	B202	10.0	7.0	180	Male

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	B204	10.0	7.3	170	Male
-	B215	10.0	7.1	170	Male
-	A222	9.9	7.0	183	Female
-	A337	9.9	7.6	191	Female
-	A278	9.9	7.0	164	Female
-	A276	9.9	7.4	180	Female
-	B230	9.9	7.0	207	Male
-	A262	9.9	6.9	180	Female
-	A258	9.9	7.6	185	Female
-	B237	9.9	7.1	162	Male
-	A225	9.8	6.8	174	Female
-	A308	9.8	7.0	124	Female
-	A306	9.8	7.0	168	Female
-	A305	9.8	6.7	146	Female
-	B229	9.8	7.1	167	Male
-	A334	9.8	7.2	179	Female
-	A340	9.8	7.0	167	Female
-	B216	9.8	7.6	181	Male
-	B239	9.8	6.9	154	Male
-	B264	9.8	6.9	192	Male
-	B253	9.8	7.2	193	Male
-	A208	9.7	6.9	146	Female
-	A282	9.7	7.2	174	Female
-	A288	9.7	7.2	155	Female
-	A274	9.7	7.1	177	Female
-	B217	9.7	6.8	151	Male
-	B270	9.7	7.2	186	Male
-	A255	9.7	7.2	159	Female
-	A291	9.7	7.7	187	Female
-	A296	9.7	6.9	174	Female
-	B269	9.7	7.0	177	Male
-	A324	9.7	7.0	162	Female
-	B225	9.7	7.1	189	Male
-	B260	9.7	6.9	178	Male
-	A233	9.6	6.8	178	Female
-	A206	9.6	6.9	155	Female
-	A216	9.6	6.8	163	Female
-	A281	9.6	7.1	155	Female
-	A219	9.6	6.8	158	Female
-	A218	9.6	6.8	146	Female
-	A251	9.6	6.8	173	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	A333	9.6	6.8	167	Female
-	A299	9.6	7.1	182	Female
-	A239	9.6	7.5	160	Female
-	B254	9.6	6.9	171	Male
-	B250	9.6	7.8	186	Male
-	A224	9.5	6.8	165	Female
-	A212	9.5	7.1	155	Female
-	A201	9.5	6.9	173	Female
-	A231	9.5	6.8	189	Female
-	A202	9.5	6.7	149	Female
-	A289	9.5	6.4	144	Female
-	A244	9.5	6.0	107	Female
-	B221	9.5	7.2	180	Male
-	B228	9.5	6.9	167	Male
-	A249	9.5	6.5	162	Female
-	A267	9.5	7.0	177	Female
-	A294	9.5	6.9	147	Female
-	A293	9.5	7.0	169	Female
-	A313	9.5	7.0	148	Female
-	A335	9.5	6.6	162	Female
-	A321	9.5	7.7	153	Female
-	B205	9.5	6.7	138	Male
-	B246	9.5	6.5	157	Male
-	B243	9.5	7.3	184	Male
-	B268	9.5	6.8	158	Male
-	A207	9.4	6.8	152	Female
-	A223	9.4	7.1	148	Female
-	A307	9.4	6.6	133	Female
-	A309	9.4	6.7	143	Female
-	A248	9.4	6.7	152	Female
-	A256	9.4	6.7	138	Female
-	A279	9.4	7.0	158	Female
-	B213	9.4	6.8	158	Male
-	B203	9.4	7.0	180	Male
-	A263	9.4	6.6	137	Female
-	A292	9.4	6.3	147	Female
-	A300	9.4	6.7	144	Female
-	B262	9.4	6.9	169	Male
-	A330	9.4	6.9	146	Female
-	A236	9.4	6.5	147	Female
-	B211	9.4	6.8	164	Male

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	B247	9.4	6.5	146	Male
-	A228	9.3	6.8	156	Female
-	A250	9.3	6.5	164	Female
-	A286	9.3	6.8	128	Female
-	A297	9.3	6.7	141	Female
-	B261	9.3	6.7	160	Male
-	A325	9.3	6.4	125	Female
-	A271	9.2	7.0	166	Female
-	A215	9.2	6.5	147	Female
-	A273	9.2	6.6	149	Female
-	A242	9.2	6.4	138	Female
-	B224	9.2	6.5	143	Male
-	B242	9.2	6.5	152	Male
-	A204	9.1	6.3	139	Female
-	A283	9.1	6.7	127	Female
-	A284	9.1	6.3	130	Female
-	A272	9.1	6.9	154	Female
-	A238	9.1	6.6	138	Female
-	A261	9.1	6.2	145	Female
-	A266	9.1	6.2	135	Female
-	A287	9.1	6.5	148	Female
-	A318	9.1	7.0	155	Female
-	B249	9.1	6.7	125	Male
-	A214	9	6.5	154	Female
-	A210	9.0	6.5	138	Female
-	A226	9.0	6.3	148	Female
-	A310	9.0	6.7	118	Female
-	A237	9.0	6.4	136	Female
-	A254	9.0	6.4	141	Female
-	A264	9.0	6.5	139	Female
-	A319	9.0	6.5	140	Female
-	A312	9.0	6.5	137	Female
-	A290	8.9	6.1	135	Female
-	A295	8.9	6.2	125	Female
-	A245	8.8	6.3	132	Female
-	A265	8.8	9.4	160	Female
-	A227	8.7	6.3	119	Female
-	B206	8.7	7.0	159	Male
-	A269	8.6	6.5	122	Female
-	B235	8.6	6.8	136	Male
-	A270	8.5	6.3	135	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	A326	8.4	6.2	112	Female
-	A331	8.4	5.5	104	Female
-	A327	8.3	6.4	127	Female
-	A329	8.0	5.8	95	Female

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Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
Gr. 6	-	11.4	7.7	214	Male
Y.49	431	11.3	7.9	240	Male
Gr. 16	-	10.9	7.2	204	Female
Gr.19	280	10.9	7.1	155	Female
Y.18	580	10.9	7.3	191	Male
Y.86	-	10.9	7.3	233	Female
Y.91	569	10.9	7.1	227	Male
R.6	358	10.9	7.4	187	Female
Gr. 5	366	10.8	6.8	174	Male
R.59	573	10.8	6.9	157	Male
Gr.62	509	10.8	7.8	180	Female
Gr.65	-	10.8	8.1	213	Male
Gr.74	566	10.8	7.8	178	Male
Gr.77	500	10.8	7.7	188	Female
Y.13	497	10.8	7.7	174	Male
Y.39	461	10.8	6.1	132	Male
Gr. 9	343	10.7	6.5	171	Female
Gr.60	442	10.7	6.9	169	Male
Gr.63	450	10.7	6.8	140	Female
Y.12	-	10.6	7.6	197	Female
Y.21	418	10.6	6.1	180	Male
Y.83	-	10.6	6.3	127	Female
R.86/W.53	503	10.6	7.9	186	Female
Gr. 7	389	10.5	7.7	201	Female
Gr.28	395	10.5	7.3	184	Female
Gr.54	427	10.5	7.2	161	Female
Gr.55	522	10.5	7.7	185	Female
Y.35	465	10.5	7.8	211	Female
Y.36	457	10.5	7.7	134	Female
W.28	486	10.5	7.0	185	Male
Gr. 8	385	10.4	7.1	194	Female
Gr.31	349	10.4	7.2	172	Female
Gr.72	481	10.4	6.7	157	Male
Y.2	598	10.4	7.4	210	Male
W.90	426	10.4	7.1	190	Female
W.8	441	10.4	6.8	169	Female
Gr. 1	371	10.3	6.9	156	Male
Gr. 12	353	10.3	6.9	183	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
B. 6	-	10.3	7.5	170	Male
Gr.17	542	10.3	7.4	186	Male
Gr.27	313	10.3	7.6	117	Male
Gr.37	-	10.3	6.7	154	Male
Gr.49	572	10.3	7.3	187	Female
Gr.50	406	10.3	7.3	176	Male
Gr.58	479	10.3	7.1	167	Male
Gr.69	588	10.3	7.1	159	Female
Gr.75	412	10.3	6.9	160	Male
Y.32	502	10.3	6.9	180	Male
Y.50	491	10.3	7.2	189	Female
Y.62	423	10.3	7.3	208	Female
W.52	398	10.3	7.2	165	Female
R.83	DEAD	10.3	7.2	178	Male
W.72	391	10.3	7.5	191	Male
Gr. 11	374	10.2	7.5	192	Male
Gr.24	365	10.2	7.3	165	Female
Gr.35	-	10.2	7.2	161	Male
Gr.36	-	10.2	7.4	174	Male
Gr.66	411	10.2	7.1	158	Male
Y.3	446	10.2	7.1	197	Male
Y.11	543	10.2	7.1	155	Female
Y.15	492	10.2	7.3	177	Male
Y.31	532	10.2	6.7	169	Male
Y.65	403	10.2	7.3	167	Male
Y.79/B.54	459	10.2	7.3	185	Male
B.62	495	10.2	7.1	151	Male
W.83	356	10.2	7.2	149	Female
Gr.40	513	10.1	7.8	186	Male
Gr.26	394	10.1	7.2	158	Male
Gr.64	584	10.1	7.7	198	Female
Gr.68	592	10.1	7.2	162	Male
Gr.76	466	10.1	7.3	171	Male
Y.14	478	10.1	7.4	156	Female
W.1	539	10.1	6.9	149	Female
W.50	373	10.1	7.2	169	Female
W.51	339	10.1	7.1	160	Female
B.34	323	10.1	7.4	160	Female
W.62	348	10.1	7.0	163	Male
B.1	548	10.0	7.2	174	Female
Y.30	550	10.0	7.2	169	Female
Y.58	678	10.0	7.8	168	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
W.33	599	10.0	6.9	171	Male
W.54	322	10.0	6.9	149	Female
Gr.44	401	9.9	7.3	158	Female
Gr.46	-	9.9	6.8	169	Female
Gr.52	425	9.9	6.6	156	Female
Y.16	579	9.9	6.9	162	Male
Y.10	473	9.9	7.0	173	Male
R.52	474	9.9	7.1	169	Male
Y.43	537	9.9	6.7	173	Female
Y.75	555	9.9	6.7	172	Male
Y.84	476	9.9	6.5	164	Male
Y.95	-	9.9	6.6	141	Female
W.15	437	9.9	6.8	158	Female
W.17/W.83	545	9.9	6.9	153	Female
Gr.42/W.36	546	9.9	6.9	153	Female
W.75	368	9.9	6.8	141	Male
W.84	-	9.9	7.1	185	Male
Gr.42	507	9.9	6.9	171	Female
Gr. 3	370	9.8	6.9	165	Female
Gr. 4	325	9.8	7.1	178	Female
Gr. 10	397	9.8	7.1	158	Male
Gr.25	369	9.8	6.4	119	Male
Gr.38	332	9.8	7.1	147	Male
R.16	307	9.8	7.1	151	Male
Gr.41	540	9.8	6.8	157	Female
Gr.56	-	9.8	6.7	152	Male
Gr.70	581	9.8	6.8	145	Male
Gr.71	470	9.8	6.9	145	Male
Gr.73	557	9.8	6.5	135	Male
Gr.82	562	9.8	7.4	144	Male
Y.23	407	9.8	7.1	164	Female
Y.26	420	9.8	7.3	192	Male
Y.33	448	9.8	6.7	150	Female
Y.51	514	9.8	6.8	169	Female
Y.72	422	9.8	7.0	154	Male
Y.73	421	9.8	6.7	155	Male
W.7	464	9.8	6.8	149	Male
W.10	480	9.8	7.0	168	Male
W.19	491	9.8	6.7	166	Male
W.46	-	9.8	6.8	139	Female
R.82	547	9.8	6.6	138	Female
W.61	390	9.8	6.8	150	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
W.64	-	9.8	6.6	152	Female
W.68	333	9.8	6.7	160	Male
B.27	-	9.8	7.0	170	Female
Gr.30	338	9.7	6.9	144	Male
Gr.51	528	9.7	7.1	172	Male
Gr.79	471	9.7	6.7	137	Female
Y.1	443	9.7	6.8	159	Female
Y.19	488	9.7	7.1	158	Female
Y.22	-	9.7	7.3	181	Male
Y.27	536	9.7	7.2	164	Male
Y41	552	9.7	6.7	167	Female
Y.63	430	9.7	7.1	153	Female
Y.66	-	9.7	6.7	160	Female
Y.80	531	9.7	6.8	165	Male
Y.89	451	9.7	6.9	158	Female
Y.93	416	9.7	6.4	153	Female
W.12	408	9.7	7.0	167	Male
W.26	436	9.7	6.8	143	Female
W.66	372	9.7	6.8	168	Male
W.71	354	9.7	6.8	142	Female
Gr. 15	396	9.6	6.8	158	Female
Gr.29	308	9.6	6.7	146	Female
Gr.32	283	9.6	6.7	132	Female
Gr.59	554	9.6	6.8	152	Male
B.64	-	9.6	7.1	175	Female
Gr.81	-	9.6	6.7	136	Female
Y.6	592	9.6	6.7	168	Female
Y.64	600	9.6	6.6	178	Male
Y.85	475	9.6	-	127	Male
W.3	733	9.6	6.5	157	Male
W.6	560	9.6	6.9	167	Male
W.20/Gr	593	9.6	6.6	173	Female
R.88	483	9.6	6.9	165	Female
R.43	326	9.6	6.6	148	Male
W.77	383	9.6	6.6	150	Male
W.85	340	9.6	6.9	140	Male
Gr. 14	315	9.5	6.6	136	Male
Gr.34	334	9.5	5.9	107	Male
Gr.57	440	9.5	6.7	152	Male
Gr.80	489	9.5	7.3	152	Female
Y.5	574	9.5	6.6	137	Female
Y.8	571	9.5	6.7	144	Male

