Communal or Separate Rearing of Families in Selective Breeding of Common Carp (*Cyprinus carpio* L.)

Thesis submitted for the Degree of Doctor of Philosophy

By

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Declaration

I hereby declare that this thesis has been composed entirely by myself and is a result of my own investigations. It has neither been accepted nor submitted for any other degree. All sources of information have been duly acknowledged.

Signature of Candidate: _____

Signature of Supervisor: _____

Signature of Supervisor: _____

Date: _____

Abstract

This study reports on investigation of ways of improving the breeding programme for growth-related traits in common carp in Vietnam. The base population was synthesized following a single pair mating scheme from six carp stocks: (1) 2nd generation of family selection; (2) Hungarian 6th generation of mass selection; (3) Hungarian scaled carp; (4) Indonesian yellow 6th generation of mass selection; (5) Indonesian yellow carp; and (6) Vietnamese 6th generation of mass selection. The next two selected generations were produced using a partial factorial mating scheme, with each family being split and reared using communal early rearing (CER) or separate early rearing (SER) methods. The second generation (G_2) was produced from selected fish from the CER G_1 group. The total number of selection, control and reference families was 135 in the G_1 and 101 in the G_2 respectively. The control and reference (Hungarian P33 line) families were produced by single pair mating (reference families with the G_2 only). Seven microsatellite loci were used for parentage assignment in the CER groups: 96.8% of the offspring (1284 individuals) and 96.2% offspring (1341 individuals) were unambiguously assigned to 113 families (selection, control) in the G_1 and 99 families (selection, control and reference) in the G_2 generations, respectively. Restricted maximum likelihood in the individual model was used to estimate phenotypic and genetic parameters. In CER, the estimated heritability values of common carp were from 0.20 ± 0.04 to 0.29 ± 0.05 for both weight and length at final harvest, indicating substantial additive genetic variation for selection on growth-related traits. The overall obtained maternal and common environmental effects were consistently close to zero. The average of direct response to selection for body weight was 15.0% per generation. In SER, the number of families in the G_1 and G_2 were 135 (selection and control) and 101 (selection, control and reference), respectively. The

heritability estimates were from 0.20 ± 0.07 to 0.31 ± 0.08 at final measurement. Common environmental (full-sib family) effect were all lower at tagging and slightly higher at last measurement, ranging from 0.05 to 0.22. The response in each generation of selection as the difference between the selection and control lines was 8.1% on average for weight at final harvest, lower than under CER. The high genetic correlations of growth-related traits between the third (one year old, mature) and second (7 months old) measurements could allow selection to be based on the earlier assessment, reducing handling stress close to spawning. The benefits of using microsatellite markers to ascertain parentage, achieve greater growth rate (close to farming systems), shorten time to maturity and selection, and the overall relative merits of using CER v's SER in this genetic improvement programme are discussed.

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Chapter 1. General Introduction

1.1. Common carp biology and aquaculture

1.1.1. Biology of common carp

Linnaeus (1758) reported that there was only one species in Europe namely *Cyprinus carpio* which was Danubian wild carp. Later, Kirpichnikov (1967) described four sub-species of wild common carp, the European and Transcaucasian *Cyprinus carpio carpio*, the Middle East *Cyprinus carpio aralensis*, the East Asian *Cyprinus carpio haematopterus* and the South Chinese and Vietnamese *Cyprinus carpio viridiviolaceus*. The two findings suggested that wild common carp can be divided into four distinct groups of geography: (1) the European wild carp represented in the region of the river Danube; (2) the wild carp from central Asian regions; and (3) the East Asian wild carp from Siberia and China and (4) the South-East Asian populations.

More recently, Balon (1995) and Kirpichnikov (1999) only identified two subspecies, the European wild carp *C.c. carpio* from the western region (Europe, Caucasus and Central Asia) and the Asian wild carp *C.c. haematopterus* from the eastern region of Eurasia. These two sub-species are differentiated by morphology, mainly by the number of gill rakers. Kohlmann et al. (2005) reported the analysis of wild and domesticated populations of common carp of different geographical origins using three types of genetic markers (allozymes, microsatellites and mitochondrial DNA). The results grouped common carp into two highly divergent clusters in Europe/Central Asia and East/South-East Asia, which also supported the two

subspecies *C.c. carpio* (Europe/Central Asia) and *C.c. haematopterus* (East-Asia), formerly distinguished only on the basis of morphological differences.

The species' habitat is in the middle and bottom water level in rivers, lakes and reservoirs. The best optimal growth of common carp is obtained at 23-30^{0}C water temperature and pH of 6.5-9.0. However, the fish can tolerate much colder (even ice on water surface) or hotter conditions. Salinity tolerance of common carp is up to about 5‰; the fish can survive at oxygen concentrations as low as 0.3-0.5mg/l (Flajshans and Hulata, 2006).

Common carp is an omnivorous fish and as a bottom feeder its main food is benthic organisms like aquatic insects, insect larvae, worms, molluscs and zooplankton. In addition, the fish also consumes leaves and seeds of aquatic and terrestrial plants and a range of other items. The carp finds much of its food by digging in the bottom, causing turbidity in the water. Common carp grows by 2-4% of its body weight daily and typically reaches 0.8kg to 1.5kg per fish after one season in subtropical and tropical regions in polyculture systems.

Female common carp matures later than male and spawning starts in spring when the temperature reaches over 17^{0}C. The fecundity of common carp is quite high, 150,000-200,000 eggs per kg body weight. The eggs are adhesive and stick to the substrate after release. Incubation takes 60 to 70 degree days depending on temperature. The hatched fry consumes its yolk and develops a swim bladder, so they can swim and eat external food after three days post-hatch at 20^{0}C.

1.1.2. Genetic variety of common carp

The common carp is one of the cultured fish species which has the longest history of domestication (Steffens, 1980). The fish has been cultivated in ponds in China as a food fish for nearly three thousand years (Hoffman, 1934). Its present cultivation extends throughout mainland China and South-East Asia. In Europe the common carp has been cultivated in ponds for several hundred years (Hickling, 1962), and its present cultivation extends from Siberia to the Mediterranean (Kirpichnikov, 1971). The Chinese and European races of the common carp have been separated from each other for a very long time, and they are known to differ in many characteristics, among them: body shape, growth rate, seine escapability, fecundity and hardiness (Hulata et al., 1974; 1976; 1980; 1982; 1985).The differences between the European and the Chinese races of carp were explained in terms of their respective adaptive evolution in the diverse carp farming practices (Wohlfarth et al., 1975) as following:

Fast growth rate appears to be highly favoured by natural selection for the following major reasons: (i) During the first few weeks after hatching, mortality of fish fry is very high because they are highly susceptible to diseases and parasites and are limited to food of very small particle size. Individuals that escape this critical stage, by fast early growth gain a considerable advantage especially in China. (ii) Another selective advantage of fast growth rate is due to the high correlation between fertility and body weight in fish. (iii) European breeders regularly selected the largest fish for breeding, a practice which gave fast growth rate a further advantage in Europe but not in China.

The disadvantages of large body weight and hence fast growth rate, are the following: (i) Larger fish are more susceptible to low oxygen concentration in the water. This factor is relatively more important under high (China) than low (Europe) density. (ii) In China,

where harvesting was done primarily by seining, smaller fish could escape the nets more easily than larger ones. This made fish size over a certain critical value highly unadaptive in China but not in Europe. In view of the above considerations it appears that the best evolutionary solution that optimally balances the advantages and disadvantages of fast growth rate in China would be a fast growth rate during early life, but maintenance of a relatively small adult body size. In Europe, on the other hand, the selection pressure for early (juvenile) fast growth rate is lower than that in China, but factors disfavouring post-juvenile fast growth rate are relatively unimportant. This explains why in the European carp early growth rate is slower, but later growth rate and adult size are much higher.

Specific adaptation of growth rate of the Chinese carp to poor pond conditions and of the European carp to favourable pond conditions are in terms of the different pond conditions to which the two races were exposed. Natural selection strongly favours full scale cover and demonstrated that scale reduction in the carp is associated with domestication, i.e. higher protection from physical damage and artificial selection.

Harvesting by seining in China as contrasted with pond drainage in Europe accounts for the high ability of the big belly to escape seining nets and their relatively long body which is the best shape for passing maximum body weight through the seine's holes. Selection of high fish by the European breeders, as described earlier, would work in the opposite direction, to create the relatively high and roundish European carp.

The wild common carp is characterized by an elongated torpedo-shaped body completely covered by scales. In Europe as well as in Asia, a large number of so-called breeds, local races and lines have been derived from it, mainly for human nutrition. An exception from utilization as food fish is the Japanese Koi carp that is

reared as an ornamental fish in garden ponds and tanks (Kohlmann et al., 2005). The various geographical races of common carp which differ in appearance and performance in aquaculture are treated as different stocks. Many varieties of common carp are distinguished based on the scaling pattern, including fully scaled carp, mirror carp and leather carp. This clarification is applied in aquaculture but is not justified taxonomically, since the basic scaling patterns are a result of simple Mendelian inheritance of two genes (Kirpichnikov, 1967).

Likewise, sub-classification based only on colour of common carp is also not justified for similar reasons. Colour variation in common carp is highly diverse. Blue common carp appears more frequently in domesticated varieties than in wild stocks and this is inherited as a simple recessive trait (Balon, 1995). Gold, red and orange are recessive traits and are found in many countries in both cultivated strains and wild populations. Some other colour variation has been changed due to selection, like Xingguo red carp and red purse carp developed by Chinese fish breeders (Wang et al., 2006). In Japan, many varieties of Koi carp (ornamental common carp) have been established by selective breeding and crossbreeding of colour mutants. Some single-colour types are inherited in a simple way, while multi-colour patterns seem to have a complex recessive inheritance (Sifa, 1999).

Planned selective breeding in common carp and other aquaculture species is dealt with in detail in sections 1.3 – 1.5.

1.1.3. Common carp aquaculture

1.1.3.1. Carp production

Common carp is one of the most popular aquaculture species, and contributed 13% (3,172,448 tonnes) of the total global freshwater aquaculture production in 2006 (FAO, 2007). Annually, the total production of common carp increased by 10.4% during the period from 1993 to 2004. The major producing region is Asia, where China produced 70% of the 2004 world production of carp.

In European countries, common carp production was 146,840 tonnes in 2004 and showed a substantial reduction from the highest production of over 402,000 tonnes obtained in 1990. The European market mostly requires live or freshly dressed fish, processed product would increase the price to less competitive levels. Otherwise, common carp culture is used for leisure, such as angling and pet fish.

1.1.3.2. Culture practices

Artificial seed production techniques have been well developed and implemented for common carp in hatcheries. Broodfish are normally kept separated by sex to avoid uncontrolled spawning which would happen if males and females were stocked together. Pituitary gland, pituitary extract or a mixture of GnRH/Dopamine antagonist can be injected to effectively induce and synchronize ovulation and spermiation (Drori et al., 1994). The adhesiveness of eggs may be eliminated in different ways such as using salt or urea and tannic acid bath, milk treatment or enzymatic treatment (Flajshans and Hulata, 2006). Artificial incubation is carried out in jars with circulated water until hatch. The hatched fry are most commonly nursed to fingerling size in

shallow ponds in monoculture system base on available zooplankton and supplementary feeding of zooplankton and starter feeds. Grow-out of common carp can be in extensive or semi-intensive ponds, in monoculture or in polyculture systems in combination with other species like tilapias, cyprinids and so on using natural and supplementary foods. Some common carp intensive monoculture systems use complete artificial food in cages, irrigation reservoirs, running water ponds and tanks or in recirculation systems. Integrated systems with animal husbandry and plant production are also applied in many countries over the world (Peteri, 2006).