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
Y.9	487	9.5	6.7	155	Female
Gr.38/Y.17	404	9.5	6.3	170	Male
Y.56	468	9.5	6.7	168	Male
W.30	517	9.5	6.6	158	Female
W.40	-	9.5	6.7	144	Male
W.70	318	9.5	6.8	141	Male
W.81/Gr.17	376	9.5	6.8	137	Female
Gr.2	381	9.4	7.1	169	Female
Gr. 13	-	9.4	6.5	138	Male
Gr.20	400	9.4	6.7	140	Male
Gr.22	357	9.4	6.5	126	Male
B.2	320	9.4	6.9	143	Female
Gr.61	516	9.4	8.8	121	Male
Gr.83	597	9.4	6.4	124	Female
Y.38	463	9.4	6.1	138	Female
Y.54	538	9.4	7.0	142	Female
Y.77	504	9.4	6.9	148	Female
Y.96	428	9.4	6.8	128	Male
W.11	591	9.4	6.0	129	Female
W.37	405	9.4	6.6	108	Female
W.39	419	9.4	6.9	148	Male
W.55	335	9.4	6.9	142	Female
W.58	309	9.4	6.6	147	Male
Gr.21	-	9.3	6.6	126	Male
Gr.45	586	9.3	6.4	132	Male
Gr.47	544	9.3	6.6	108	Male
Y.4	402	9.3	6.5	141	Female
Y.29	511	9.3	6.6	135	Female
Y.46	515	9.3	6.3	136	Female
Y.59	452	9.3	6.6	141	Female
R.70	472	9.3	6.8	134	Male
Y.78	447	9.3	6.2	142	Female
Y.82	583	9.3	6.8	149	Male
Y.90	458	9.3	6.6	160	Male
Y.99	553	9.3	6.3	109	Male
W.14	582	9.3	6.2	143	Male
W.27	485	9.3	6.2	143	Male
W.48	375	9.3	6.2	131	Male
W.57	347	9.3	6.4	117	Male
B.57	663	9.3	6.7	153	Male
W.73	399	9.3	6.6	133	Female
W.80	341	9.3	6.7	148	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
Gr.18	345	9.2	6.8	128	Female
Gr.33	321	9.2	6.1	119	Male
Gr.53	568	9.2	6.7	156	Male
B.97	505	9.2	6.2	126	Female
Gr.78	549	9.2	6.2	118	Female
R.55	527	9.2	6.8	165	Male
Y.20	410	9.2	6.8	161	Male
Y.25	-	9.2	6.7	158	Male
Y.34	-	9.2	6.5	134	Female
Y.37	414	9.2	6.4	125	Male
Y.45	508	9.2	6.4	146	Male
R.1	-	9.2	6.7	172	Male
Y.70	576	9.2	6.5	142	Female
Y.87/Gr.50	570	9.2	6.2	135	Male
Y.97	518	9.2	6.8	135	Male
W.2	565	9.2	6.8	142	Male
W.5	535	9.2	5.9	114	Male
W.9	506	9.2	6.7	141	Male
W.49	319	9.2	6.2	125	Female
Y.48	561	9.1	6.5	136	Male
Y.61	429	9.1	6.4	142	Male
Y.67	484	9.1	6.4	127	Female
W.16	417	9.1	6.5	127	Female
W.21	530	9.1	6.3	119	Female
W.41	559	9.1	6.6	137	Male
W.60	362	9.1	7.1	170	Male
W.59	312	9.1	6.5	133	Female
Gr.62/W86	384	9.1	6.0	114	Female
B.53	-	9.0	6.3	104	Female
Y.7	563	9.0	6.1	107	Female
Y.42	493	9.0	6.4	133	Female
W.29	590	9.0	6.2	120	Female
W.31	594	9.0	6.9	128	Male
W.45	-	9.0	5.9	118	Female
W.47	379	9.0	6.4	118	Male
W.56	352	9.0	6.4	115	Male
W.65	329	9.0	6.6	127	Male
Gr.43	510	8.9	6.5	158	Female
Gr.67	424	8.9	6.3	116	Male
Y.24	494	8.9	6.5	114	Male
Y.69	456	8.9	6.6	126	Male
Y.71	449	8.9	6.4	117	Male

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
Y.74	-	8.9	6.7	133	Female
W.44	361	8.9	5.9	104	Female
W.67	DEAD	8.9	6.6	138	Male
W.74	314	8.9	6.4	104	Male
Gr.48	482	8.8	6.1	112	Female
B.54	477	8.8	5.4	111	Female
W.4	360	8.8	6.4	111	Female
W.13	577	8.8	6.1	116	Male
W.22	432	8.8	6.4	117	Male
W.23	526	8.8	6.0	129	Female
W.38	498	8.8	6.2	101	Female
W.43	499	8.8	6.0	106	Female
W.69	392	8.8	6.3	113	Male
W.79	684	8.8	6.1	122	Male
Gr.39	328	8.7	6.1	174	Male
Y.44	409	8.7	6.2	113	Female
Y.76	462	8.7	6.1	120	Male
Y.81	521	8.7	6.2	114	Female
Y.88	525	8.7	6.1	132	Female
Y.94	330	8.7	5.8	93	Female
W.25	556	8.7	6.1	105	Female
W.34	-	8.7	5.8	125	Male
W.42	524	8.7	6.3	115	Male
W.76	337	8.7	5.6	101	Female
Y47	-	8.6	6.2	106	Female
Y.55	460	8.6	6.2	126	Male
Y.68	512	8.6	6.1	107	Male
W.78	-	8.6	6.5	122	Male
Gr.23	317	8.5	6.2	104	Female
Y.28	578	8.5	6.2	119	Male
Gr.21/Y.92	-	8.5	6.9	116	Female
W.24	534	8.5	5.5	101	Female
Y.53	435	8.4	5.9	109	Male
Gr.33/Y.52	558	8.4	5.5	114	Male
Y.57	585	8.4	5.9	109	Female
R.90	587	8.4	6.4	128	Male
W.35	454	8.4	6.2	101	Male
Y.98	523	8.3	5.8	102	Female
W.32	575	8.2	5.9	104	Male
Y.40	520	8.1	5.7	95	Male
W.82	324	8.1	5.6	94	Female
Y.60	551	7.9	5.5	84	Female

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Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	F53	13.3	11.2	461	Female
-	F52	13.0	10.8	454	Female
-	F41	12.8	9.5	348	Female
-	F79	12.6	9.1	352	Female
-	F31	12.5	9.7	330	Female
-	M2	12.5	9.2	304	Male
-	F81	12.3	9.8	359	Female
-	F40	12.2	9.1	321	Female
-	F63	12.2	9.3	354	Female
-	M11	12.2	9.3	329	Male
-	M14	12.2	8.3	320	Male
-	F97	12.1	9.5	306	Female
-	F88	12.1	9.1	332	Female
-	F64	12.0	8.9	288	Female
-	M4	12.0	8.9	281	Male
-	F10	11.9	8.6	340	Female
-	F6	11.9	9.1	324	Female
-	F29	11.9	8.8	296	Female
-	M10	11.8	9.3	327	Male
-	M20	11.8	9.2	302	Male
-	F69	11.7	8.5	297	Female
-	M3	11.7	9.6	283	Male
-	F2	11.6	8.9	287	Female
-	F8	11.6	8.9	274	Female
-	F16	11.6	8.9	284	Female
-	F47	11.6	8.8	282	Female
-	F68	11.6	8.2	273	Female
-	F101	11.6	9.0	293	Female
-	M17	11.6	8.9	298	Male
-	F70	11.5	9.0	299	Female
-	F54	11.5	8.6	295	Female
-	M26	11.5	9.0	307	Male
-	M7	11.5	8.2	282	Male
-	F25	11.4	8.4	270	Female
-	F110	11.4	8.6	293	Female
-	F44	11.4	8.8	287	Female
-	F60	11.4	9.3	289	Female
-	F55	11.4	8.4	277	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	F56	11.4	8.4	256	Female
-	M15	11.4	8.7	306	Male
-	M12	11.4	8.7	-	Male
-	F45	11.3	9.2	289	Female
-	M19	11.3	8.2	263	Male
-	F102	11.2	8.4	261	Female
-	M29	11.2	8.2	251	Male
-	F5	11.2	8.4	279	Female
-	F18	11.2	8.4	252	Female
-	F98	11.2	8.6	252	Female
-	M39	11.2	8.4	263	Male
-	M35	11.2	8.8	260	Male
-	M33	11.2	8.6	287	Male
-	F76	11.1	8.6	264	Female
-	F71	11.1	8.3	282	Female
-	F58	11.1	8.2	265	Female
-	M21	11.1	8.9	273	Male
-	F22	11.0	9.7	289	Female
-	F42	11.0	8.4	275	Female
-	F73	11.0	8.4	295	Female
-	F94	11.0	7.4	236	Female
-	F85	11.0	8.4	224	Female
-	M28	11.0	8.1	257	Male
-	M22	11.0	8.2	244	Male
-	F32	11.0	7.8	247	Female
-	F20	10.9	8.2	243	Female
-	F4	10.9	8.6	249	Female
-	F37	10.9	8.2	267	Female
-	F17	10.9	7.9	257	Female
-	F15	10.9	8.3	246	Female
-	F41	10.9	8.0	268	Female
-	F48	10.9	8.0	257	Female
-	F90	10.9	8.1	263	Female
-	F67	10.9	8.2	249	Female
-	F57	10.9	8.1	245	Female
-	F91	10.9	8.1	266	Female
-	M37	10.9	8.2	269	Male
-	M30	10.9	8.1	246	Male
-	M13	10.9	8.3	263	Male
-	F50	10.8	7.6	253	Female
-	F65	10.8	8.1	231	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	F77	10.8	7.9	242	Female
-	F82	10.8	8.2	251	Female
-	M1	10.8	8.1	274	Male
-	F9	10.7	8.1	247	Female
-	F46	10.7	7.6	260	Female
-	F96	10.7	7.6	224	Female
-	M32	10.7	8.0	261	Male
-	M23	10.7	7.7	220	Male
-	F39	10.6	8.2	229	Female
-	F74	10.6	8.3	238	Female
-	F93	10.6	7.9	221	Female
-	M34	10.6	8.0	230	Male
-	M9	10.6	8.2	240	Male
-	F12	10.5	8.0	250	Female
-	F19	10.5	7.7	237	Female
-	F14	10.5	8.2	229	Female
-	M42	10.5	7.9	248	Male
-	F83	10.5	7.8	227	Female
-	M25	10.5	7.9	258	Male
-	F11	10.4	7.3	248	Female
-	F103	10.4	8.1	238	Female
-	M31	10.4	8.2	253	Male
-	M43	10.4	8.0	270	Male
-	M16	10.4	8.8	230	Male
-	M24	10.4	7.9	228	Male
-	F13	10.3	7.6	218	Female
-	F3	10.3	8.3	228	Female
-	F30	10.3	7.8	229	Female
-	F38	10.3	8.3	257	Female
-	F105	10.3	7.9	243	Female
-	M2	10.3	7.7	216	Male
-	F72	10.2	7.7	214	Female
-	F61	10.2	7.9	227	Female
-	F99	10.2	7.7	188	Female
-	F95	10.2	7.8	237	Female
-	M44	10.2	8.2	224	Male
-	M18	10.2	8.2	156	Male
-	F92	10.1	8.8	227	Female
-	M38	10.1	8.2	238	Male
-	F111	10.1	7.2	194	Female
-	F7	10.0	8.3	234	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
-	F27	10.0	7.5	196	Female
-	F43	10.0	7.2	217	Female
-	F59	10.0	7.6	186	Female
-	F78	10.0	7.3	173	Female
-	F23	9.9	7.4	187	Female
-	F104	9.9	7.6	212	Female
-	F100	9.8	7.4	218	Female
-	F33	9.7	7.4	180	Female
-	F107	9.7	7.3	205	Female
-	F84	9.7	7.5	197	Female
-	M8	9.7	7.8	198	Male
-	M41	9.6	7.5	1812	Male
-	F62	9.5	7.3	186	Female
-	F106	9.5	7.2	211	Female
-	M40	9.5	7.4	204	Male
-	F42	9.0	7.5	201	Female
-	F66	8.5	6.2	130	Female

Roman Bay

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
R.34	-	11.4	8.6	218	Female
Gr.13	-	11.1	8.2	211	Female
Gr.94	-	11.0	8.0	184	Female
R.53	-	11.0	8.4	202	Male
Gr.29	-	10.7	7.4	165	Female
R.13	-	10.7	7.1	157	Female
R.54	-	10.7	7.4	170	Male
R.58	-	10.7	7.7	165	Male
R.71	-	10.7	7.5	166	Male
Gr.90/R.90	-	10.7	7.5	138	Female
Gr.38	-	10.6	7.4	172	Female
Gr.96	-	10.6	7.9	172	Female
R.1	-	10.6	7.5	169	Female
R.22	-	10.6	7.4	169	Female
Gr.67	-	10.5	7.4	159	Female
R.23	-	10.5	7.5	1667	Female
R.40	-	10.5	7.4	171	Female
R.68	-	10.5	7.8	183	Male
Gr.33	-	10.4	7.0	163	Female
Gr.41	-	10.4	7.6	177	Female
Gr.42	-	10.4	7.6	174	Female
Gr.54	-	10.4	7.7	162	Female
R.6	-	10.4	7.7	176	Female
R.11	-	10.4	7.7	167	Female
R.51	-	10.4	7.5	177	Male
R.57	-	10.4	7.4	172	Male
R.66	-	10.4	7.9	172	Male
R.79	-	10.4	7.5	161	Male
Gr.75/R.75	-	10.4	7.1	155	Female
Gr.32	-	10.3	7.0	162	Female
Gr.40	-	10.3	7.2	151	Female
Gr.90	-	10.3	6.9	163	Female
R.12	-	10.3	7.4	179	Female
R.28	-	10.3	7.2	156	Female
R.42	-	10.3	7.7	171	Female
R.43	-	10.3	7.7	163	Female
R.52	-	10.3	7.5	153	Male
R.69	-	10.3	7.4	166	Male

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
R.85	-	10.3	7.1	141	Male
R.87	-	10.3	7.5	150	Male
R.89	-	10.3	7.4	144	Male
Gr.12	-	10.2	7.3	151	Female
Gr.44	-	10.2	7.9	182	Female
Gr.68	-	10.2	6.8	163	Female
Gr.71	-	10.2	6.6	150	Female
Gr.78	-	10.2	7.0	141	Female
Gr.83	-	10.2	6.9	156	Female
Gr.98	-	10.2	6.9	155	Female
R.10	-	10.2	7.9	180	Female
R.56	-	10.2	7.5	169	Male
R.72	-	10.2	7.4	152	Male
Gr.44/R.44	-	10.2	7.2	149	Female
Gr.7	-	10.1	7.1	143	Female
Gr.10	-	10.1	7.0	160	Female
Gr.14	-	10.1	7.1	168	Female
Gr.60	-	10.1	7.1	154	Female
Gr.79	-	10.1	7.6	149	Female
Gr.91	-	10.1	7.0	149	Female
Gr.92	-	10.1	7.1	158	Female
R.44	-	10.1	7.0	142	Female
R.55	-	10.1	7.1	151	Male
R.61	-	10.1	7.3	170	Male
R.63	-	10.1	7.6	153	Male
R.91	-	10.1	7.5	169	Male
Gr.16/R.16	-	10.1	7.4	158	Female
Gr.24/R.24	-	10.1	7.3	141	Female
Gr.30/R.30	-	10.1	7.1	150	Female
Gr.64/R.64	-	10.1	7.0	123	Female
Gr.66/R.66	-	10.1	7.2	142	Female
Gr.81/R.81	-	10.1	6.8	138	Female
Gr.8	-	10.0	7.5	145	Female
Gr.21	-	10.0	7.0	151	Female
Gr.35	-	10.0	7.2	161	Female
Gr.43	-	10.0	6.8	151	Female
Gr.65	-	10.0	7.6	143	Female
R.8	-	10.0	7.2	163	Female
R.9	-	10.0	7.3	1523	Female
R.29	-	10.0	7.3	160	Female
R.31	-	10.0	7.6	165	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
R.33	-	10.0	7.2	144	Female
R.76	-	10.0	7.1	170	Male
R.78	-	10.0	7.2	144	Male
R.83	-	10.0	7.3	167	Male
Gr.38/R.38	-	10.0	7.2	138	Female
Gr.43/R.43	-	10.0	7.0	127	Female
Gr.52/R.52	-	10.0	7.0	141	Female
Gr.9	-	9.9	6.8	147	Female
Gr.22	-	9.9	7.1	154	Female
Gr.25	-	9.9	6.8	146	Female
Gr.30	-	9.9	7.8	143	Female
Gr.31	-	9.9	7.2	156	Female
Gr.39	-	9.9	7.1	146	Female
Gr.47	-	9.9	7.4	155	Female
Gr.57	-	9.9	6.7	142	Female
Gr.64	-	9.9	6.7	142	Female
Gr.86	-	9.9	7.3	131	Female
Gr.97	-	9.9	7.0	144	Female
R.5	-	9.9	6.8	146	Female
R.21	-	9.9	7.3	164	Female
R.26	-	9.9	7.0	135	Female
R.27	-	9.9	7.2	162	Female
R.37	-	9.9	7.1	153	Female
R.46/W.27	-	9.9	6.8	131	Female
R.47	-	9.9	7.1	133	Female
R.48	-	9.9	7.3	139	Female
R.60	-	9.9	7.3	147	Male
R.64	-	9.9	7.0	148	Male
R.75	-	9.9	6.6	145	Male
R.94	-	9.9	6.9	148	Male
R.98	-	9.9	7.1	134	Male
Gr.31/R.31	-	9.9	6.7	133	Female
Gr.80/R.80	-	9.9	7.2	137	Female
Gr.91/R.91	-	9.9	7.0	123	Female
Gr.98/R.98	-	9.9	7.2	138	Female
Gr.4	-	9.8	7.0	162	Female
Gr.6	-	9.8	7.3	156	Female
Gr.17	-	9.8	7.1	149	Female
Gr.19	-	9.8	7.5	158	Female
Gr.27	-	9.8	6.8	151	Female
Gr.46	-	9.8	6.7	134	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
Gr.61	-	9.8	7.5	144	Female
Gr.66/B.28	-	9.8	7.4	164	Female
Gr.84	-	9.8	6.7	132	Female
Gr.88	-	9.8	7.4	158	Female
Gr.99	-	9.8	7.3	133	Female
R.2	-	9.8	7.3	160	Female
R.4	-	9.8	7.0	142	Female
R.19	-	9.8	6.7	149	Female
R.32	-	9.8	7.4	134	Female
R.39	-	9.8	7.6	147	Female
R.70	-	9.8	7.3	157	Male
R.73	-	9.8	6.9	155	Male
R.77	-	9.8	7.3	146	Male
R.82	-	9.8	7.5	159	Male
Gr.18/R.18	-	9.8	7.1	130	Female
Gr.41/R.41	-	9.8	7.1	153	Female
Gr.47/R.47	-	9.8	6.8	136	Female
GR.53/R.53	-	9.8	7.2	135	Female
Gr.57/R.57	-	9.8	6.8	143	Female
Gr.71/R.71	-	9.8	7.4	119	Female
Gr.5	-	9.7	7.0	132	Female
Gr.16	-	9.7	6.8	155	Female
Gr.23	-	9.7	6.9	120	Female
Gr.26	-	9.7	7.1	153	Female
Gr.49	-	9.7	6.7	133	Female
Gr.50	-	9.7	6.4	131	Female
Gr.56	-	9.7	7.1	151	Female
Gr.58	-	9.7	6.7	149	Female
Gr.73	-	9.7	6.7	145	Female
Gr.75	-	9.7	6.7	147	Female
Gr.77	-	9.7	6.9	129	Female
Gr.93	-	9.7	6.6	138	Female
R.14	-	9.7	6.9	142	Female
R.16	-	9.7	6.8	133	Female
R.24	-	9.7	7.5	155	Female
R.25	-	9.7	6.9	131	Female
R.36	-	9.7	6.8	123	Female
R.41	-	9.7	6.9	157	Female
R.74	-	9.7	6.9	137	Male
R.80	-	9.7	7.0	146	Male
R.86	-	9.7	7.0	143	Male