1.1.3.3. Vietnamese common carp culture

Polyculture is the most common culture method for common carp in Vietnam. The fish is stocked with other carp species (grass carp, silver carp, bighead carp, etc.) and tilapia in a variety of aquaculture production systems (VAC system, rice-fish culture system, sewage-fed, etc.). The VAC system consists of three components: V is garden (horticulture), A is pond (aquaculture) and C is animal shed/pen (livestock husbandry). By-products of garden and livestock husbandry are available resources that can be applied to increase cultured fish production. There are many kinds of potential fertilizers for fish pond fertilization, including inorganic fertilizer and organic fertilizer (manure, green fertilizer, sewage). Manures from livestock and agricultural by-products are used more often and applied directly to the fish ponds.

A fish pond, especially a fresh water pond, usually produces a variety of food organisms in different layers of the water. Therefore, stocking species (or different sized classes of a given species) that have complementary feeding habits, or that feed in different zones, efficiently utilizes space and available food in the pond and increases total fish production. Moreover to maximize fish production with available

food organisms in ponds, polyculture, with a variety of fish in different feeding niches, has been commonly practiced. Tang (1970) described multispecies polyculture as a harmonious system where the available fish foods and stocked fish species are balanced, however the yield is low.

As stated in the study of Rothuis et al. (1998b), in Vietnam, rice culture remains the major agricultural activity, and fish production in rice fields is determined by rice management factors rather than by a fish polyculture strategy. Farm management is basically aimed at maximizing rice production. The developing rice and the low water level in the rice-field (3-5cm initially, 20cm at rice harvest) have an impact on the aquatic environment, and as such, on the fish. Frequent fertilization of the rice early in the crop cycle and a low plant density at this stage stimulates the development of phytoplankton and zooplankton. Afterwards, progressive shading by growing rice plants, and a limited nutrient availability, diminish plankton development. Consequently, the rice-field environment is characterized by large fluctuations in temperature and oxygen, and a limited availability of natural food resources for fish (Rothuis et al., 1998a). Inputs for fish are usually restricted to on-farm food resources, which are used in limited quantities, particularly during the early phase of fish rearing. Since fish production depends to a great extent on natural food, the choice of the fish species is determined by their capacity to utilize available food efficiently, as well as by their tolerance towards prevailing water quality conditions. Therefore, fish production depends to a great extent on naturally occurring food resources in the rice-field. At present, farmers stock a wide variety of species in polyculture, but the dominant species is common carp.

In common carp culture practices in Vietnam, fingerling is usually stocked in late February to March each year and harvested using large drag nets or pond drainage approximately 10 to 12 months later. The harvesting time is usually at the end of the lunar year since market price seems to be optimal at this time. The harvesting is also timed to enable ponds to be prepared for a new stocking season in the spring. In addition, the fish can reach the preferred marketable size of over 1 kg on average after 10 to 12 months.

1.2. Molecular genetic markers for selective breeding in aquaculture

1.2.1. Molecular genetics in aquaculture

The use of molecular genetic techniques in fisheries research has developed over the past forty years, particularly in the last decade, and now offers better opportunities for studying genetic variation at the molecular level by using a rapidly expanding range of technologies. The development of molecular markers started firstly from allozymes (enzyme) and then later to nucleic acid (DNA) that created a whole new set of questions and greater chances for genetic studies. Developments in molecular genetics are largely due to the increased availability of techniques and an improved awareness of the value of genetic data. Recently, molecular genetic research in fisheries has covered a wide range of topics from the development of markers for stock identification to the genetics of pathogenic organisms of commercially important species and the expression of genes.

Genetic approaches can provide valuable information and better understanding of the animals (Verspoor, 1998). Genetic markers can be used as a useful tool to assess

whether the genetic goals of the culture programme have been achieved. Molecular marker approaches combined with biometrical methods can improve the efficiency of breeding programmes for aquaculture species. In addition, genetic markers can be applied for genotyping and identifying of individuals and family groups that allows them to be stocked together in order to simplify experimental designs (Ferguson, 1995).

Many DNA markers are being used more frequently and effectively, and the amount of variation detected within and among populations and individuals may differ according to the type of markers. In general, mtDNA shows less variation within populations but more variation between populations than nuclear DNA because of its maternal inheritance and no known recombination (Hillis et al., 1996). The previous and existing studies of molecular variation in a wide range of farmed fish species show that molecular markers are mostly fairly easy to develop and identify. Furthermore, potential genetic markers for specific genes may be identified independently or from a survey of the existing literature.

While enzyme screening may be able to identify suitable (polymorphic) markers, it shows some limitation about sample collection and storage. Protein electrophoresis also surveys on a small portion of the genome that sufficient variation may not exist in assayable loci to discriminate between diverged populations. The development of PCR techniques is very useful that allows successful study on variation at DNA level (mitochondrial DNA-mtDNA and nuclear DNA-nDNA). Higher levels of variability at satellite (nDNA) and mtDNA loci make for better assessment of genetic change, particularly with regard to allelic diversity. However, it may be necessary to use a

combination of molecular markers (e.g., allozymes, mtDNA, nDNA), measurement of various traits (e.g., growth rate, behaviour) and hatchery records (if appropriate records have been kept) to assess genetic variance of captive aquaculture species (Penman, 1999).

1.2.2. The nature of genetic variation

Genetic variation is the basic background and fundamental material for the success of any selective breeding programme. The objectives of a selective breeding programme in fish are improvement of specific traits such as fast growth, high food conversion ratio and disease resistance. Such a programme should start from a base population with high genetic variation. During selection, the genetic material (gene pool) of the base population is changed directionally and reduced variance due to replacement of "negative" alleles for the traits concerned by "positive" alleles.

The nature of genetic variation in a population may be caused by different reasons like inbreeding, genetic drift, gene flow, mutation and natural selection that increase or reduce the level of variability. For instance, mutation usually contributes a very low frequency of genetic variation while both genetic drift and inbreeding always cause decreases in the amount of variation. The trend of selection and gene flow may either increase or decrease genetic variation depending on the particular situation.

It is an assumption that multiple genes control quantitative traits (Tave, 1993). Each gene that helps to produce a quantitative phenotype has different levels of variance depending on its alleles. The quantitative phenotypes exhibit continuous variation, firstly because each nuclear gene is inherited following Mendelian principles so that a gamete receives only one of two alleles at each locus segregated during meiosis. In

addition, many loci are involved in the production of a quantitative phenotype and each locus is undergoing segregation simultaneously and independently of all others, unless they are linked. As a result, the genetic make up of gametes and potential offspring varies to some degree so that the phenotype produces an approximately normal distribution in a population. Secondly, all such phenotypes are also influenced by environmental factors so different environmental conditions affect the production of individual phenotypic variabilities (Ferguson, 1995). The environment as radom variation factor, therefore, plays an important role in contributing to the production of continuous distributions of quantitative phenotypes in a population.

1.2.3. Molecular genetic analysis

There are three types of molecules that provide potential sources of genetic markers; these are DNA, mRNA and proteins. Of these, DNA, the genetic material itself, is the molecular basis of heredity with over 99% resident in the nucleus of the cell (nDNA). The remaining DNA is mitochondrial DNA (mtDNA) which is found in cellular mitochondria, small cytosolic organelles involved in energy production (Verspoor, 1998).

Isozyme (protein) electrophoresis was the dominant genetic markers and first applied in fish study in 1970s. The technique was primary used as molecular tool to characterize population genetic variation in various fish species (Carvalho and Pitcher, 1995). This technique is suitable for population studies as it is relatively inexpensive and requires little specialized equipment; it is also a rapid procedure to perform on a fairly large scale. However, allozyme markers do involve some problems, for instance, tissue collection and storage are very importance because protein electrophoresis can only assay enzymatically active proteins and many

important loci are assayed from organs such as the heart or liver, thus requiring the fish to be killed (Morizot et al., 1990). In addition, the marker is complex inheritance, linkage in a few group and difficult to standadize.

The DNA methods have generated increasingly more interest because the potential amount of genetic variation detectable by DNA methods vastly exceeds the amount detectable by protein methods. In practice, mtDNA is easily extracted and amplified from fresh, frozen, or alcohol-stored tissue. The mtDNA has found favour and is generally assumed to be more powerful than allozyme analysis for population study. Because the mtDNA is haploid and maternally inherited, it therefore has an effective population size only one quarter that of nDNA. Furthermore, the mtDNA seems to accumulate mutations more rapidly than do single copy nuclear genes. These have contributed to the popularity of mtDNA as a genetic marker in fish populations (Verspoor, 1998). Significant disadvantages of mtDNA analysis are that it is usually treated as a single character, whereas allozyme electrophoresis permits the examination of many independent characters known as loci. The ability to examine many independent loci is an important advantage of nDNA analysis, and may compensate in population analyses for the slower rate of evolution of nDNA genes compared with mtDNA genes. Because different regions of the mitochondrial genome evolve at different rates, certain regions of the mtDNA have been targeted for certain types of studies. For instance, many studies of mtDNA have used restriction fragment length polymorphism (RFLP) and sequencing of specific fragments of the mtDNA genome to interpret levels of divergence within and between fish populations.

One group of nuclear DNA sequences, microsatellite loci, are currently used for a very wide range of applications, from population genetics studies to linkage mapping.

Microsatellite loci are highly polymorphic repeated sequences and are distributed throughout the nuclear genome. The high mutation rates at microsatellites make them basically distinct from other nuclear DNA polymorphisms because of the fact that changes in allele frequency are more frequently affected by mutation as well as by genetic drift.

The introduction of the polymerase chain reaction (PCR) technique contributed strongly to the widespread use of DNA sequencing and fragment analysis, because it allows rapid amplification of particular DNA segments. The technique can be applied to both nuclear and mitochondrial encoded genes. As described by Dowling et al. (1996), differences among individuals in the number and/or pattern of DNA fragments can arise from a number of distinct processes, including changes in the amount of DNA, the structure of DNA, or the number or distribution of specific sites. The polymerase chain reaction (PCR) has rapidly developed as the most convenient way for the application of DNA marker technology since it requires only very small amounts of DNA for analysis (Utter, 1994). Also, most types of DNA analysis, even those based on larger quantities of DNA like the Southern transfer, can be done without killing fish, unlike allozymes, where particular tissues were needed.

1.2.4. Microsatellite markers for assessment of genetic variation

1.2.4.1. Molecular basis of microsatellites

Nuclear DNA is a valuable source of genetic information that researchers in fish genetics have only recently started to exploit. Many studies have been looking at nucleotide variation in the nuclear genome using different approaches, for instance, examining introns, looking at repetitive sequences and so on. Even though these

approaches and their implementation are very complicated, the potential for detecting variation is much more powerful than the mtDNA and isozyme analyses. Moreover if genetic variability exists, nuclear DNA studies are more likely to detect it. Microsatellite markers are currently the most commonly used polymorphic nuclear DNA marker in aquaculture and fisheries studies (Liu and Cordes, 2004).