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
R.90	-	9.7	6.7	145	Male
R.92	-	9.7	7.1	142	Male
R.93	-	9.7	7.7	147	Male
R.95	-	9.7	7.2	158	Male
R.97	-	9.7	7.3	161	Male
Gr.51/Gr.52	-	9.7	6.8	148	Male
Gr.6/R.6	-	9.7	6.7	131	Female
Gr.12/R12	-	9.7	6.9	135	Female
Gr.14/R.14	-	9.7	7.7	120	Female
Gr.28/R.28	-	9.7	6.7	122	Female
Gr.32/R.32	-	9.7	6.7	139	Female
Gr.33/R.33	-	9.7	6.9	135	Female
Gr.42/R.42	-	9.7	6.5	127	Female
Gr.46/R.46	-	9.7	6.6	127	Female
Gr.61/R.61	-	9.7	7.0	137	Female
Gr.67/R.67	-	9.7	6.7	132	Female
Gr.73/R.73	-	9.7	6.7	129	Female
Gr.82/R.82	-	9.7	7.3	141	Female
Gr.83/R.83	-	9.7	7.1	133	Female
Gr.84/R.84	-	9.7	6.3	132	Female
Gr.89/R.89	-	9.7	6.9	130	Female
Gr.93/R.93	-	9.7	7.0	146	Female
Gr.99/R.99	-	9.7	6.6	125	Female
Gr.3	-	9.6	6.6	142	Female
Gr.11	-	9.6	7.3	157	Female
Gr.45	-	9.6	6.8	144	Female
Gr.52	-	9.6	6.9	164	Female
Gr.55	-	9.6	6.7	144	Female
Gr.69	-	9.6	6.8	141	Female
Gr.82	-	9.6	6.7	149	Female
Gr.85	-	9.6	7.1	142	Female
Gr.87	-	9.6	7.6	150	Female
Gr.89/B.17	-	9.6	7.1	158	Female
Gr.95	-	9.6	6.8	133	Female
R.30	-	9.6	6.8	161	Female
R.35	-	9.6	7.3	147	Female
R.45	-	9.6	7.0	150	Female
R.65	-	9.6	7.1	147	Male
R.67	-	9.6	6.9	150	Male
R.81	-	9.6	6.8	144	Male
Gr.2/Gr.54	-	9.6	7.4	133	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
Gr.5/Gr.57	-	9.6	6.3	122	Female
Gr.7/R.7	-	9.6	6.8	138	Female
Gr.29/R.29	-	9.6	7.0	126	Female
Gr.39/R.39	-	9.6	7.2	138	Female
Gr.50/R.50	-	9.6	7.0	139	Female
Gr.69/R.69	-	9.6	6.6	137	Female
Gr.72/R.72	-	9.6	6.7	137	Female
Gr.74/R.74	-	9.6	6.8	128	Female
Gr.77/R.77	-	9.6	6.5	139	Female
Gr.79/R.79	-	9.6	6.9	125	Female
Gr.87/R.87	-	9.6	6.9	131	Female
Gr.88/R/88	-	9.6	6.9	136	Female
Gr.92/Gr.92	-	9.6	7.1	125	Female
Gr.97/R.97	-	9.6	6.8	126	Female
Gr.58/Gr.59	-	9.6	7.1	126	Female
Gr.15	-	9.5	7.4	151	Female
Gr.34	-	9.5	6.5	129	Female
Gr.36	-	9.5	7.2	147	Female
Gr.37	-	9.5	7.0	146	Female
Gr.48	-	9.5	6.8	151	Female
Gr.63	-	9.5	7.1	144	Female
R.7	-	9.5	7.2	151	Female
R.17	-	9.5	6.6	136	Female
R.49	-	9.5	6.1	124	Female
R.62	-	9.5	7.0	140	Male
Gr.3/Gr.55	-	9.5	7.0	130	Female
Gr.20/R.20	-	9.5	6.9	127	Female
Gr.21/R.21	-	9.5	6.9	138	Female
Gr.22/R.22	-	9.5	6.5	1267	Female
Gr.23/R.23	-	9.5	6.9	121	Female
Gr.25/R.25	-	9.5	6.7	123	Female
Gr.37/Gr.37	-	9.5	6.8	130	Female
Gr.40/R.40	-	9.5	6.8	117	Female
Gr.55/R.55	-	9.5	6.5	133	Female
Gr.60/R.60	-	9.5	7.1	133	Female
Gr.76/R.76	-	9.5	6.9	130	Female
Gr.78/R.78	-	9.5	7.0	139	Female
Gr.85/R.85	-	9.5	6.8	128	Female
Gr.94/R.94	-	9.5	6.7	125	Female
Gr.95/R.95	-	9.5	6.5	117	Female
Gr.1	-	9.4	7.0	146	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
Gr.24	-	9.4	6.6	129	Female
Gr.53	-	9.4	6.7	130	Female
Gr.80	-	9.4	6.4	125	Female
Gr.81	-	9.4	7.3	142	Female
R.15	-	9.4	7.6	144	Female
R.20	-	9.4	6.5	128	Female
R.59	-	9.4	7.3	154	Male
R.99	-	9.4	6.8	147	Male
Gr.4/Gr.56	-	9.4	6.8	132	Female
Gr.9/R.9	-	9.4	7.0	121	Female
Gr.11/R.11	-	9.4	6.6	127	Female
Gr.15/R.15	-	9.4	7.0	136	Female
Gr.27/R.27	-	9.4	6.7	120	Female
Gr.36/R.36	-	9.4	6.9	125	Female
Gr.45/R.45	-	9.4	6.9	133	Female
Gr.48/R.48	-	9.4	6.9	115	Female
Gr.51/R.51	-	9.4	6.3	124	Female
Gr.54/R.54	-	9.4	6.4	108	Female
Gr.59/R.59	-	9.4	6.8	124	Female
Gr.62/R.62	-	9.4	6.7	127	Female
Gr.63/R.63	-	9.4	7.0	124	Female
Gr.68/R.68	-	9.4	6.7	118	Female
Gr.2/B.60	-	9.3	7.1	128	Female
Gr.20	-	9.3	7.4	144	Female
Gr.28	-	9.3	7.1	136	Female
Gr.62	-	9.3	6.8	125	Female
Gr.72	-	9.3	7.2	152	Female
Gr.74	-	9.3	6.3	122	Female
R.88	-	9.3	6.9	132	Male
Gr.1/Gr.53	-	9.3	6.8	122	Female
Gr.8/R.8	-	9.3	7.0	120.	Female
Gr.13/R.13	-	9.3	6.5	122	Female
Gr.17/R.17	-	9.3	6.3	112	Female
Gr.35/R.35	-	9.3	6.4	106	Female
Gr.49/R.49	-	9.3	7.1	129	Female
Gr.56/R.56	-	9.3	6.9	126	Female
Gr.58/R.58	-	9.3	6.9	131	Female
Gr.65/R.65	-	9.3	7.0	132	Female
Gr.86/R.86	-	9.3	6.5	121	Female
Gr.70	-	9.2	6.1	-	Female
R.38	-	9.2	7.0	138	Female

Temporary tag ID	Permanent ID	Length (cm)	Width (cm)	Weight (g)	Sex
Gr.10/R.10	-	9.2	7.7	125	Female
Gr.19/R.19	-	9.2	6.4	114	Female
Gr.34/R.34	-	9.2	6.6	120	Female
Gr.96/R.96	-	9.2	6.6	119	Female
Gr.18	-	9.1	6.7	133	Female
Gr.51	-	9.1	6.4	122	Female
Gr.59	-	9.1	6.5	121	Female
Gr.76	-	9.1	6.6	129	Female
R.18	-	9.1	6.7	127	Female
R.50	-	9.1	6.4	137	Female
R.96	-	9.1	6.4	122	Male
Gr.26/R.26	-	9.1	7.0	135	Female
Gr.70/R.70	-	9.1	7.0	116	Female
R.3	-	9.0	6.4	1123	Female
R.84	-	9.0	6.6	121	Male

APPENDIX B

GENOTYPICALLY SELECTED BROODSTOCK

Abagold	Aquafarm	HIK	I&J	Roman Bay
AB78F	AQFA328	HIK431	IJF79	16_10
AB6F-F	AQFB209	HIK358	IJF31	15_42
AB69F	AQFB259	HIK366	IJF40	15_49
AB_14M	AQFB212	HIK573	IJM11	17_22
AB5F-F	AQFA213	HIK461	IJM14	16_4
AB80F	AQFA322	HIK442	IJF64	14_47
AB96F	AQFA260	HIK418	IJF16	16_19
AB64F	AQFA280	HIK389	IJF54	17_12
AB59F	AQFB207	HIK385	IJF110	17_31
AB93F	AQFA316	HIK349	IJF102	15_41
AB25M	AQFB252	HIK313	IJF5	14_23
AB106F	AQFA304	HIK406	IJF98	16_50
AB41M	AQFA332	HIK479	IJF22	15_48
AB29M	AQFB233	HIK411	IJF73	17_7
AB7F-F	AQFB241	HIK532	IJF20	17_35
AB109F	AQFB244	HIK459	IJF17	18_16
AB51M	AQFA342	HIK495	IJF67	15_11
AB47M	AQFA230	HIK394	IJM13	17_30
AB88F	AQFB202	HIK592	IJM23	17_8
AB98F	AQFB215	HIK476	IJM31	18_33
AB66F	AQFB230	HIK545	IJF27	15_50
AB54M	AQFA262	HIK368	IJF43	16_28
AB53M	AQFA306	HIK581	IJF104	17_43
AB107F	AQFB217	HIK536	IJF106	19_4
AB48M	AQFA219	HIK475	IJM10	19_2
AB16M	AQFA231	HIK357	IJF69	19_24
AB63F	AQFA249	HIK538	IJF68	19_26
AB33M	AQFA207	HIK504	IJF55	15_36
AB44M	AQFA228	HIK493	IJF50	15_47
AB71F	AQFA273	HIK329	IJF74	19_23
AB10F	AQFA238	HIK_449	IJM9	17_48
AB83F	AQFA261	HIK328	IJF92	18_14
AB61F	AQFB249	HIK578	IJF59	-
AB_103F	AQFB218	HIK481	IJF84	-
AB57M	AQFB238	HIK572	IJF62	-
AB46M	AQFA209	HIK412	IJF45	-
AB28M	AQFA243	HIK423	IJF15	-
AB85F	AQFB263	HIK466	IJF91	-
AB105F	AQFB266	HIK323	IJF38	-
AB32M	AQFB255	HIK348	IJF7	-
AB31M	AQFA257	HIK401	IJM8	-
AB22M	AQFA225	HIK473	IJF76	-
AB110F	AQFB270	HIK555	IJF65	-
-	AQFB225	HIK369	IJM16	-
-	AQFA299	HIK420	-	-
-	AQFA307	HIK308	-	-
-	AQFA279	HIK320	-	-

Abagold	Aquafarm	HIK	I&J	Roman Bay
-	AQFB203	HIK597	-	-
-	AQFA325	HIK544	-	-
-	AQFA214	HIK472	-	-
-	AQFA245	HIK561	-	-
-	AQFA270	HIK432	-	-
-	AQFA247	HIK502	-	-
-	AQFA275	HIK537	-	-
-	AQFA337	HIK437	-	-
-	AQFA208	HIK332	-	-
-	AQFA212	HIK514	-	-
-	AQFA202	HIK488	-	-
-	AQFA297	HIK436	-	-
-	AQFB261	HIK483	-	-
-	AQFB224	HIK334	-	-
-	AQFA226	HIK489	-	-
-	AQFA295	HIK663	-	-
-	AQFA329	HIK535	-	-
-	AQFB204	HIK563	-	-
-	AQFA282	HIK557	-	-
-	AQFB268	HIK464	-	-
-	AQFA236	HIK552	-	-
-	AQFA326	HIK463	-	-
-	AQFA206	HIK591	-	-
-	AQFB211	HIK309	-	-
-	-	HIK505	-	-
-	-	HIK527	-	-
-	-	HIK482	-	-
-	-	HIK392	-	-
-	-	HIK390	-	-
-	-	HIK468	-	-
-	-	HIK586	-	-
-	-	HIK512	-	-
-	-	HIK375	-	-
-	-	HIK345	-	-
-	-	HIK499	-	-

APPENDIX C

PHENOTYPICALLY SELECTED BROODSTOCK

Abagold	Aquafarm	HIK
AB4F_F	A201	HIK309
AB5F_F	A202	HIK315
AB6F_F	A203	HIK333
AB7F_F	A206	HIK344
AB8F_F	A208	HIK346
AB9F_F	A209	HIK347
AB10F_F	A211	HIK351
AB10F	A213	HIK357
AB14M	A218	HIK363
AB25M	A220	HIK371
AB29M	A222	HIK372
AB30M	A225	HIK374
AB32M	A230	HIK375
AB33M	A234	HIK377
AB36M	A235	HIK394
AB39M	A241	HIK403
AB40M	A243	HIK410
AB42M	A247	HIK418
AB43M	A252	HIK428
AB45M	A258	HIK431
AB51M	A259	HIK432
AB59F	A271	HIK448
AB60F	A272	HIK458
AB62F	A276	HIK459
AB63F	A280	HIK468
AB64F	A292	HIK472
AB69F	A298	HIK478
AB70F	A302	HIK481
AB72F	A304	HIK490
AB75F	A314	HIK492
AB80F	A315	HIK495
AB82M	A316	HIK506
AB87F	A318	HIK535
AB88F	A320	HIK559
AB89F	A322	HIK562
AB90F	A325	HIK566
AB91F	A328	HIK569
AB93F	A332	HIK586
AB95F	A333	HIK594
AB96F	A334	HIK663
AB105F	A335	HIK312
AB109F	A340	HIK314
-	A342	HIK319
-	B201	HIK325
-	B202	HIK331
-	B204	HIK335
-	B206	HIK339

Abagold	Aquafarm	HIK
-	B208	HIK340
-	B209	HIK343
-	B210	HIK345
-	B212	HIK353
-	B216	HIK356
-	B217	HIK370
-	B218	HIK373
-	B219	HIK376
-	B223	HIK389
-	B231	HIK390
-	B233	HIK396
-	B238	HIK398
-	B240	HIK399
-	B241	HIK402
-	B244	HIK406
-	B245	HIK416
-	B251	HIK423
-	B252	HIK427
-	B255	HIK436
-	B256	HIK441
-	B257	HIK451
-	B258	HIK457
-	B259	HIK483
-	B263	HIK488
-	B265	HIK489
-	B266	HIK491
-	B268	HIK503
-	B269	HIK504
-	B270	HIK509
-	A219	HIK522
-	-	HIK525
-	-	HIK530
-	-	HIK545
-	-	HIK550
-	-	HIK557
-	-	HIK571
-	-	HIK572
-	-	HIK574
-	-	HIK576
-	-	HIK584
-	-	HIK590
-	-	HIK592
-	-	HIK593

APPENDIX D

SIMULATED POPULATIONS

Top 32 genotypic animals

Abagold	Aquafarm	HIK	I&J	Roman Bay
AB78F	AQFA328	HIK431	IJF79	16_10
AB6F-F	AQFB209	HIK358	IJF31	15_42
AB69F	AQFB259	HIK366	IJF40	15_49
AB5F-F	AQFB212	HIK573	IJM11	17_22
AB80F	AQFA213	HIK461	IJM14	16_4
AB96F	AQFA322	HIK442	IJF64	14_47
AB64F	AQFA260	HIK418	IJF16	16_19
AB59F	AQFA280	HIK389	IJF54	17_12
AB93F	AQFB207	HIK385	IJF110	17_31
AB25M	AQFA316	HIK349	IJF102	15_41
AB106F	AQFB252	HIK313	IJF5	14_23
AB41M	AQFA304	HIK406	IJF98	16_50
AB29M	AQFA332	HIK479	IJF22	15_48
AB7F-F	AQFB233	HIK411	IJF73	17_7
AB109F	AQFB241	HIK532	IJF20	17_35
AB51M	AQFB244	HIK459	IJF17	18_16
AB47M	AQFA342	HIK495	IJF67	15_11
AB88F	AQFA230	HIK394	IJM13	17_30
AB98F	AQFB202_1	HIK592	IJM23	17_8
AB66F	AQFB215	HIK476	IJM31	18_33
AB54M	AQFB230	HIK545	IJF27	15_50
AB53M	AQFA262	HIK368	IJF43	16_28
AB107F	AQFA306	HIK581	IJF104	17_43
AB48M	AQFB217	HIK536	IJF106	19_4
AB16M	AQFA219	HIK475	IJM10	19_2
AB63F	AQFA231	HIK357	IJF69	19_24
AB33M	AQFA249	HIK538	IJF68	19_26
AB44M	AQFA207	HIK504	IJF55	15_36
AB71F	AQFA228	HIK493	IJF50	15_47
AB10F	AQFA273	HIK329	IJF74	19_23
AB83F	AQFA238	HIK449	IJM9	17_48

32 Randomly selected animals

Abagold	Aquafarm	HIK	I&J	Roman Bay
AB103F	AQFA201	20_13	IJF105	13_44
AB76F	AQFA253	21_9	IJM1	15_33
AB78F	AQFA254	B_27	IJM10	15_34
AB43M	AQFB203	Gr_81	IJM4	17_6
AB52M	AQFB252	HIK318	IJF43	15_8
AB71F	AQFB253	HIK324	IJF12	18_44
AB95F	AQFB265	HIK325	IJF25	15_16
AB9F-F	AQFA263	B_53	IJF77	14_1
AB65F	AQFA211	HIK417	IJM16	17_19
AB21M	AQFA204	HIK374	IJM8	19_7
AB104F	AQFA260	DOOD_1	IJM30	19_14
AB42M	AQFA305	Y_83	IJF95	18_24
AB71F	AQFB232	O-2_29	IJF76	17_17
AB84M	AQFB241	W_46	IJF60	16_2
AB93F	AQFB264	HIK600	IJF19	16_10
AB3M	AQFA227	HIK548	IJF66_2	15_19
AB68F_2	AQFB204	HIK394	IJF99	14_21
AB92F	AQFA212	HIK580	IJM34	13_46
AB50M	AQFA279	20_19	IJM42	15_24
AB6F-F	AQFA285	Y_74	IJF47	17_40
AB92F	AQFA328	HIK452	IJF14	19_14
AB107F	AQFA339	HIK353	IJF15	14_9
AB55M	AQFB267	HIK369	IJF106	13_52
AB87F	AQFA251	Gr_16	IJF8	14_30
AB44M	AQFA267	HIK565	IJF61	15_10
AB10F-F	AQFA323	HIK547	IJM40	16_7
AB52M	AQFB237	HIK411	IJF47	18_10
AB53M	AQFA329	HIK424	IJM32	16_19
AB87F	AQFB244	HIK552	IJF56	18_31
AB50M	AQFA279	Y_86	IJF4	16_5
AB93F	AQFA220	HIK366	IJM9	17_42
AB81F	AQFA325	Gr_81	IJF83	19_16

APPENDIX E

ALLELE FREQUENCY DATA

HmD59

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
100	0.005	-	0.006	-	-	-	-	-	-	-
105	0.005	-	-	-	-	-	-	-	-	-
106	0.011	0.052	0.012	-	0.039	0.021	0.029	0.008	0.044	0.061
109	0.022	-	-	-	-	-	-	-	-	-
110	0.071	0.015	0.040	0.008	0.043	0.078	-	-	0.044	0.100
111	0.022	-	-	-	-	-	0.043	-	-	-
112	0.054	0.037	0.069	0.039	0.053	0.049	0.071	0.101	0.087	-
113	0.016	-	-	-	-	-	-	-	-	-
114	0.016	0.008	0.029	0.069	0.039	0.012	0.014	0.034	-	0.002
115	0.011	-	-	-	-	-	-	-	-	-
116	0.049	0.090	0.029	0.077	0.072	0.115	0.014	-	0.065	0.120
117	0.016	-	-	-	-	-	-	-	-	-
118	0.016	0.037	0.126	0.085	0.058	0.008	0.057	0.017	0.065	0.028
119	0.011	-	-	-	-	-	0.029	-	-	-
120	0.044	0.105	0.052	0.054	0.077	0.082	0.014	0.105	0.065	0.007
121	0.022	-	-	-	-	-	0.029	-	-	-
122	0.114	0.112	0.149	0.085	0.111	0.217	0.200	0.416	0.174	0.096
123	0.016	-	-	-	-	-	0.029	-	-	-
124	0.152	0.202	0.190	0.100	0.115	0.152	0.171	0.135	0.130	0.252
125	0.005	-	-	-	-	-	-	-	-	-
126	0.092	0.060	0.058	0.262	0.082	0.066	0.043	-	0.065	-
127	0.005	-	-	-	-	-	0.014	-	-	-
128	0.054	0.082	0.052	0.100	0.048	0.049	0.014	0.046	0.065	0.007
130	0.114	0.090	0.069	0.100	0.058	0.021	0.043	0.109	0.044	0.052
131	-	-	-	-	-	-	0.014	-	-	-
132	0.005	-	0.012	0.015	0.039	0.021	0.057	-	-	0.011
134	0.011	-	0.017	-	0.039	0.041	0.029	-	0.065	0.189
135	-	-	-	-	-	-	0.014	-	-	-
136	0.011	0.097	0.029	0.008	0.039	0.021	0.014	-	0.022	0.004
137	-	-	-	-	-	-	0.014	-	-	-
138	0.005	-	0.006	-	0.019	-	0.014	-	-	-
140	-	-	0.012	-	0.063	0.049	-	-	0.022	0.028
142	-	-	0.012	-	0.005	-	-	-	-	-

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
144	0.005	-	0.017	-	-	-	-	0.025	-	0.015
146	-	-	0.017	-	0.005	-	0.029	-	-	-
148	0.005	0.015	-	-	-	-	-	-	0.044	-
154	0.005	-	-	-	-	-	-	0.004	-	0.002
184	0.005	-	-	-	-	-	-	-	-	-

HmidPS1.870

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
91	0.011	-	-	-	0.010	-	-	-	-	-
99	0.203	-	0.155	0.139	0.172	0.130	0.139	0.043	0.239	0.393
101	0.017	-	0.035	0.172	0.020	0.004	0.042	0.111	-	-
103	-	-	-	0.016	0.005	-	-	-	-	-
105	0.011	-	0.023	-	0.005	-	0.014	0.004	0.065	0.018
107	0.022	0.008	0.029	0.008	0.049	0.021	0.042	0.111	0.044	-
109	0.011	0.008	0.029	0.008	0.010	0.004	-	0.004	0.044	0.020
111	0.011	-	0.017	0.016	0.010	0.004	0.014	0.004	0.065	0.081
113	0.055	-	0.029	-	0.064	0.097	0.056	0.090	0.065	0.022
115	0.022	0.082	0.103	0.123	0.093	0.109	0.111	0.030	0.087	0.024
117	0.055	0.030	0.017	0.008	0.054	0.084	0.056	0.009	-	0.013
119	0.044	0.030	0.029	0.033	0.039	0.034	0.056	0.064	0.044	0.191
121	0.126	0.202	0.081	0.041	0.093	0.101	0.111	0.060	0.109	0.035
123	0.071	0.075	0.126	0.082	0.054	0.080	0.069	0.094	0.044	0.037
125	0.071	0.067	0.063	0.123	0.059	0.063	0.097	0.111	0.022	0.004
127	0.033	0.060	0.103	0.016	0.064	0.029	0.042	0.064	0.065	0.132
129	0.055	0.015	0.035	0.066	0.088	0.139	0.069	0.077	0.065	0.004
131	0.088	0.097	0.069	0.082	0.039	0.017	0.042	0.124	-	-
133	0.033	0.097	0.017	0.025	0.049	0.067	-	-	0.044	0.018
135	0.011	0.022	-	-	0.005	-	0.014	-	-	0.009
137	0.017	-	-	-	0.010	0.004	-	-	-	-
139	0.006	-	0.006	0.008	0.005	-	0.014	-	-	-
140	-	-	0.017	0.033	-	-	-	-	-	-
143	-	-	-	-	-	-	0.014	-	-	-
145	0.006	-	-	-	-	-	-	-	-	-
149	-	-	0.006	-	-	-	-	-	-	-
151	0.022	0.022	-	-	0.005	0.013	-	-	-	-
153	-	-	0.012	-	-	-	-	-	-	-

HmidPS1.967

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
131	-	-	-	0.012	0.005	-	0.022	-	0.008	-
135	0.241	0.307	0.339	0.287	0.260	0.319	0.283	0.276	0.223	0.196
137	0.389	0.307	0.226	0.259	0.230	0.292	0.174	0.351	0.262	0.196
139	0.019	0.016	0.032	0.035	0.029	0.014	0.044	0.030	0.008	0.021
141	0.222	0.258	0.285	0.259	0.324	0.306	0.391	0.299	0.323	0.442
143	0.093	0.097	0.097	0.115	0.093	0.014	0.065	0.045	0.146	0.092
145	0.019	-	0.022	0.023	0.029	0.056	-	-	-	0.054
147	0.019	0.016	-	0.006	0.025	-	0.022	-	0.031	-
159	-	-	-	0.006	0.005	-	-	-	-	-

HmRS129

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
241	0.031	0.057	0.007	0.009	0.020	-	-	-	0.071	0.005
243	-	-	-	-	0.005	-	-	-	-	-
253	-	-	-	-	-	0.009	-	-	-	0.002
257	0.006	0.008	-	0.017	-	-	0.016	0.069	-	-
259	0.177	0.189	0.125	0.119	0.107	0.196	0.125	0.034	0.238	0.199
261	0.049	0.049	0.033	0.009	0.046	0.009	0.047	0.098	-	-
262	0.006	-	-	-	-	-	-	-	-	0.005
263	0.116	0.295	0.112	0.051	0.128	0.113	0.094	0.069	0.071	0.137
265	0.006	0.008	0.079	0.059	0.051	0.065	0.016	-	-	-
266	0.006	-	-	-	-	-	0.016	-	-	-
267	0.043	0.041	0.026	0.076	0.092	0.065	0.047	0.015	-	-
269	0.006	0.025	0.033	0.076	0.026	0.013	0.031	0.025	-	-
270	0.012	-	-	-	-	-	-	-	-	-
271	0.024	0.131	0.040	0.085	0.041	0.061	-	0.029	0.024	0.002
272	0.006	-	-	-	-	-	0.016	-	-	-
273	0.031	0.008	0.053	0.093	0.077	0.100	0.031	0.039	-	0.019
274	0.012	-	-	-	-	-	0.063	-	-	-
275	0.061	0.025	0.046	0.042	0.036	0.009	0.047	0.064	0.071	0.002
276	0.006	-	-	-	-	-	-	-	-	-
277	0.031	-	0.072	0.009	0.051	0.048	0.063	0.074	0.119	0.199
279	0.024	0.090	0.066	0.051	0.056	0.004	0.016	0.054	0.095	0.092
280	0.018	-	-	-	-	-	0.016	-	-	-
281	0.055	0.066	0.059	0.009	0.066	-	0.031	0.069	0.071	0.228
282	0.024	-	-	-	-	-	0.031	-	-	-
283	0.079	-	0.079	0.009	0.041	0.044	0.125	0.108	0.048	0.017
284	0.024	-	-	-	-	-	-	-	-	-
285	-	-	-	-	-	-	-	0.010	0.024	0.076
286	0.024	-	-	-	-	-	0.031	-	-	-
287	0.043	-	0.020	0.009	0.056	0.191	-	0.015	0.048	-
288	0.006	-	-	-	-	-	-	-	-	-
289	0.006	-	0.026	0.203	0.066	0.035	0.078	0.083	0.048	0.002
290	0.012	-	-	-	-	-	-	-	-	-
291	0.018	0.008	0.026	0.034	0.015	0.013	0.016	-	0.048	0.014