Microsatellites are short regions of tens to hundreds of base pairs of DNA composed of repeated motifs (two to six base pairs, but generally dinucleotide, trinucleotide or tetranucleotide repeats are selected as markers). Microsatellites have attractive characteristics that can be developed as effective genetic markers for numerous applications in aquaculture and fisheries research (Wright and Bentzen, 1995). First, microsatellites are highly abundant in eukaryotic genomes, so sufficient markers can be readily identified and screened for a wide variety of research objectives. Secondly, many microsatellites exhibit extremely high levels of allelic variation, especially beneficial to a variety of research contexts. Third, microsatellite alleles are codominant markers following Mendelian inheritance and so are more informative in pedigree studies. Genotypes conform to Hardy-Weinberg expectations. Finally, because microsatellites are flanked by unique DNA sequences and can be synthesized by PCR, only small amounts of sample are required for analysis.

Since microsatellites are short tandom repeat sequences, they can be identified and observed by both manual and automated procedures. The most common observed microsatellite DNA is CA repeat in complement with GT. In order to find microsatellites composed of CA repeats, a synthesized complementary DNA fragment is used as a probe to screen for microsatellites. The target genomic DNA is digested by restriction enzymes to generate small fragments with an average length of 400bp

before cloning into an M13 phage vector. The phages containing $(CA)_n/(GT)_n$ insertion are identified by hybridizing with a $(CA)_n/(GT)_n$ probe. Positive clones are chosen for sequencing and PCR primer pairs are designed on the basis of flanking sequences. Then, the designed primers are used to amplify DNA from a genome template. A specific pair of primers will only amplify complementary sequences, revealing any size variants for different alleles and individuals (Griffiths et al., 1999).

1.2.4.2. The high variability of microsatellite loci

The amount of genetic variation in a population is measured by the number of alleles, their frequency and the level of heterozygosity at specific loci. If one allele of a locus is present at very high frequency and all others are at nearly zero, then there will be little heterozygosity because, by probability, most individuals will be homozygous for the common allele (Griffiths et al., 1999). The rate of mutations generating microsatellite repeat number variation is highest among all studied types of nuclear DNA markers; estimations for dinucleotide repeats range from 10^{-2} to 10^{-4} per generation. It is reported that variability at the molecular level occurs due to the addition or subtraction of single repeat units after mispairing of the two DNA strands during the replication process. It has been shown, however, that the stepwise mutation model does not fully explain observed allele frequency distributions within populations. Although allelic variation at dinucleotide repeat loci is predominantly due to single step mutations, rare changes of more than one repeat unit may occur as well. Furthermore, unequal crossing-over or recombination during meiosis may also cause polymorphism at the microsatellite loci (Sultmann and Mayer, 1997).

1.2.4.3. Application of microsatellite markers

Microsatellites are the most common DNA marker using to analyse mating systems and population genetic structure, despite the fact that their pattern of mutation is still poorly understood (Kocher and Stepien, 1997).

They may be especially useful for studies of fishes with low levels of allozyme or mtDNA variability resulting from inbreeding or strong reductions in population size, or where gene flow or recent isolation has limited genetic divergence. These genetic markers are potentially capable of detecting genetic structure on small spatial scales and over short periods of time. Microsatellite markers have rapidly developed as a very powerful tool for the analysis of mating systems and population structure because they are (1) highly variable markers even in species lacking polymorphism at allozyme loci; (2) codominant markers for which allele sizes can be scored exactly; and (3) amplified by PCR that makes it possible to work with a wide variety of types of samples.

The microsatellite markers utilise the feature of high mutation rate of short tandemly repeated sequences so that they are useful for studying the relationships at the individual, population and (closely related) species levels (Griffiths et al., 1999). For example, microsatellites revealed life-history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.) (Hansen et al., 2000) and population structure of Atlantic salmon (*Salmo salar* L.) (King et al., 2001). In the case of Atlantic cod (*Gadus morhua*), microsatellite DNA analysis showed that the population over-wintering in the inshore waters of Newfoundland is genetically distinct from the population that over-winters offshore (Ruzzante et al., 1997).

1.2.5. Microsatellite markers for parentage assignment

1.2.5.1. Tracability of microsatellite markers

DNA fingerprinting was proposed as a tool for reconstructing the pedigree of communally reared aquaculture populations, which would allow high intensity selection programmes to take place in production fish farms (Doyle and Herbinger, 1994). According to Rodzen et al. (2004), the development of DNA profiling techniques for family identification can reduce the problem of the introduction of environmental effects common to full sibs since fish are communal reared at very early stage in the same environmental condition. Selective breeding programmes based on a family design require the different families to be kept separately until the fry are big enough to be tagged (5-10g). Consequently, the length of this period is substantial. The consequences of this delay in tagging are both reduced selection accuracy and lower response to selection due to influence of confounding common environmental effects. Identification of families by their specific fingerprint allows the families to be kept together from fertilization. This will eliminate the problems related to common environmental effects and yield a higher selection response (Fjalestad et al., 2003). The use of genetic markers for parentage testing and pedigree reconstruction in aquaculture situations has also been suggested by many authors (Ferguson and Danzmann, 1998; Hara and Sekino, 2003; Sekino et al., 2003).

Parentage analyses based on DNA markers are increasingly being applied to retain pedigree information under communal aquaculture rearing situations (Estoup et al., 1998; Norris et al., 2000; Walker et al., 2002; Jerry et al., 2004). A major benefit of DNA parentage determination is that large numbers of progeny from many families can be pooled at very early stages of development without the requirement to

physically tag individuals, and without the influence of confounding early environmental effects on final trait expression (Doyle and Herbinger, 1994). The power of assignment tests depends on a number of factors including genetic differentiation among populations, the number of population samples, the degree of polymorphism at the loci, the number of loci studied and sample sizes (Bernatchez and Duchesne, 2000; Hansen et al., 2001). Currently, parentage testing using genetic markers in domestic animals is mostly based on exclusion techniques. Exclusion is a simple and efficient method for assigning parents to an offspring that uses incompatibilities between parents and offspring base on Mendelian inheritance rules. A major drawback of exclusion is that a single mismatch between parent and offspring genotypes is enough to exclude a potential parent, thus making this technique extremely sensitive to genotyping errors or mutations (Jones and Ardren, 2003).

Microsatellites are a valuable tool in breed identification and family selection programmes in which genetic tagging will allow different genotypes to be reared together, thus greatly reducing the impact of environmental variance and the number of replicate ponds needed in some contexts (Garcia de Leon et al., 1998). Microsatellite DNA loci have already been isolated and characterized in several fish species including salmon (O'Reilly et al., 1998), rainbow trout (Herbinger et al., 1995; Estoup et al., 1998), turbot (Estoup et al., 1998), sea bream (Perez-Enriquez et al., 1999), tilapia (Lee and Kocher, 1996) and common carp (Crooijmans et al., 1997; Aliah et al., 1999). The use of microsatellite markers in breeding programmes allows the identification of parental effects on offspring performance from very early life stages. It also suggests that microsatellites may greatly improve experimental selection protocols as they allow designs in communal environments (Garcia de Leon

et al., 1998). Several studies have empirically used microsatellite loci to successfully reconstruct pedigrees in fish populations with families mixed from hatching (Herbinger et al., 1995; Estoup et al., 1998; O'Reilly et al., 1998; Perez-Enriquez et al., 1999; Norris et al., 2000). They have been used successfully to reassign progeny from mixed pools to their parents in several species, including sea bass *Dicentrarchus labrax* (Garcia de Leon et al., 1998); turbot *Scophthalmus maximus* (Estoup et al., 1998); channel catfish *Ictalurus punctatus* (Waldbeiser and Wolters, 1999); 15 microsatellite loci for 93% parentage assignment in rainbow trout, *Oncorhynchus mykiss* (Fishback et al., 1999); 14 loci for 92% parentage assignment in chinook salmon, *Oncorhynchus tshawytscha* (Olsen et al., 2001); 8 microsatellite loci for 98% parentage assignment in Atlantic salmon, *Salmo salar* (O'Reilly et al., 1998; Norris et al., 2000); 4 microsatellite loci for 73% parentage assignment in red sea bream, *Pagrus major* (Perez-Enriquez et al., 1999); 8 microsatellite loci for 95% parentage assignment in Hungarian mirror carp (Vandeputte et al., 2004); 8 microsatellite loci for 90% parentage assignment in Japanese shrimp, *Penaeus japonicus* (Jerry et al., 2005).

1.2.5.2. Microsatellite markers and parentage assignment for common carp

The microsatellite markers of the poly (CA) type in common carp have been isolated from a common carp library and sequenced. These loci for common carp are valuable as genetic markers for use in population, breeding, and evolutionary studies (Crooijmans et al., 1997). Desvignes et al. (2001) used allozyme and microsatellite markers in genetic variability studies on cultured stocks of common carp comprising six strains from extensive aquaculture in two French regions and five strains from the Czech Republic stemming from artificial selection and maintained in the Research

Center of Vodnany. The genetic variability of microsatellites for the whole data set was considerably higher than that for allozymes. In the study of Tanck et al. (2000), microsatellite allele frequencies showed that the common carp from Anna Paulowna Polder in the Netherlands were significantly different from a group of carp originating from several different domesticated strains.

The use of microsatellite markers for parentage assignment of common carp has been assessed. About 95% of 550 carp offspring were assigned exactly to single parental pairs in a full factorial cross of 10 dams x 24 sires using eight microsatellite markers with the mean number of 7.75 alleles (Vandeputte et al., 2004). Using two multiplex PCRs of five microsatellite loci each, with the mean number of 18.2 alleles per locus, 93.2% and 98% of offspring were allocated to single families in groups coming from 28 pairs and 26 pairs of parents, respectively (Gheyas, 2006). Such parentage assignment in common carp may allow more precise estimation of the genetic parameters in a breeding programme using factorial designs which separate additive, dominace and maternal components of variances without environmental bias.

1.3. Selection methods and genetic improvement analysis for aquaculture species

1.3.1. Selection methods

A selection programme is carried out to identify and select individuals with better additive genetic merit for the traits in question as parents for the next generation, and to continue this over several generations to improve performance for these traits. The effect of selection is to change gene frequencies, that are observed by the change of

the population mean. However, it is also necessary to minimise inbreeding in the population during selection.

There are many selection methods that have been applied to fish, that all aim at estimating true additive genetic merit and applying this. The most commonly used selection methods in fish are individual selection, family selection and combined selection, which are described in some detail as follows:

1.3.1.1. Individual selection

Individual selection (so-called mass selection) is only based on the phenotype/performance of individuals. This is a very popular method of selection used in animal breeding as well as for many aquaculture species. Individual selection is a simple method with many advantages for implementation, such as low cost and rapid response, however there can be serious drawbacks due to environmental and age differentiation, and uncontrolled inbreeding.

In addition, individual selection can only measure and select individuals that are alive, so it is problematic for selection of traits such as meat quality and disease resistance, which have low heritabilities or for which individuals need to be killed/infected for assessment. It is reported that mass selection of fish species is more practical on traits of fair or high heritability, like growth rate (h^2 values often 0.2-0.4).

To apply individual selection it is very important to keep all individuals in the same environment and consolidate other factors at any stage of the life cycle. Also, it is very important to this selection method to try to keep Ne as high as possible. Doing

these will reduce errors and inbreeding depression and thus increase the selection gain.

1.3.1.2. Family-based selection

Family selection is a method of selection in which parents for the next generation are selected on the basis of the ranking of the mean performance of each family so selection decisions are made for the whole family, hence it is also called between-family selection. The families are primary kept separate and then individuals from all families are tagged as early as possible before communal stocking and rearing. Family selection uses information from families so environmental conditions for all families should be standardised to minimize common environmental variation. The individuals selected as breeders for the next generation may be derived from all individuals within the selected families or randomly chosen from all selected families. Family selection only uses the individual's information after determination of the family mean.

The main advantage of communal rearing is to eliminate environmental differences between families, enabling us to overcome one of the main challenges in family selection that allows us to deal better with traits where animals need to be killed for assessment or where h^2 is low such as threshold traits, carcass quality traits and disease resistance. Furthermore, the efficiency of family selection depends on the number of individuals in each family, or family size. The larger the family size, the closer the phenotypic and genetic means. The estimation of breeding values is based on phenotypic observations only on full-sib and half-sib families in family selection, which is very useful for traits which are cannot be measured on individuals selected as parents for the next generation. The high cost of operation may be a major issue, since families are normally reared separately up to tagging.

1.3.1.3. Combined selection

If more than one selection method is used in a breeding programme, it is called combined selection. This aims to maximize the rate of genetic gain. The advantages of combined selection are to combine and optimize available sources of information that can be useful for breeding value estimation such as information on individuals, information about full-sibs and half-sibs, progenies and pedigree information. It is therefore the most effective selection method for a breeding programme. The most popular methods usually combined are between-family and within-family selection that make use of information on both family deviations and mean phenotypic values of individuals.

1.3.2. Genetic improvement analysis

1.3.2.1. Traits for selection

A breeding programme focuses on the accumulation of a series of short-term genetic changes in the population, so initial definition of traits for selection is a priority considered in all animal selective breeding programmes. The most preferable criteria for inclusion of traits in the breeding programme are their current value and future potential. The traits for selection should meet criteria such as economic and ethical importance, genetic variance, possibility of measuring and evaluating at a reasonable cost.

1.3.2.2. Genetic parameters and estimation

Effective breeding plans are based on the knowledge of genetic and phenotypic parameters in a particular population. Genetic parameters are characteristics of the

particular population and it is very important to measure them and make decisions for selection based on this. Genetic parameters are functions of the covariance and variance components of the traits. Therefore the calculation of genetic parameters means the estimation of variance components.

Genetic parameters are not constant: they may be changed due to selection or management over years (Koots et al., 1994). The estimation of genetic parameters differs depending on breeds (Trus and Wilton, 1988; Koots et al., 1994), methods of estimation (Mohiuddin, 1993), data origin, management (Tess et al., 1984) and over time (Koots et al., 1994). For example, Ferrera et al. (1999) reported that genetic parameters estimated from sire models are lower than from full animal models or sire-dam models, while full animal models gave similar results to sire-dam models.

Heritability is a very important parameter that measures the strength of relationship between phenotypic and genetic values for a trait (Bourdon, 2000). Heritability in selection is the proportion of additive genetic variance in the total phenotypic variance so that the magnitude of the heritability determines the expected response to selection in a population (Van Vleck et al., 1987). The prediction of breeding values and selection response are based on genetic heritability. The heritability of a trait is unstable and may change due to differences of populations and environment. As a result, estimation of heritability for traits of economic importance in a particular population can only show the genetic progress expected from selection for improvement of the specified trait in that population in the given environment.

A higher heritability for a trait indicates true breeding value of an animal with better performance record, because when heritability is higher the prediction of breeding

values will be more accurate. There are some ways to increase heritability such as making the environment more uniform, measuring the traits more accurately and adjusting the known environmental effects (Bourdon, 2000).

Genetic correlation is a parameter to measure the relationship between breeding values of two traits (Bourdon, 2000). A genetic correlation represents the correlation between the additive breeding values for two traits or between the sums of additive effects of the genes influencing both of the traits. Genetic correlations of traits can result from a single gene affecting more than one trait and/or linkage effects, that is, the occurrence of two or more loci that affect the same trait on the same chromosome. Therefore, when two traits are genetically correlated, selection for one will cause genetic changes in the other. Furthermore, the breeding value of one trait can be predicted based on the observed performance of another trait that is strongly genetically correlated with that trait. Knowledge of the magnitude of genetic and phenotypic correlations is important for multiple trait evaluation, particularly when predicting correlated responses to selection (Falconer and Mackay, 1996). Significant negative or positive correlations indicate that selection for or against a trait would influence other correlated traits.

1.3.2.3. Methods for estimation of genetic parameters

Genetic evaluation involves collecting and forming the available information into a single value for each individual that can be used to rank the population for selection. Tools for genetic evaluation have been developed and applied to estimate breeding values and provide more accurate predictions of genetic merit of animals for economically important traits. There are several methods for estimation of genetic

parameters, from simple analyses such as Analysis of Variances (ANOVA) to more complex ones such as Maximum Likelihood (ML) and its modification, Restricted Maximum Likelihood (REML) (Meyer, 1989). Breeding values are very important for final decisions about which animals are to be selected as parents for the next generation. In order to achieve estimates of breeding values for animals for traits of interest, (co)variance components have been calculated with better accuracy by using several practical procedures, as below:

Best Linear Unbiased Prediction (BLUP): Henderson (1953) firstly developed a procedure for predicting breeding value for selection which was later named best linear unbiased prediction (BLUP). BLUP was developed and is used as a standard procedure to predict breeding values (BV's) for selection. As a result, BLUP has found widespread application in genetic evaluation in animal breeding programmes because of its desirable statistical properties to estimate breeding values close to the true breeding values (BV's) of animals by using a simple linear mixed model. The equation of BLUP is:

$$y = Xb + Zu + e$$

where

y = vector of animal records,

b = vector of unknown fixed effects,

u = vector of unknown random BV's belonging to the animals making the records,

e = vector of unknown random residual effects,

X = known incidence matrix relating records to fixed effects in vector **b**,

Z = known incidence matrix relating records to BV's in vector **u**.

The above linear model has been developed and applied for animal breeding as the "Animal Model" that can be modified based on certain assumptions and characteristics of the data.

Animal model: The model aims at estimating breeding values of all animals based on their own data and/or data of their relatives, based on assumptions that the animals are derived from a single population and may have more than one record (Elzo, 1996).

Data used for genetic evaluation have their own structure of records. For instance, some parents have no records, and some dams are related. So the animal model can be made to fit the estimation depending on the types of data. Quaas and Pollak (1980) developed the reduced animal model which allowed equations to be set up only for parents. These modified versions include the Sire Model and the Sire Dam Model (Elzo, 1996).

Sire model: The purpose of sire model is to evaluate only sire effects using progeny information. This model is applied for the condition that parents have no data and dams are unrelated. This model is simpler than the animal model but dam effects are not calculated.

Sire-dam model: The purpose of this model is to evaluate maternal effects in addition to sires. This model can be used when parents have no records of their own.

Relationship matrix: The relationship matrix represents the relationships among any number of animals which are created from the pedigree file. This matrix contains the additive relationship between any two individuals, explaining the probability that the two alleles at a random locus are identical by descendent in the two individuals (Elzo,

1996). Therefore, the information from relatives is important particularly for traits that have low heritability and sex effects (Wood et al., 1991).

Connectedness: Connectedness can be defined as a measurement of the genetic relationships among populations that could affect the accuracy of covariance component estimates of a trait in one or more populations in relation to that of another. Mathur et al. (1998) reported that the higher the degree of connectedness, the more accurate the comparisons of estimated breeding values (EBV's) across populations will be. Data with good connectedness should result in better accuracy of genetic variance estimation (Schaeffer, 1975)**.**

Computational software: As more complex models have been developed for genetic evaluation, more advanced computer softwares have been required. Several computer software packages have been used to estimate genetic parameters such as Least-Squares Analyses (Harvey, 1960), Variance and Covariance components (Henderson, 1977), Maximum Likelihood (Harville, 1977), Restricted Maximum Likelihood (Patterson and Thompson, 1971), Expectation-Maximization (Dempster et al., 1977), Derivative-Free Restricted Maximum Likelihood (Graser et al., 1987), Multiple Trait Derivative-Free Restricted Maximum Likelihood (Boldman et al., 1995), ASREML (Gilmour et al., 2002) and SAS (SAS Institute Inc., 2002).

1.4. Selective breeding in aquaculture

1.4.1. Selective breeding in aquaculture species

Scientifically-based selective breeding of aquaculture species has been carried out only very recently and is thus new compared to livestock species (Gjedrem, 2005), although the domestication of fish has been practiced since 4000 to 5000 years ago in

China. Positive selection responses were found for improvement of disease resistance in brook trout (Cipriano et al., 2002), increasing early spawning, egg number and yearling weight in rainbow trout in USA. The first estimate of heritability in fish was for body weight of rainbow trout published by Aulstad et al. (1972) then breeding programmes of Atlantic salmon, coho salmon, chinook salmon and brown trout were later carried out. The traits for selection were body weight, age at sexual maturation, fat percentage, flesh colour and disease resistance in rainbow trout (Rye et al., 1990), coho salmon (Hershberger et al., 1990; Beacham and Evelyn, 1992), chinook salmon (Winkelman and Peterson, 1994), Atlantic salmon (Rye et al., 1990; Rye and Refstie, 1995; Rye and Gjerde, 1996). In Asia, most selective breeding programmes were for growth traits of fish, which were improved successfully in silver barb (Anon, 2002; Hussain et al., 2002), rohu (Anon, 2002) and blunt snout bream (Li and Cai, 2003). The most common selection methods were individual and family selection. While individual selection was more efficient for high heritability such as growth traits, it was less reliable for traits of low heritability. Family selection with pedigree records was applied in many selective breeding programmes, particularly for traits of low heritability and those difficult to measure, for instance, flesh quality trait, age at sexual maturity and survival. Two large successful selective breeding programmes in fish were demonstrated by AKVAFORSK for salmon and ICLARM (International Center for Living Aquatic Resources Management) for tilapia.

The breeding programme of Atlantic salmon and rainbow trout in AKVAFORSK, Norway started in 1971. The base populations were collected from different locations and populations then they were crossed to produce synthetic populations. Growth rate was the primary selected trait in the first two generations, and after that age at maturity was included from third generation and disease resistance and meat quality

were added from the fifth generation. The breeding programme applied family selection method for all traits while individual selection was also implemented within families for growth rate. All estimates of heritability for body weight were over 0.2 which indicated good prospects of genetic improvement for this trait. Evaluation of genetic gain demonstrated 13-14.4% per generation for growth trait in rainbow trout and Atlantic salmon, respectively (Gjerde, 1986). The improved Atlantic salmon grew 83.9% faster after 6 generations of selection compared to the wild stock or the genetic gain was estimated to be about 14% per generation for growth rate and a reduction of 12.5 units in sexual maturity or 8% per generation (Gjerde and Korsvoll, 1999).

The Genetic Improvement of Farmed Tilapia (GIFT) programme was started in the late 1980s by ICLARM. The selective breeding programme was initiated by documentation of tilapia genetic resources from Asia and Africa. Promising strains of Nile tilapia from Africa and available Asian cultured stocks were collected and established to be evaluated with cultured stocks in the Philippines in a wide range of farming systems and agroclimatic conditions. The breeding goal of the GIFT programme was to develop more productive stocks of tilapia by selection for high growth rate and other economically important traits (e.g. disease resistance and maturation rate) (Pullin et al., 1991). After five generations of selection, the GIFT fish had obtained genetic gain from 12% to 17% per generation and a cumulative increase of 85% in growth compared to base populations. Testing of the second generation in four other countries (Bangladesh, China, Vietnam and Thailand) also revealed higher growth rates and better survival rates than in local strains although the GIFT tilapia did not obtained performance as estimated.