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
292	0.006	-	-	-	-	-	-	-	-	-
293	0.024	-	0.007	-	0.010	0.004	-	0.123	-	-
295	-	-	0.013	0.025	-	-	-	-	-	-
296	-	-	-	-	-	-	0.016	-	-	-
297	-	-	0.033	0.009	0.005	-	0.016	0.015	0.024	-
299	-	-	-	-	0.005	-	-	0.005	-	-
301	0.006	-	0.020	-	-	-	-	-	-	-
303	-	-	0.013	0.009	-	-	0.016	0.005	-	-
307	-	-	0.013	-	-	-	-	-	-	-

HmRS27

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
235	-	-	-	-	0.010	0.004	-	-	-	-
239	0.006	0.015	0.012	0.008	0.015	0.051	0.029	0.005	-	-
243	0.023	0.008	0.047	-	0.020	0.004	-	-	0.022	-
247	0.056	0.046	0.024	-	0.039	0.106	0.014	0.019	0.044	-
251	0.011	0.008	0.053	0.050	0.039	0.017	0.029	0.009	0.044	0.014
255	0.034	0.053	0.035	-	0.025	0.025	0.043	0.056	0.044	-
259	0.028	-	0.071	0.042	0.059	0.034	0.071	0.005	0.109	0.039
263	0.067	0.038	0.053	0.025	0.069	0.064	0.043	0.093	0.022	0.096
267	0.062	0.136	0.018	0.075	0.029	0.013	0.086	0.042	0.022	0.027
271	0.017	0.008	0.012	0.042	0.010	0.119	-	-	0.044	0.148
275	0.017	-	0.006	-	0.069	-	0.014	-	-	-
279	0.006	0.015	0.018	0.008	0.010	-	-	0.014	-	0.002
283	0.011	-	0.012	-	0.005	-	0.014	0.005	-	-
287	0.017	-	-	-	0.020	0.025	-	-	-	-
291	0.006	-	0.024	0.017	0.005	-	-	-	0.022	-
295	0.006	-	0.012	-	-	-	-	-	-	-
299	0.011	-	0.012	0.008	0.010	0.009	-	-	-	-
303	0.017	0.038	0.018	0.033	0.029	0.004	0.029	-	-	-
307	0.028	0.083	0.018	0.183	0.005	-	0.029	0.032	-	-
309	-	-	0.006	-	0.015	0.004	-	-	-	-
311	0.017	-	0.006	-	0.034	0.038	-	-	-	0.018
313	-	-	0.006	0.017	-	-	0.029	-	-	-
315	0.039	0.061	0.018	-	0.039	0.034	0.043	0.009	-	-
317	0.006	-	-	-	-	-	-	-	-	-
319	0.017	-	0.029	0.025	0.010	-	-	-	0.065	0.052
323	0.028	0.030	0.035	0.017	0.020	-	0.043	-	0.087	0.023
325	-	-	0.006	0.008	-	-	-	-	-	-
327	0.028	0.008	0.035	0.017	0.010	-	0.014	0.005	0.022	0.068
331	0.073	0.023	0.029	0.050	0.044	0.064	0.043	0.056	0.065	-
335	0.039	0.030	0.029	0.117	0.034	0.051	0.014	0.116	0.065	0.146
339	0.039	0.061	0.035	0.042	0.044	0.004	0.071	0.065	0.044	0.011
341	-	-	0.006	-	-	-	-	-	-	-

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
343	0.028	0.023	0.041	0.008	0.039	0.034	0.014	0.023	0.044	0.248
347	0.028	-	0.053	0.033	0.029	0.009	0.086	0.046	0.022	0.005
351	0.034	0.008	0.029	0.025	0.029	0.051	0.014	-	0.022	0.014
355	0.028	0.061	0.053	0.050	0.015	-	0.029	0.037	0.065	0.002
357	0.006	-	-	-	-	-	-	-	-	-
359	0.028	-	0.024	0.033	0.049	0.081	0.029	0.019	-	0.021
361	-	-	-	-	-	-	-	0.097	-	-
363	0.039	0.076	0.024	-	0.029	0.017	0.086	0.208	0.044	0.023
365	-	-	-	-	0.005	0.042	-	-	-	-
367	0.023	0.030	0.024	0.042	0.025	0.017	0.014	0.009	0.044	0.039
371	0.011	0.091	0.018	0.025	0.020	0.013	0.043	0.028	-	0.002
373	-	-	0.012	-	-	-	0.014	-	-	-
375	0.017	0.015	0.006	-	0.010	0.030	-	-	-	-
377	0.006	-	0.006	-	-	-	0.014	-	-	-
379	0.006	-	0.006	-	-	-	-	-	0.022	0.005
381	-	-	0.006	-	-	-	-	-	-	-
383	-	-	0.006	-	0.005	0.004	-	-	-	-
385	0.006	-	-	-	-	-	-	-	-	-
387	0.006	-	-	-	0.010	0.030	-	-	-	-
389	0.006	-	-	-	-	-	-	-	-	-
391	-	-	-	-	0.015	0.004	-	-	-	-
397	-	-	0.012	-	-	-	-	-	-	-
399	0.006	-	-	-	-	-	-	-	0.022	-
403	0.006	0.023	-	-	-	-	-	-	-	-
404	0.011	0.015	-	-	0.005	-	-	-	-	-

HmRS80

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
170	0.005	-	-	-	-	-	-	-	-	-
174	0.011	-	0.023	0.008	0.005	-	-	-	-	-
176	-	-	0.012	-	-	-	-	-	-	-
178	0.011	-	0.017	-	0.019	-	-	-	-	-
180	0.011	0.008	0.017	-	0.024	-	-	-	-	-
182	0.033	-	0.006	0.039	0.024	0.041	0.043	0.021	0.022	0.042
183	0.011	-	-	-	0.005	-	-	-	-	-
184	0.049	0.146	0.058	-	0.067	0.066	0.029	0.021	0.087	0.123
186	0.005	-	-	-	0.010	0.004	-	-	-	-
188	0.049	0.069	0.081	0.023	0.067	0.098	0.071	0.009	0.022	-
190	-	0.008	0.006	0.008	0.005	-	-	-	-	0.002
192	0.120	0.154	0.069	0.078	0.087	0.103	0.100	0.086	0.109	0.203
196	0.120	0.115	0.138	0.172	0.120	0.053	0.129	0.316	0.174	0.029
198	-	-	-	-	-	-	-	-	0.022	-
200	0.092	0.146	0.126	0.094	0.149	0.213	0.143	0.128	0.044	0.108
202	0.016	-	0.006	0.008	0.005	0.008	0.029	-	-	-
204	0.049	0.069	0.086	0.078	0.058	0.008	0.086	0.060	0.044	0.161
206	0.011	-	0.006	-	-	-	0.029	0.021	-	-
208	0.076	0.077	0.069	0.094	0.096	0.066	0.086	0.086	0.087	0.152
212	0.103	0.054	0.046	0.141	0.072	0.062	0.071	0.004	0.022	0.015
216	0.103	0.054	0.103	0.180	0.106	0.172	0.086	0.184	0.196	0.049
220	-	0.100	0.075	0.070	0.048	0.045	0.043	-	0.065	0.018
222	-	-	-	-	-	-	0.014	0.004	-	-
224	-	-	0.017	0.008	-	-	-	0.060	-	0.002
226	-	-	-	-	-	-	0.014	-	-	-
228	0.011	-	0.012	-	-	0.012	-	-	-	0.004
230	0.022	-	0.023	-	0.010	0.004	-	-	0.044	0.002
232	0.005	-	-	-	0.014	0.037	0.014	-	-	-
236	-	-	-	-	0.005	0.008	-	-	-	-
240	0.005	-	0.006	-	0.005	-	0.014	-	0.044	0.015
244	0.005	-	-	-	-	-	-	-	-	-
248	-	-	-	-	-	-	-	-	0.022	0.075

HmNR106

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
342	0.011	-	-	0.024	0.026	0.029	0.029	0.025	-	-
344	-	-	0.011	-	0.016	0.008	-	-	-	-
346	0.177	0.091	0.347	0.294	0.284	0.343	0.229	0.240	0.364	0.507
348	0.097	0.061	0.063	0.111	0.077	0.079	0.100	0.244	0.068	0.145
350	0.043	0.167	-	-	0.062	0.017	0.029	-	0.068	-
352	0.011	0.015	0.023	0.016	-	-	-	-	-	-
354	-	-	-	-	0.010	-	-	-	-	-
360	0.011	-	-	-	-	-	-	-	-	-
366	0.011	0.008	-	-	-	-	-	-	-	-
368	-	-	0.006	-	-	-	-	-	0.023	-
370	0.005	-	0.011	0.016	-	0.004	-	-	-	-
372	-	0.008	0.017	-	-	-	-	-	-	0.002
374	0.016	-	0.006	0.008	0.010	0.017	0.014	0.008	-	-
376	0.043	-	0.023	0.016	0.021	0.017	0.014	-	0.023	0.002
378	0.081	0.197	0.063	0.024	0.052	0.099	0.157	0.185	0.046	0.002
380	0.038	-	0.017	0.008	0.057	0.046	0.043	0.042	0.023	0.046
382	0.145	0.114	0.097	0.064	0.067	0.070	0.043	0.076	0.091	0.007
384	0.048	0.038	0.028	0.008	0.036	0.012	0.086	0.013	-	0.002
386	0.145	0.129	0.108	0.127	0.150	0.112	0.071	0.101	0.114	0.123
388	0.054	0.144	0.085	0.119	0.057	0.095	0.100	0.013	0.136	0.141
390	0.043	0.030	0.051	0.135	0.031	0.012	0.043	0.038	0.046	0.004
392	0.011	-	0.028	0.008	0.041	0.041	0.029	0.017	-	0.018
394	0.011	-	-	0.024	-	-	-	-	-	-
396	-	-	0.006	-	0.005	-	-	-	-	-
398	-	-	0.006	-	-	-	0.014	-	-	-
400	-	-	0.006	-	-	-	-	-	-	-

HmNR120

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
223	0.005	-	-	-	-	-	-	-	-	-
235	0.016	-	0.017	0.015	0.015	0.004	0.014	0.034	0.065	0.009
239	0.011	0.030	0.006	-	0.015	0.016	-	-	-	-
243	-	0.008	-	0.023	-	-	-	-	-	0.002
247	0.100	0.149	0.058	0.062	0.123	0.193	0.083	0.071	0.174	0.360
251	-	-	-	-	0.005	-	-	-	-	-
255	-	-	0.012	-	0.005	-	0.028	0.088	-	0.002
259	0.047	0.075	0.041	0.031	0.044	0.012	0.028	0.004	0.022	0.011
263	0.063	0.052	0.023	0.023	0.020	0.008	0.014	-	0.022	0.029
267	0.063	0.015	0.064	0.123	0.064	0.094	0.097	0.105	0.022	0.007
271	0.037	0.052	0.052	0.115	0.020	0.004	0.042	0.050	0.044	0.110
275	0.063	0.030	0.047	0.015	0.034	0.078	0.069	0.092	0.022	0.002
279	0.032	0.105	0.064	0.069	0.044	0.029	0.028	0.004	0.065	0.265
283	0.026	0.067	0.017	0.008	0.025	0.045	0.069	0.042	0.044	0.022
287	0.037	0.112	0.023	0.008	0.069	0.098	0.042	0.067	0.109	0.026
291	0.021	0.008	0.070	0.046	0.069	0.037	0.069	0.008	0.065	0.053
295	0.090	0.060	0.058	0.092	0.064	0.062	0.042	0.004	0.044	-
299	0.037	0.015	0.041	-	0.059	0.033	0.069	0.227	0.065	0.002
303	0.068	0.060	0.058	0.085	0.093	0.074	0.125	0.046	0.044	-
305	-	-	-	-	-	0.037	-	-	-	-
307	0.021	-	0.064	0.031	0.044	0.004	0.014	-	0.022	0.015
309	-	-	-	-	0.005	0.057	-	-	-	-
311	0.068	-	0.041	0.039	0.054	0.045	0.014	-	0.065	0.002
315	0.037	0.037	0.076	0.039	0.015	0.008	0.014	0.017	0.022	0.018
319	0.042	0.037	0.052	0.008	0.005	0.004	0.042	0.008	-	0.004
323	0.037	-	0.023	0.008	0.025	0.021	-	-	-	0.002
325	-	-	-	-	0.005	0.004	0.014	0.038	-	-
327	0.032	0.022	0.041	0.154	0.034	-	0.014	0.029	0.022	-
331	0.005	0.008	0.006	-	0.015	-	0.014	-	0.044	0.050
335	-	-	0.012	0.008	0.010	-	0.014	0.013	-	-
339	0.016	0.045	-	-	0.015	0.033	0.014	0.021	0.022	-
343	0.016	-	-	-	0.005	-	0.014	-	-	-
347	-	-	0.017	-	-	-	0.014	-	-	0.009