The experiences gained from selection in salmonids and tilapia has resulted in similar programmes in other fish and shellfish species, which often showed 10% to 15% genetic gain for growth rate per generation and similar responses could be obtained for other traits of interest. As result, about 1% to 2% of farmed fish and shellfish were produced from breeding programmes today, while this figure was 65% in Norway (Gjedrem, 2000). So selective breeding is a very powerful tool for production improvement in aquaculture.

1.4.2. Selective breeding in common carp

Mass selective breeding programme for better growth and cold tolerance in common carp was firstly analyzed and reported in Russia by Kirpichnikov et al. (1974). The programme carried out crossing of cold tolerant Amur wild carp with the fast growing Galician carp and applied individual selection for five generations. There was no evidence for the efficiency of growth rate selection, but the fish improved from 30% to 77% survival over winter. Another mass selection programme in Russia increased 0.5% to 1.4% per generation in growth trait (Kirpichnikov, 1993).

In Israel, the first selective breeding programme focused on growth rate for five generations (Moav and Wohlfarth, 1973). The selected line showed a very small response to selection in the first generation and then decreased even lower compared to the control line in the fifth generation. Realized heritability was 0.3 in the first three generations and declined to zero in generation 4 and 5. The authors indicated that this stock had already reached a selection plateau for fast growth rate and inbreeding depression could be happened in the selection population. Another study looked at selecting for body shape (height/length ratio) in one generation (Ankorion et al.,

1992). This showed high realized heritability (over 0.33) for the selected trait but no evidence for clear correlation with growth rate.

Many strains/varieties of common carp have been developed during its long history of culture in China. Of which, Jian carp was selected through a six generations combined breeding programme involving family selection, inter-line crossing and gynogenesis. Improvement of the growth rate in pond culture over several generations has been developed although a highly significant genotype × environment interaction was observed when growth was compared in pond and cages. The Jian carp were shown to growth faster than several other varieties of common carp in China (Penman et al., 2005). Further selection of Jian carp for one more generation during 1999-2000 resulted in increased growth by 6% and 9% in mono and polyculture, respectively (Anon, 2002).

Individual selection for growth rate of carp in Vietnam produced an increase in body weight over two generations and estimated realized heritabilities of 0.2 to 0.29 only in one line out of three selected lines. Growth rate of selected fish had increased 33% compared to the base population after five generations of selection (Thien, 1996). However, realized heritability decreased to nearly zero by the sixth generation so the author suggested that family selection should be applied in the breeding programme.

Recently, more efficient carp selection programmes have been set up, including control of pedigree information for family selection by applying physical tagging and genetic tagging methods. Wang et al. (2006) provided estimates for growth-related traits in Oujiang colour common carp population, with heritabilities of 0.14-0.30. In other studies, microsatellite markers were applied to assign early communal rearing of progeny to their parents in selective breeding of carps. The estimation of genetic

parameters based on parentage assignment showed that heritabilities for weight and length in European selective mirror carp ranged from 0.33 to 0.37 (Vandeputte et al., 2004) and even over 0.5 (Kocour et al., 2007). The findings showed relatively high positive genetic and phenotypic correlations between the traits of interest. Therefore, it is suggested that genetic improvement of common carp for growth-related traits is feasible and it should be possible to efficiently achieve a positive response to selection in Vietnamese carp.

1.5. Genetic resources of common carp and selective breeding in Vietnam

1.5.1. Common carp genetic resources

Common carp is one of the most important traditional aquaculture species in Vietnam. The fish is cultured in a variety of farming systems in different agroecological zones. Tuan (1986) reported that there were eight races or local strains of common carp based largely on morphology but the main cultured strain was Vietnamese white carp. However these fish show very slow growth performance even though they have high survival rate and good disease resistance.

In the 1970s, Hungarian scaled carp and Indonesian yellow carp were introduced to Vietnam. Crossing between three landrace carps (Vietnamese, Hungarian and Indonesian) was carried out to improve survival rate and growth of hybrids. The results showed higher survival and better growth performance of hybrids compared to crossing within landraces.

1.5.2. Overview of carp selection in Vietnam

To overcome the difficulty in keeping/maintaining pure *C. carpio* strains for hybridization, mass selection of common carp was conducted to develop three lines having fast growth. This programme was started in 1985 from three base stocks, Vietnamese white carp, Hungarian scale carp and Indonesian yellow carp. These carp strains were crossed with each other to produce different offspring combinations and carried out selection (Figure 1.1). Four generations of selection were carried out over six years (1985-1991). The realized heritability estimates were 0.29, 0.20 and 0.05 in generations 1, 2 and 4 respectively. After five generations of mass selection, the growth rate of selected fish had increased 33% compared to the base population (Thien, 1993). A decline in the response to selection was noticed by the F_5 generation and a decision was made to change from mass selection to family selection.

Family selection of common carp was conducted at Research Institute for Aquaculture No.1 (RIA 1) from 1998 to 2001 for growth and survival rate traits. Using the fifth generation of common carp from mass selection as the initial material, the programme succeeded in producing two new generations. However, the first generation was selected from only the five best families out of 24 evaluated. After two generations of family selection, these fish were estimated to show 7% faster growth compared to the base population, but it was realised that the numbers of families involved was two small for continued selection.

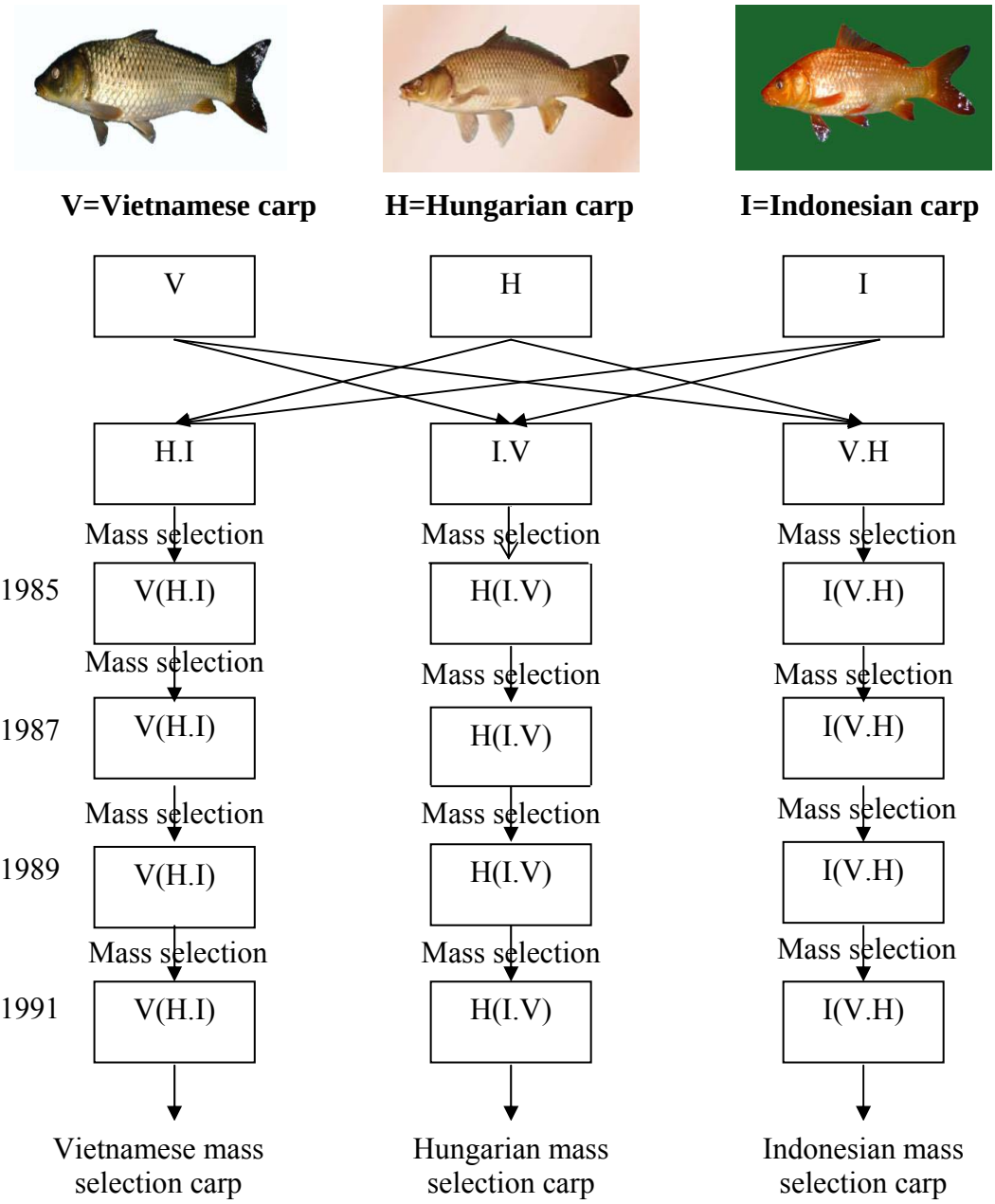

Figure 1.1. Mass selection of common carp in Vietnam from 1985 to 1991.

1.5.3. On-going selective breeding programme

The selected lines derived from mass and family selection have shown problems such as low survival rate, high abnormality percentage and colour variation for the last few years which could be the result of bad management or inbreeding during selective breeding. For instance, the progeny exhibited a high percentage of yellow colour which was inherited from Indonesian yellow carp or violet colour originating from one special race of Vietnamese carp. Such colour traits are recessive in common carp (Trong, 1967; Komen, 1990). Furthermore, greater productivity of common carp is requested by farmers. It is therefore necessary to carry out a genetic improvement programme for growth rate and uniform wild type colour in common carp by using a combination of between and within family selection methods. This selection programme was supported by the WorldFish Center, through Asian Development Bank (ADB) funding, from 2004. Six common carp lines were used to found a base population. Ranked by their gene pool contribution (number of brooders), these were: (1) 2nd generation of family selection; (2) Hungarian 6th generation of mass selection; (3) Hungarian scale carp; (4) Indonesian yellow 6th generation of mass selection; (5) Indonesian yellow carp; and (6) Vietnamese 6th generation of mass selection. One hundred and one full-sib families were produced and nursed in separate hapas. Eighty six families were available for PIT tagging and communal rearing in pond. Fifteen families were lost due to mortality. Subsequently, 250 individuals (150 females and 100 males) were selected from the best performing 63 families. Furthermore, a control population was established by keeping 100 fish (60 females and 40 males) from 30 families with performance close to the overall mean of the 86 families.

1.6. Aims of the Thesis

The common carp has a long history of domestication and development, thus its culture technologies and artificial seed production have been studied intensively. Some family-based selective breeding programmes on common carp have been undertaken but a major challenge for these has been the difficulty of keeping a large number of progeny groups in separate hapas, tanks or pond rearing units until the fish are big enough to be physically tagged, normally at about 10g. Stocking and rearing multiple families in separate units requires expensive facility investment and is labour intensive, and furthermore the results are not always as expected due to poorer performance in hapa and tank conditions compared to pond culture, because common carp is a bottom feeder and prefers to live in earthen ponds. Due to limitations of facilities, labour cost and poor fish growth in separate family rearing, the application of communal pond rearing techniques offers a potential solution for selective breeding of common carp. Communal rearing techniques can reduce the number of rearing units necessary for production of many families while increasing the number of families or groups that can be compared (Moav and Wohlfarth, 1974; McGinty, 1987; Macbeth, 2005). Additionally, by rearing all families in the same environment, the environmental component of phenotypic variation among families can be reduced. This enables the accurate evaluation of the additive genetic component for growth and other commercially important performance traits.

Therefore, this study aims at using molecular genetic techniques to investigate ways of further improving the on-going breeding programme in common carp in Vietnam. More specifically, parallel experiments of separate early rearing (SER) and communal early reaing (CER) methods were carried out to compare for the accuracy of

estimation of additive genetic parameters for growth traits in the selective breeding programme of common carp. The objectives of this study were to:

(1) Estimate the potential of genetic improvement through heritability assessment of the base population, produced from crosses of six carp lines.

(2) Investigate the possibility of using microsatellites as genetic markers for parentage assignment in the Vietnamese common carp selective breeding programme.

(3) Estimate genetic and phenotypic parameters, selection responses for growth traits (weight, length and height) after communal early rearing (genetic tagging) and separate early rearing (physical tagging) common carp for two generations of selection.

(4) Investigate the effectiveness of separate early rearing and communal early rearing methods in selective breeding of common carp for growth performance traits (weight, length and height).

Chapter 2. General Materials and Methods

2.1. Background of experimental design

Most large breeding programmes for genetic improvement of aquaculture species are based on pair mating of each individual with one or more individuals of the opposite sex. Full-sib family groups of offspring are raised separately in hapas or tanks until reaching a suitable size for physical tagging. A large number of progeny in each family are then stocked together, ongrown and scored for estimation of breeding values. It is assumed that a large number of families are produced for the estimates to be reliable. This obviously needs intensive investment in facilities and labour. In addition, estimation of quantitative genetic parameters may possibly be confounded with environmental biases.

It has been suggested that the development of molecular markers, especially microsatellites, has provided a solution to overcome some of the problems of more traditional selection programmes, because using such markers can assign communally reared progeny to their parents. The success of parentage assignment using microsatellite markers was demonstrated in rainbow trout (Herbinger et al., 1995), sea bream (Batargias et al., 1999), Atlantic salmon (O'Reilly et al., 1998), common carp (Vandeputte et al., 2004) and many other aquaculture species. The heritability estimates obtained were very high for fish and shellfish. Therefore, the comparison of separate and communal rearing methods was designed to test their relative efficiency in the context of the Vietnamese common carp selective breeding programme.

2.2. The flow of experiments and data

The base population (G_0) was progeny of six available lines (six founder stocks) of common carp kept in the live gene bank programme at the National Broodstock Center, Research Institute for Aquaculture No.1, Vietnam. The G_1 generation was produced from selected parents of the G_0 generation. Each family in the G_1 generation was divided into two different rearing methods: separate early rearing (SER, G_1-SER) and communal early rearing (CER, G_1-CER). Furthermore, these two methods of rearing were applied again in the second selection generation, G_2 for separate family rearing (G_2-SER) and G_2 for communal rearing (G_2-CER), which were derived from selected parents of the G_1-CER because this method produced fish of large enough size for maturity at one year old. There were three measurements in each generation and the timing of the measurements were close between CER and SER. However differences in the environmental conditions between years affected the growth of fish and subsequently changed the age of measurements between the two selection generations. There was a large difference in the timing of the third measurement between the two selection generations, because better growth and earlier maturity in the CER so the measurement was much earlier to reduce stress on maturing fish that was experienced in the first generation. A summary of the experiments and data are presented in Figure 2.3 and Table 2.1 and 2.2.

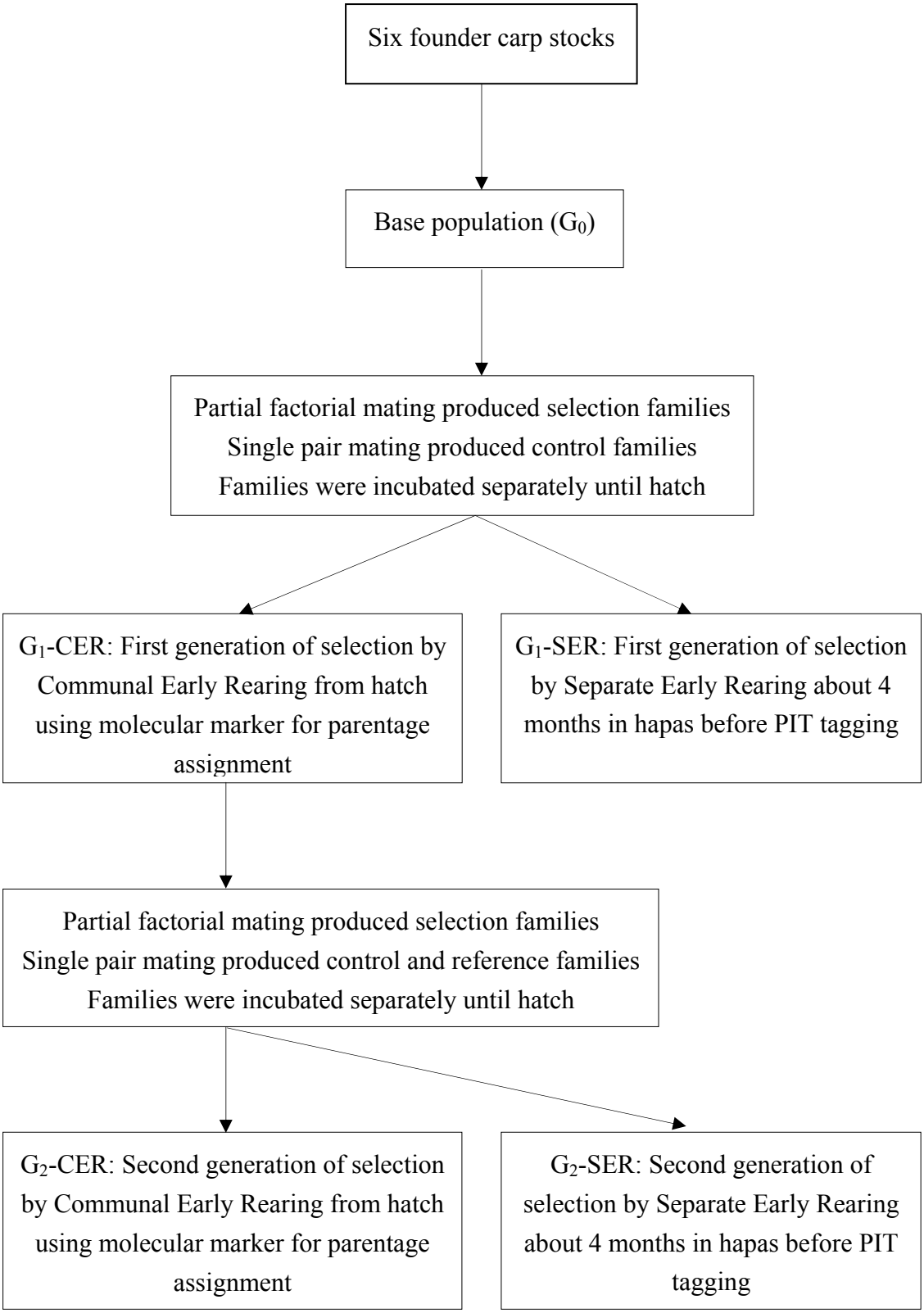

Figure 2.1. The flow of experiments and data for two selection generations of selective breeding programme in common carp.

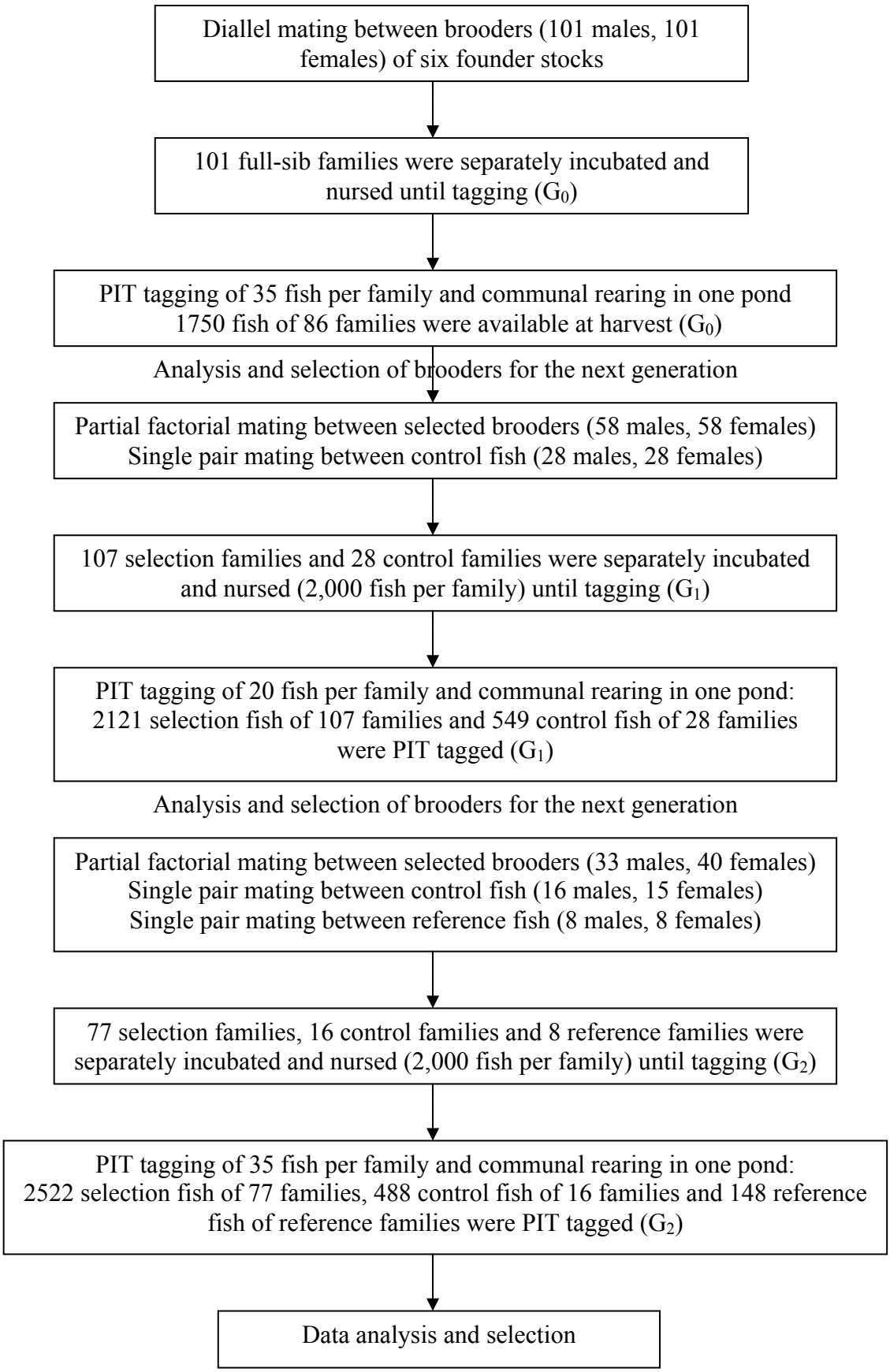

Figure 2.2. Experimental scheme for selective breeding and assessment of separate early rearing (SER) method. The selected breeders that were used to produce the G_2 came from the CER fish (as shown in Figure 2.1).