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
351	0.005	-	-	-	-	-	-	-	-	-
355	0.005	0.015	0.012	-	-	-	-	-	-	-
361	-	-	0.006	-	-	-	-	-	-	-
367	-	-	-	-	0.005	-	-	-	-	-

HmNR20

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
183	-	-	-	-	-	0.004	-	-	-	-
187	-	-	0.024	0.031	0.005	-	-	-	0.022	-
189	0.032	-	0.006	0.008	0.029	0.037	0.042	0.034	-	-
191	-	-	-	-	0.005	-	-	-	-	-
193	0.095	0.105	0.125	0.177	0.128	0.145	0.111	0.178	0.087	0.113
197	-	-	0.006	0.015	0.015	0.008	0.042	0.042	0.022	0.109
199	0.011	-	0.012	-	-	-	0.014	0.004	-	-
203	0.032	0.008	-	-	-	-	-	-	-	-
205	0.016	-	0.018	-	-	-	0.028	0.064	-	-
209	0.005	-	0.006	-	-	-	-	-	-	-
211	-	-	-	-	-	-	0.014	0.009	-	-
215	-	-	-	-	-	-	0.014	0.110	-	0.002
217	0.032	0.030	0.012	-	0.020	0.008	-	-	0.022	0.087
221	0.037	0.157	0.060	0.123	0.093	0.157	0.014	-	0.022	0.076
223	0.026	0.015	0.054	0.077	0.064	0.017	0.069	0.051	0.044	0.011
227	0.121	0.127	0.089	0.077	0.103	0.120	0.069	0.030	0.130	0.048
229	0.053	0.090	0.060	0.046	0.059	0.054	0.083	0.182	0.022	0.017
231	-	-	-	-	-	-	-	-	-	0.002
233	0.216	0.142	0.226	0.185	0.167	0.087	0.181	0.161	0.217	0.209
237	0.053	0.037	0.012	-	0.010	0.004	0.042	0.034	-	-
239	0.095	0.037	0.071	0.023	0.078	0.116	0.139	0.025	0.087	0.178
243	0.005	-	0.030	0.023	0.005	-	0.014	-	-	-
245	0.079	0.097	0.155	0.115	0.123	0.116	0.083	0.038	0.109	0.017
249	0.026	0.008	0.006	0.092	0.039	0.066	-	-	0.065	0.117
251	0.011	-	0.018	-	0.025	0.004	-	-	0.065	0.002
255	0.005	0.022	-	-	-	-	0.014	-	-	-
261	-	-	0.006	0.008	-	-	-	-	-	-
279	-	-	-	-	-	-	-	-	0.022	-
285	-	-	-	-	0.005	0.012	-	-	-	-
287	0.005	-	-	-	0.005	0.017	0.014	0.034	-	-
305	0.005	-	-	-	-	-	-	-	-	-
311	0.021	0.097	-	-	0.010	-	-	-	0.022	0.002
315	-	-	-	-	-	-	-	-	0.022	-

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
319	0.021	0.030	0.006	-	0.015	0.029	0.014	0.004	0.022	0.009

HmNS19

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
173	0.013	0.008	0.006	0.025	0.011	-	-	0.018	-	-
177	-	-	-	0.017	0.006	-	-	-	-	-
178	0.006	-	-	-	-	-	0.029	-	0.023	-
180	-	-	-	-	-	-	-	-	0.023	0.002
182	-	-	-	0.008	0.017	0.005	-	-	-	-
185	-	-	-	-	-	-	0.015	0.004	0.023	-
186	0.019	-	0.055	-	0.066	0.045	0.059	0.071	0.046	0.018
187	-	-	-	-	0.011	-	-	-	-	-
188	-	-	-	-	0.011	-	-	-	-	-
189	0.006	-	-	-	-	-	0.015	0.004	-	-
191	0.006	-	0.012	0.025	-	-	-	-	-	-
192	0.019	-	0.006	0.008	0.028	0.050	-	0.004	-	0.005
193	0.019	-	0.012	0.008	0.006	-	-	-	-	-
195	-	-	0.006	-	-	-	0.015	0.009	-	-
196	-	-	-	-	-	0.010	0.029	0.004	0.023	0.009
197	-	-	-	-	0.011	-	-	-	-	-
198	0.013	-	0.031	0.033	0.006	-	0.015	0.031	0.046	0.106
199	0.006	-	0.006	-	0.006	0.015	0.015	-	-	-
200	-	-	-	-	-	-	-	-	0.023	0.112
201	-	-	-	-	-	-	-	-	-	0.005
202	0.006	-	0.012	-	0.006	0.010	-	-	0.023	0.007
203	-	-	0.018	0.017	0.017	-	-	-	-	0.007
204	-	-	0.018	-	0.006	-	-	-	0.023	-
205	0.006	-	-	-	-	-	-	-	0.023	0.002
206	-	-	0.006	0.033	0.006	-	-	-	-	-
207	-	-	0.006	-	-	-	-	-	0.023	-
208	0.013	-	-	-	0.017	0.030	0.029	0.013	0.023	0.025
209	0.006	-	-	-	0.011	-	0.029	0.009	-	0.002
210	0.031	0.040	0.012	-	-	-	0.015	0.004	0.023	0.002
211	0.013	-	0.024	-	0.011	0.035	0.015	0.097	0.023	-
214	0.006	0.008	0.012	0.100	0.011	0.015	-	-	-	-
215	0.006	-	0.006	0.092	-	-	-	-	-	-
216	0.013	-	0.031	-	0.017	0.030	-	-	-	0.016

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
217	0.050	0.024	0.012	0.033	0.066	0.040	0.029	0.080	0.046	0.016
218	0.025	0.024	0.006	0.017	0.022	0.025	-	-	-	0.007
220	-	-	-	-	0.011	-	0.015	0.035	-	0.002
221	0.025	-	-	-	0.022	-	-	-	-	0.007
222	-	-	0.006	-	0.006	-	-	-	0.046	-
223	0.013	-	0.031	0.025	0.028	-	0.029	0.093	-	-
224	0.069	0.040	0.092	0.100	0.060	0.050	0.074	0.013	0.091	0.094
225	0.025	-	0.043	0.017	0.006	0.015	0.044	0.066	0.046	0.089
226	-	-	0.006	-	0.011	-	-	-	-	0.002
228	0.006	0.040	0.031	-	0.006	0.010	-	-	-	-
229	0.006	-	0.006	-	0.006	-	-	-	-	-
230	0.031	0.113	0.037	-	0.022	0.005	0.029	0.062	-	-
231	0.113	0.347	0.055	0.025	0.071	0.079	0.103	0.133	0.046	0.009
232	0.019	0.008	0.018	0.008	0.028	0.059	0.029	0.009	-	-
233	0.013	-	0.031	-	-	-	0.015	-	0.023	-
234	-	-	0.006	-	-	-	-	-	-	-
235	0.013	-	0.018	0.050	-	-	-	-	-	-
236	0.013	0.008	0.012	0.025	0.017	0.059	0.059	0.009	-	-
237	0.006	0.016	0.006	-	0.011	0.030	-	-	0.023	-
238	0.050	0.016	0.067	0.067	0.060	0.064	0.044	0.053	0.046	0.018
239	0.025	0.008	0.012	0.008	0.022	0.005	-	-	0.023	0.028
240	0.006	0.032	0.012	0.025	0.006	-	-	-	-	-
242	-	0.008	0.006	-	-	-	-	-	-	-
243	0.019	0.008	0.006	0.008	0.006	0.005	0.044	-	-	-
244	0.019	0.008	0.012	-	-	-	-	-	-	-
245	0.013	0.008	0.012	-	0.017	0.005	0.015	-	-	0.021
246	0.094	0.121	0.031	0.017	0.044	0.040	0.059	0.146	0.114	0.163
247	0.013	0.048	-	-	-	-	-	-	-	-
248	-	-	0.006	-	0.006	0.010	-	-	-	-
250	0.006	-	0.006	-	0.017	0.010	-	-	-	-
251	0.006	-	0.012	0.008	0.006	0.035	0.015	-	-	-
252	0.019	0.008	0.043	0.017	0.011	0.035	0.015	-	-	0.184
253	0.019	0.024	0.043	0.017	0.028	0.055	0.029	0.004	0.046	0.012
254	-	-	0.006	0.058	-	-	-	-	-	-
255	-	-	-	-	0.006	-	-	-	-	-

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
257	0.006	-	-	-	0.011	0.005	0.015	0.018	-	-
258	0.006	-	-	-	0.017	0.010	-	-	-	-
259	0.019	0.008	-	-	0.006	-	-	-	-	-
260	-	0.008	0.006	0.017	0.017	0.025	-	-	0.046	0.009
261	-	-	0.006	-	-	-	0.015	-	-	-
264	-	-	-	0.025	-	-	-	-	-	-
265	-	-	0.006	0.017	0.006	-	-	-	-	-
266	0.013	-	-	0.025	0.011	0.015	0.015	-	-	-
267	0.013	-	-	-	-	-	0.015	0.004	-	-
268	0.019	0.016	-	-	-	-	-	-	0.046	0.021
273	-	-	0.006	0.008	0.006	-	-	-	-	-
275	0.006	-	0.006	-	0.011	0.010	-	-	-	-
281	-	-	-	-	0.028	0.059	-	-	-	-
288	-	-	-	-	-	-	0.015	0.004	-	-

HmidPS1.818

Allele	Broodstock: Abagold	Offspring: Abagold	Broodstock: Aquafarm	Offspring: Aquafarm	Broodstock: HIK	Offspring: HIK	Broodstock: I&J	Offspring: I&J	Broodstock: Roman Bay	Offspring: Roman Bay
140	-	-	-	-	0.005	-	-	-	-	-
142	-	-	-	-	-	-	0.014	0.013	-	-
144	0.037	0.091	0.023	0.131	0.014	0.045	0.042	0.050	-	-
145	0.021	-	0.023	0.031	0.010	-	0.042	-	0.044	-
146	0.068	0.030	0.073	0.139	0.058	0.057	0.069	0.008	0.044	0.004
148	0.005	-	0.006	0.015	0.005	-	0.042	-	-	-
150	0.090	0.061	0.124	0.031	0.139	0.062	0.167	0.118	0.130	0.057
152	0.053	0.038	0.017	0.031	0.014	0.029	-	-	0.044	0.011
153	-	-	0.006	-	-	-	-	-	-	-
154	0.053	0.038	0.096	0.077	0.115	0.115	0.056	0.046	0.022	0.009
157	0.274	0.379	0.225	0.215	0.135	0.148	0.208	0.248	0.217	0.472
158	0.158	0.076	0.146	0.100	0.159	0.131	0.139	0.097	0.174	0.306
159	0.021	-	0.028	0.031	0.019	0.004	0.042	0.025	0.152	0.079
160	0.084	0.061	0.096	0.023	0.135	0.111	0.083	0.177	0.087	0.039
161	-	-	-	-	-	-	-	0.004	-	-
163	0.037	0.083	0.034	0.023	0.034	0.074	-	-	-	-
165	0.026	0.106	0.079	0.100	0.043	0.045	0.028	0.038	0.044	0.020
166	0.005	-	0.011	-	0.019	0.033	-	-	0.022	-
167	0.058	0.030	-	0.015	0.034	0.041	0.014	0.029	0.022	0.002
169	0.005	0.008	-	-	0.053	0.103	0.014	-	-	-
172	0.005	-	0.011	0.031	0.010	0.004	0.028	0.147	-	0.002
175	-	-	-	0.008	-	-	-	-	-	-
176	-	-	0.006	-	-	-	-	-	-	-
180	-	-	-	-	-	-	0.014	-	-	-

APPENDIX F

PARENT PAIR LOD SCORES

Offspring ID	Parent pair LOD score
17_43	5.50E+01
IJM14	4.93E+01
B_64	4.82E+01
IJF42_1	4.79E+01
IJF68	4.70E+01
AQFA264	4.63E+01
AQFA270	4.63E+01
AQFB203	4.58E+01
HIK581	4.58E+01
Y_66	4.56E+01
15_46	4.54E+01
IJF69	4.50E+01
HIK585	4.41E+01
18_13	4.40E+01
AQFB266	4.38E+01
HIK511	4.36E+01
AQFB224	4.27E+01
AB109F	4.21E+01
HIK464	4.21E+01
HIK438	4.20E+01
AQFB244	4.15E+01
HIK407	4.13E+01
HIK591	4.11E+01
Y_89	4.11E+01
HIK334	4.10E+01
18_18	4.09E+01
Y_12	4.08E+01
HIK459	4.08E+01
HIK592	4.08E+01
IJM31	4.06E+01
AB93F	4.05E+01
AQFA226	4.04E+01
HIK549	4.04E+01
HIK537	3.98E+01
HIK313	3.96E+01
AB25M	3.92E+01
AB48M	3.91E+01