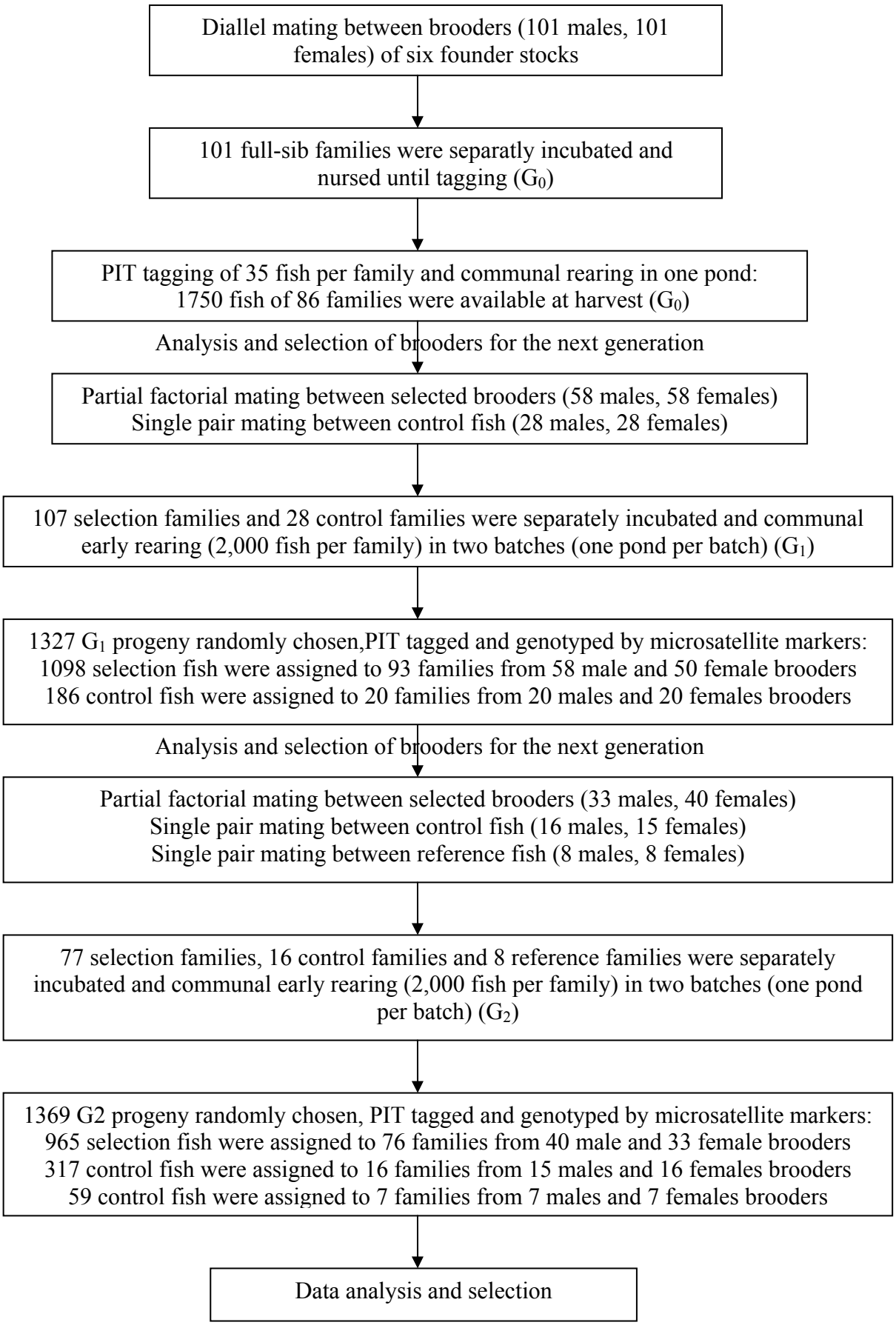

Figure 2.3. Experimental scheme for selective breeding and assessment of communal early rearing (CER) method.

Table 2.1. Family production in the G_0, G_1 and G_2 generations of common carp selective breeding programme in SER method.

	Lines	G_0	G_1	G_2
Number of contributing male brooders in	Selection	101	58	33
	Control	-	28	15
	Reference	-	-	8
Number of contributing female brooders in	Selection	101	58	40
	Control	-	28	16
	Reference	-	-	8
Number of families produced for	Selection	101	107	77
	Control	-	28	16
	Reference	-	-	8
Number of families with survivors at tagging in	Selection	86	107	77
	Control	-	28	16
	Reference	-	-	8
Number of tagged fish (at the first measurement) in	Selection	-	2121	2522
	Control	-	549	488
	Reference	-	-	214
Number of tagged fish available at the second measurement in	Selection	-	1991	2338
	Control	-	494	446
	Reference	-	-	157
Number of tagged fish available at the third measurement (at harvest) in	Selection	1750	1898	2176
	Control	-	454	410
	Reference	-	-	148
Age at the first measurement (days post hatch)		-	137	97
Age at the second measurement (days post hatch)		-	204	183
Age at the third measurement (days post hatch)		383	414	257

Table 2.2. Family production in the G_0, G_1 and G_2 generations of common carp selective breeding programme in CER method.

	Lines	G_0	G_1	G_2
Number of contributing male brooders (expected number is in brackets) in	Selection	101	58 (58)	33 (33)
	Control	-	20 (28)	15 (15)
	Reference	-	-	7 (8)
Number of contributing female brooders (expected number is in brackets) in	Selection	101	58 (58)	40 (40)
	Control	-	20 (28)	16 (16)
	Reference	-	-	7 (8)
Number of families with assigned progeny (expected number is in brackets) in	Selection	101	93 (107)	76 (77)
	Control	-	20 (28)	16 (16)
	Reference	-	-	7 (8)
Number of assigned and tagged fish (at the first measurement) in	Selection	-	1098	965
	Control	-	186	317
	Reference	-	-	59
Number of tagged fish available at the second measurement in	Selection	-	1081	940
	Control	-	183	308
	Reference	-	-	52
Number of tagged fish available at the third measurement (at harvest) in	Selection	1750	1056	874
	Control	-	174	287
	Reference	-	-	46
Age at the first measurement (days post hatch)		-	135	88
Age at the second measurement (days post hatch)		-	201	184
Age at the third measurement (days post hatch)		383	396	317

2.3. Broodstock management and spawning

2.3.1. Husbandry management

All the male and female broodstock in each generation were injected with PIT tags to identify individuals and construct the pedigree record. The males and females were raised separately in different ponds with the stocking density of 1 fish per $3m^2$ to obtain sexual maturation and avoid natural breeding in ponds. This type of husbandry management was normally undertaken from September to February the following year. In this period, the fish were fed twice daily at approximately 5% body weight (BW) with pelleted food containing 25% crude protein (CP). The pond environment was well managed and the maturation condition of the fish was checked once per month. Most of the males gave good quality sperm while about 80% of females were mature and ready for spawning by the end of this period. The spawning season of common carp takes place in spring, generally from March to April, in the North of Vietnam.

2.3.2. Spawning induction and incubation

Early in the spawning season, sexually mature breeders were selected for production of families through artificial spawning. Males and females were kept separately in 10 fibreglass tanks, each with 10 spawners per $3m^3$ (2m×1m×1.5m). The brood fish were twice injected with doses of hormone (LHRH-a) at an interval of 5 hours. About 8-10 hours after the second injection, the release of eggs started and stripping was done immediately for each family. Sperm collected from each male was then added to fertilize the eggs of females according to the breeding plan. The sperm of some males were stored for a short time at 4^0C in a refrigerator if they were to be bred with two

different females. Fertilization of eggs was by the dry method. After removing the stickiness of the fertilized eggs by treatment with 5% extracted pineapple juice (Thai and Ngo, 2004), the eggs were transferred to upwelling incubation jars (each 10 l). The eggs of each full-sib family were incubated in a separate jar. At 21-22^{o}C water temperature, hatching mainly took place after 48-50 hours.

2.4. Experimental fish production

2.4.1. Founder population

The founder population was the parents of the G_0 generation. They were two years old at the time of breeding and were derived from 6 carp stocks: (1) 2nd generation of family selection; (2) Hungarian 6th generation of mass selection; (3) Hungarian scaled carp; (4) Indonesian 6th generation of mass selection; (5) Indonesian yellow carp; and (6) Vietnamese 6th generation of mass selection.

One hundred and one sires and 101 dams, selected at random among the six stocks, were used to produce full-sib families of the G_0 generation. Each sire was mated to one dam and each dam mated to only one sire, following a single pair mating scheme. There was variation in the number of pairs per cross type, ranging from 0 to 9 (Table 2.3) because of ranking for growth performance of the six stocks based on the results of on-farm testing (Tuan et al., 2005). The 2nd generation of family selection and Hungarian 6th generation of mass selection carps showed better growth compared to all other stocks therefore their contribution was increased in the base population.

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Table 2.3. Single pair mating scheme designed for producing G_0 generation (Figures in each cell represent the surviving family in each cross type. Figures in bracket represent the number of pairs mated in each cross type).

Maternal parent	Paternal parent					
	Family selection, 2nd generation	Hungarian 6th generation	Hungarian scaled carp	Indonesian 6th generation	Indonesian yellow carp	Vietnamese 6th generation
Family selection, 2nd generation	8(9)	9(9)	7(7)	5(5)	0(4)	3(3)
Hungarian 6th generation		8(9)	7(7)	4(5)	1(4)	2(3)
Hungarian scaled carp			6(7)	4(5)	4(4)	1(3)
Indonesian 6th generation				5(5)	3(3)	3(3)
Indonesian yellow carp					3(3)	3(3)
Vietnamese 6th generation						0(0)

2.4.2. G_0 generation

Due to limited facilities and asynchronous maturation of breeders, 101 families were produced in 3 batches (12, 38 and 51 families respectively) with 22 days between the 1st and 3nd batches. All the male and female spawners were injected with PIT tags to identify the individual and family number.

After 3 days old, the swim-up fry were fed with artificial food for 2 days before they were transferred to the rearing facility. The fry were placed in fine mesh hapas for separate family nursing. Family rearing from swim-up fry to the size of 5-10 g was conducted using soybean powder and small pelleted food containing 30% crude protein. During this period, some families were lost because of high mortality rates. As a result, 86 families were available for PIT tagging of 35 fish per family and communal grow-out in pond.

Broodfish (total 250, comprised of 150 females and 100 males) were selected from the best 63 families out of the 86 families that made up the G_0 generation, and these were the primary materials for the selective breeding programme of common carp. In addition, 60 females and 40 males with average overall performance were selected from 30 families for a control population.

2.4.3. G_1 and G_2 production

2.4.3.1. Selection population

Good mature females and males were selected based on observation of oocytes and sperm. Female spawners were checked every 30 minutes for egg release after the second dose of hormone application. A partial factorial mating scheme (Table 2.4)

was applied to produce the selected families. Therefore, the eggs were stripped from a female, divided into two approximately equal parts and fertilized by two males to create full-sib and half-sib families. They were mated base on their estimated breeding values, while avoiding closely related individuals.

Table 2.4. Partial factorial mating scheme designed for producing each set of G_1 and G_2 generations of the selected population.