Offspring ID	Parent pair LOD score
15_36	3.88E+01
HIK600	3.88E+01
HIK523	3.87E+01
18_33	3.86E+01
IJF92	3.84E+01
IJM34	3.82E+01
AQFB230	3.81E+01
AQFB213	3.80E+01
HIK409	3.78E+01
HIK323	3.78E+01
HIK463	3.75E+01
IJF3	3.75E+01
IJF13	3.75E+01
IJF8	3.75E+01
18_6	3.74E+01
IJF84	3.74E+01
IJF74	3.73E+01
HIK406	3.72E+01
IJM2_1	3.72E+01
16_39	3.71E+01
HIK394	3.71E+01
HIK479	3.71E+01
AQFA271	3.71E+01
16_4	3.70E+01
IJM10	3.69E+01
HIK548	3.69E+01
IJF17	3.69E+01
AB88F	3.66E+01
AB64F	3.64E+01
16_19	3.64E+01
IJF59	3.63E+01
IJM23	3.62E+01
HIK408	3.60E+01
16_28	3.58E+01
16_45	3.57E+01
HIK383	3.54E+01
19_5	3.52E+01

Offspring ID	Parent pair LOD score
19_9	3.51E+01
HIK527	3.51E+01
IJF45	3.51E+01
AQFA258	3.50E+01
19_7	3.49E+01
AQFB255	3.48E+01
IJM8	3.47E+01
AB75F	3.45E+01
HIK663	3.45E+01
O-2_1	3.45E+01
17_5	3.45E+01
IJF104	3.44E+01
16_50	3.44E+01
HIK375	3.43E+01
HIK410	3.43E+01
17_25	3.43E+01
19_1	3.43E+01
17_22	3.43E+01
17_16	3.42E+01
16_22	3.38E+01
HIK472	3.38E+01
AQFB261	3.37E+01
AQFB225	3.36E+01
IJF47	3.35E+01
19_24	3.35E+01
AQFA282	3.34E+01
AB66F	3.34E+01
HIK586	3.31E+01
HIK525	3.30E+01
AQFA280	3.30E+01
AQFA261	3.27E+01
IJF76	3.26E+01
HIK330	3.25E+01
AQFA209	3.25E+01
18_31	3.21E+01
HIK308	3.20E+01
W_19	3.19E+01

Offspring ID	Parent pair LOD score
AQFA216	3.19E+01
HIK357	3.17E+01
AQFB270	3.16E+01
IJF106	3.15E+01
HIK532	3.14E+01
17_38	3.14E+01
AB96F	3.13E+01
16_49	3.12E+01
21_39	3.12E+01
15_49	3.11E+01
IJM22	3.10E+01
AB28M	3.10E+01
HIK505	3.08E+01
IJM9	3.06E+01
IJF7	3.05E+01
AQFA275	3.05E+01
AQFA329	3.01E+01
HIK369	3.00E+01
AQFA267	2.98E+01
AQFA245	2.97E+01
HIK466	2.96E+01
AQFB253	2.96E+01
HIK495	2.95E+01
B_53	2.95E+01
Y_22	2.94E+01
AQFA260	2.93E+01
HIK481	2.93E+01
AQFA207	2.89E+01
19_8	2.89E+01
HIK488	2.88E+01
16_9	2.87E+01
17_9	2.87E+01
Y_83	2.86E+01
AQFA286	2.82E+01
AQFB252	2.82E+01
AB45M	2.82E+01
AQFB207	2.81E+01

Offspring ID	Parent pair LOD score
19_22	2.81E+01
IJF62	2.81E+01
AB8F-F	2.78E+01
HIK327	2.74E+01
16_12	2.73E+01
AQFA214	2.73E+01
18_14	2.72E+01
18_16	2.71E+01
17_24	2.71E+01
17_35	2.71E+01
17_8	2.69E+01
17_46	2.69E+01
AQFA297	2.68E+01
HIK504	2.68E+01
HIK312	2.68E+01
AQFB218	2.68E+01
HIK524	2.64E+01
AQFA243	2.62E+01
IJF110	2.61E+01
HIK319	2.58E+01
HIK437	2.58E+01
AQFA213	2.58E+01
AQFB238	2.57E+01
HIK597	2.57E+01
AB68F_2	2.54E+01
19_26	2.53E+01
AQFA306	2.53E+01
IJF43	2.52E+01
17_51	2.52E+01
IJF66_2	2.49E+01
AQFA295	2.48E+01
HIK341	2.45E+01
AB71F	2.45E+01
HIK366	2.44E+01
AQFA304	2.43E+01
AQFB241	2.42E+01
AB26M	2.42E+01

Offspring ID	Parent pair LOD score
AQFA203	2.42E+01
AB83F	2.42E+01
AQFA322	2.40E+01
15_43	2.40E+01
HIK536	2.37E+01
AQFB265	2.37E+01
IJF67	2.36E+01
Gr_65	2.35E+01
15_47	2.34E+01
AB105F	2.30E+01
AB54M	2.29E+01
IJF20	2.29E+01
AB78F	2.28E+01
AB41M	2.28E+01
16_2	2.28E+01
HIK436	2.26E+01
19_23	2.22E+01
19_2	2.22E+01
B_6	2.21E+01
HIK483	2.20E+01
AB57M	2.19E+01
AQFA257	2.16E+01
AQFA256	2.14E+01
HIK499	2.07E+01
AQFA337	2.05E+01
16_51	2.05E+01
AB5F-F	1.99E+01
DOOD_1	1.98E+01
16_5	1.97E+01
AQFB236	1.95E+01
HIK545	1.93E+01
HIK307	1.92E+01
HIK476	1.92E+01
17_31	1.91E+01
AB68F_1	1.88E+01
AB53M	1.87E+01
IJF41_1	1.86E+01

Offspring ID	Parent pair LOD score
HIK461	1.82E+01
19_19	1.77E+01
AB43M	1.77E+01
AB10F	1.76E+01
AB46M	1.76E+01
HIK349	1.75E+01
HIK443	1.72E+01
IJF41_2	1.69E+01
AQFA228	1.68E+01
16_17	1.65E+01
IJF56	1.65E+01
17_2	1.64E+01
18_4	1.64E+01
HIK557	1.63E+01
Gr_17	1.60E+01
HIK348	1.60E+01
AB51M	1.60E+01
AQFA312	1.59E+01
HIK419	1.59E+01
W_84	1.59E+01
HIK544	1.58E+01
HIK368	1.57E+01
AB85F	1.56E+01
15_51	1.56E+01
AB69F	1.55E+01
20_19	1.54E+01
HIK320	1.51E+01
HIK374	1.50E+01
HIK390	1.48E+01
HIK578	1.47E+01
AQFA249	1.47E+01
18_46	1.45E+01
HIK516	1.45E+01
HIK560	1.42E+01
IJF29	1.42E+01
AB99F	1.40E+01
18_7	1.38E+01

Offspring ID	Parent pair LOD score
HIK423	1.38E+01
AQFB263	1.35E+01
HIK572	1.35E+01
IJF55	1.33E+01
HIK508	1.31E+01
16_21	1.31E+01
15_50	1.27E+01
AB44M	1.24E+01
IJM16	1.21E+01
AB98F	1.21E+01
HIK329	1.21E+01
17_3	1.20E+01
IJF79	1.19E+01
HIK473	1.18E+01
19_14	1.14E+01
HIK283	1.10E+01
15_41	1.08E+01
IJM26	1.08E+01
16_44	1.08E+01
AB61F	1.06E+01
IJF27	1.05E+01
HIK361	1.03E+01
19_6	1.03E+01
19_20	1.03E+01
17_47	9.64E+00
IJF31	9.58E+00
HIK482	9.55E+00
AB50M	9.52E+00
AQFB211	9.52E+00
AQFB215	9.51E+00
14_6	9.38E+00
AQFB204	9.12E+00
17_32	9.02E+00
HIK489	9.00E+00
AQFA212	8.84E+00
14_46	8.58E+00
13_39	8.56E+00

Offspring ID	Parent pair LOD score
17_48	8.55E+00
AQFA231	8.41E+00
HIK587	8.37E+00
HIK493	8.29E+00
HIK535	8.23E+00
16_13	8.23E+00
AB33M	8.19E+00
AQFA230	8.19E+00
13_52	8.14E+00
HIK475	7.87E+00
AB32M	7.79E+00
AQFA325	7.79E+00
AB106F	7.77E+00
IJF22	7.73E+00
17_30	7.71E+00
AQFB268	7.59E+00
IJM32	7.41E+00
IJF23	7.35E+00
19_18	7.23E+00
AB110F	7.14E+00
HIK420	7.14E+00
15_42	7.13E+00
AQFA342	7.13E+00
HIK328	7.06E+00
AQFB243	6.76E+00
O-4_4	6.66E+00
HIK564	6.57E+00
AB22M	6.55E+00
IJF82	6.34E+00
IJF38	6.31E+00
HIK563	6.30E+00
HIK502	6.29E+00
13_49	6.24E+00
AB67F	6.24E+00
15_11	6.14E+00
IJM13	6.05E+00
AB31M	5.92E+00

Offspring ID	Parent pair LOD score
IJF102	5.91E+00
16_23	5.90E+00
AQFB249	5.88E+00
IJF54	5.81E+00
AQFA202	5.66E+00
18_2	5.51E+00
AQFA206	5.47E+00
IJF65	5.36E+00
IJM11	5.30E+00
IJF66_1	5.30E+00
20_11	5.22E+00
HIK418	5.19E+00
16_46	5.19E+00
IJM2_2	5.13E+00
AQFA238	5.07E+00
IJF99	5.06E+00
14_33	5.04E+00
18_37	5.02E+00
W_64	4.85E+00
AQFB259	4.81E+00
14_40	4.77E+00
14_39	4.66E+00
AB63F	4.61E+00
IJF91	4.60E+00
AQFA254	4.52E+00
Gr_37	4.29E+00
13_41	3.98E+00
IJF15	3.89E+00
HIK561	3.87E+00
AB16M	3.82E+00
HIK389	3.78E+00
AB14M	3.77E+00
IJF50	3.70E+00
HIK509	3.62E+00
AQFA219	3.62E+00
14_37	3.53E+00
IJF98	3.51E+00

Offspring ID	Parent pair LOD score
AQFB202_1	3.26E+00
HIK568	3.26E+00
18_30	3.21E+00
O-4_28	3.20E+00
AQFA332	3.18E+00
B_27	3.09E+00
HIK514	3.02E+00
17_7	3.01E+00
HIK412	2.93E+00
HIK478	2.91E+00
AQFA236	2.81E+00
AQFA316	2.80E+00
AQFB233	2.78E+00
HIK422	2.75E+00
HIK385	2.71E+00
HIK401	2.61E+00
HIK538	2.57E+00
AB6F-F	2.47E+00
Gr_81	2.46E+00
AQFA273	2.38E+00
HIK398	2.34E+00
HIK343	2.33E+00
HIK392	2.22E+00
HIK345	2.19E+00
16_14	2.16E+00
AB91F	2.12E+00
AQFA247	2.09E+00
18_20	1.99E+00
HIK447	1.83E+00
AB55M	1.82E+00
15_33	1.81E+00
16_48	1.79E+00
HIK417	1.66E+00
AQFA326	1.64E+00
19_16	1.62E+00
AQFA330	1.62E+00
AQFA208	1.61E+00

Offspring ID	Parent pair LOD score
IJF105	1.59E+00
14_23	1.44E+00
AB38M	1.31E+00
HIK579	1.30E+00
AQFA225	1.24E+00
IJM30	1.23E+00
IJF40	1.17E+00
Y_76	1.16E+00
AQFB228	1.12E+00
17_14	1.10E+00
AQFB223	9.66E-01
W_27	7.96E-01
IJF101	7.53E-01
15_34	7.19E-01
16_3	6.27E-01
HIK684	5.13E-01
14_3	4.93E-01
HIK432	4.13E-01
HIK435	3.87E-01
AQFA279	2.92E-01
HIK510	1.71E-01
HIK347	1.25E-01
AB95F	1.07E-01
IJM20	5.17E-02