Maternal parent	Paternal parent						
	M1	M2	M3	M4	M5	M...	Mn
F1							
F2							
F3							
F4							
F5							
F...							
Fn							

The first and second generations of selection, known as G_1 (G_1-CER and G_1-SER) and G_2 (G_2-CER and G_2-SER) were produced in the years 2006 and 2007 respectively. Partial factorial mating of one hundred and sixteen G_0 broodfish, comprising 58 females and 58 males, was carried out to produce the G_1 generation of 107 selected

families. They were produced in two sets, one week apart, and 10 male brooders used in the first set were re-used in the second set.

After one year of growing, while the SER fish were small (mean weight of 368.5 g) with very few mature females, the CER fish was approximately three times bigger (mean weight of 989.1 g) and ready for spawning. The decision was therefore made to use the CER fish only in order to produce the G_2 generation. Seventy-seven G_2 families were produced from 73 brooders (40 females and 33 males), selected from the best 57 families of the G_1 CER fish. The mating scheme and operation were similar to the production of the G_1 generation. There were fewer number of selection family than expected in the G_1 generation because of poor matured broostock and mortality at incubation.

2.4.3.2. Control population

The control families were produced for experiments in the year 2006 (G_1 generation) and 2007 (G_2 generation). One hundred fish (60 females and 40 males) in the G_0 and sixty fish (30 females and 30 males) in the G_1 generations were selected from families with performance close to the overall mean of the population to create a control population. The control population was established from individuals of 30 families in the G_0 (to produce G_1 control population) and 20 families in the G_1 (to G_2 produce control population).

There were fewer number of control brooders in the G_1 generation compare to in the G_2 generation since the fish was well management and successful culture from nature of farming and the previous generations (G_0 and G_1). However, poor matured

broodstock and environmental factors were happened during the time of producing the G_2 generation. Consequently, the number of full-sib control families produced was 28 in the G_1 generation and 16 in the G_2 generation. Similarly to the selective breeding population, the control population of the G_2 generation was produced from the CER fish because the SER fish were small with very few mature females. The control family production was also split into two sets, at the same times as the production of the selected family. They were managed in the same way as the selected families. Eggs from each female were fertilized by a single male to produce full-sib families and were separately incubation in upwelling jars.

2.4.3.3. Reference population

In early 2005 five hundred common carp fingerlings originated from 15 families of the P33 strain were introduced into the Research Institute for Aquaculture No.1, Vietnam from the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI), Hungary for the purpose of seed production. These fish were used to generate full-sib families for a reference population in 2007, for comparison to the G_2 generation of selection. The P33 reference families were only produced in 2007 (G_2 generation). Ten full-sib reference families were produced by single pair mating in order to compare the P33 performance to the G_2 selected population. There was fewer number of families than expected due to poor hatching rate and few number of unmatured brooders. The reference family production was also split into two sets, at the same times as the production of the selected and control family. The control and reference families were produced and managed by the same techniques as the selected families.

2.5. Forming CER and SER in the G_1 and G_2 generations

Fertilized eggs were incubated in jars until yolk absorption was complete. Approximately the same number of exogenous feeding larvae (2000, estimated volumetrically) in each family were taken for SER and CER comparisons.

- SER

The same number of exogenous feeding larvae in each family (selected and control families in the G_1; selected, control and reference families in the G_2) were stocked and nursed separately in hapas (one family in one hapas), in one pond until reaching the size for PIT tagging. The tagged fishes were all communal rearing in one pond. The same pond and techniques were used in each generation.

- CER

The same volume of larvae were taken from each family for each batch (selected and control families in the G_1; selected, control and reference families in the G_2) and then pooled for communal nursing and grow-out in ponds, each batch separately in one pond and without repeat. One batch was raised in one pond because there was not any other available big pond out of the one used for SER which could communally rearing all families of the two batches. PIT tagging and parentage assignment were carried out once they had reached the appropriate size. The same two ponds and techniques were used in each generation.

2.6. Nursing and grow-out of the G_1 and G_2 generations

2.6.1. Separate early rearing (SER)

2.6.1.1. Nursing from larvae to fry and fingerling

Selected, control and reference families were nursed separately from larvae to fry. Larvae were first stocked in 1 m^2 fine net hapas with 0.8 m water depth at a stocking density of about 2,000 individuals per m^2 for each family and fed egg yolk and soybean powders for 30 days at a feeding rate of approximately 20% of body weight per day. When the fish had grown to approximately 0.1 g in average, 100 individuals per family were randomly taken by using hand net and transferred to a 5 m^2 plastic hapa. They were then fed small size pelleted food (25% crude protein) at approximately 10% body weight per day.

2.6.1.2. PIT tagging and growth out

Ancestors as well as parents used in mating to produce progeny could be traced using PIT tags for pedigree management. In addition, an equal number of individuals in each family were tagged for communal rearing in pond after separate early rearing (SER).

PIT tags were put into 20 randomly chosen individuals per family in the G_1 and 35 randomly chosen individuals per family in the G_2, when these fish were about 10 g. Tags were injected intramuscularly in the back of each fish, close to the head, for G_0 and G_1 generations, but this was changed to intraperitoneal injection for the G_2 generation due to some number of tag losses in the previous generations. The tagged

fish were then communally reared in a 4,000 m^2 mono-culture grow-out earthen pond (1.5 m deep) at a stocking density of 1 fish/m^2. They were fed daily at approximately 5% body weight with pelleted feed containing 25% crude protein. The feeding rate was adjusted monthly based on sample weights. Water parameters were monitored to ensure good conditions for fish growth.

2.6.2. Communal early rearing (CER)

2.6.2.1. Nursing from larvae to fry and fingerling

Fish were communally nursed in two ponds, corresponding to the two breeding batches (1 batch/pond) at a stocking density of 2,000 individuals per m^2. After 30 days of nursing they were all harvested and kept in 25 m^2 (5m×5m×1m) cement tank which separately by pond. Pool the fish in the tank together and used hand net to randomly take and measure 1 kg of fish before counting for number of invidividual. By knowing the average weight of one fish, the total number of required fish were weighted and restocked to maintain 20 fish per m^2 (same stocking density of the nursing period in 5 m^2 plastic hapa in SER). The type of food and stocking density applied for nursing were similar to the SER group.

2.6.2.2. PIT tagging, parentage assignment and grow-out

The communally reared fingerlings in each of the two batches were harvested and restocked in earthen ponds at a stocking density of 1 individuals/m^2 (same time and stocking density in SER group). The fish were randomly sampled for PIT tag injection together with fin clip collection for parentage analysis. The randomly sampling techniques was applied as same as pooling in nursing period of CER group. Growth

performance data including weight (g), length (cm) and height (cm) of fish were measured in the CER fish together with PIT tagging and fin clip sampling storage in 95% ethanol for later DNA analysis. There were 1,400 samples and 1,500 samples for parentage assignment collected in the G_1 and G_2 respectively. The fin clip sampling only applied for the CER groups so that the collected performance data could be correctly allocated to families to estimate breeding values.

The fish were communally reared for the whole time from first feeding fry to adult; a limited number of these fish were tagged and kept together after fin clip sampling for parentage assignment at big fingerling stage. The parentage assignment in combination with PIT tags allowed pedigree management of CER fish that the later growth performance data and analysis was based on. The culture conditions and management applied were the same as for the SER groups.

2.7. Data collection for growth performance

2.7.1. Types and method of data collection

Wet weight (g), standard length (cm) (the straight line distance from the anterior most point of the head to the base of the caudal fin) and body height (cm) (the straight line distance from the anterior end of the dorsal fin to the belly) of individuals were collected three times for both CER and SER fish. A ruler with 1 mm intervals was used for length and height measurement while a digital balance with 0.1g accuracy was used to weight the fish. Data collection took place within 2-3 days for all of the fish that were to be assessed at that time in each rearing method.

2.7.2. Times of sampling and sample size

- G_0 generation

Thirty-five fish in each of 86 families were PIT tagged, making a total of 2956 fish in the G_0 generation (some families had less than 35 fish). During the grow-out, high mortality occurred when fish were about 400-500 g. Data was collected only one time on 1750 fish at the final harvest of 383 days old. This was the base population which was produced by diallel mating scheme and each parent was used only one time. The purpose was to try to combine high variation of genetic material (six stocks) for the base population rather than selection from this generation.

- G_1 generation

The first assessment of growth performance of fish was at 137 days old when 20 individuals in each family were randomly sampled and PIT tagged and counted for survival rate during the nursing stage in hapas in the SER group. The total of tagged fish was 2121 in the selected population and 549 in the control population. The next data collection was done at 204 days old on 1991 selected fish and 494 control fish. The last data collection time was carried out at final harvest of 414 days old of 1898 selected fish and 454 control fish using the PIT tags to identify individuals. In the CER group, the first data collection time was at 135 days old on 1098 assigned selected fish and 186 assigned control fish that was also the time of PIT tagging and fin clipping for molecular parentage assignment. The next set of data collection was carried out at 201 days old on 1081 selected fish and 183 control fish. The final data was collected at harvest time of 396 days on 1056 selected fish and 174 control fish.

- G_2 generation

In the SER group, data was first collected at 97 days on 2522 selected fish, 488 control fish and 214 reference fish. The second set of data was sampled at 183 days old on 2338 selected fish, 446 control fish and 157 reference fish. The final data set was at harvest of 257 days on 2176 selected fish, 410 control fish and 148 reference fish.

In the CER group, 965 selected fish, 317 control fish and 59 reference fish were assigned and data collected for the first time at 88 days old. The second set of data was sampled at 184 days old on 940 selected fish, 308 control fish and 52 reference fish. The third time of data collection was carried out at final harvest at 317 days on 874 selected fish, 287 control fish and 46 reference fish.

The size of fish at PIT tagging (first set of data) in the CER group was bigger than in the SER group in the G_1 and G_2 generations because the experiments tried to control similar sampling time, stocking density and management for the two rearing methods (SER and CER). The lower number of total fish at final harvest was due to mortality, error in reading tag numbers and tag loss. The lower number of sample for CER group was primary due to the high cost of genotyping.

The difference of age at PIT tagging between the G_1 and G_2 generations was due to change of climate or environmental condition and even selection. In consequence, fish growing in the G_2 generation reached size of PIT tagging (10 g/fish in SER group) in shorter time. The final data set in the G_2 generation was also collected at younger age because it could reduce stress on potential brooders which could be selected for breeding in the next generation that was experienced from the G_1 generation.

2.8. Selection procedure

The selective breeding programme was started in early 2005 at the Research Institute for Aquaculture No.1 (RIA 1). This was also part of the CARP-II Project coordinated by the WorldFish Centre, Malaysia and funded by the Asian Development Bank. During the selective breeding programme WorldFish Centre provided assistance for data analysis and selection decisions. In detail, each individual was measured for growth performance including body weight, standard length and height in each generation of selection. The data was checked for normality and homogeneity of variances before analysis. Their breeding values were estimated from an animal model analyzing the fish at three times using SAS (SAS Institute Inc., 2002) and ASREML (Gilmour et al., 2002). Brooders were selected based on the rank of estimated breeding values (EBVs), separately for males and females. The estimated breeding values were used to select males and females as potential brooders for the next generation. In addition, the selected spawning brooders were focused on the best ranking of about 50 males and 50 females. The pedigree records allowed avoiding mating between close relatives. Also, the brooders were selected to equalise contributions from each family, as far as possible. All the analyzed data and results were sent back to RIA1 for application and carrying out the next generation of selection for the two methods (SER and SER).

Chapter 3. Parentage Assignment of Common Carp

3.1. Introduction

3.1.1. Parentage assignment

3.1.1.1. Pedigree information in selective breeding programmes

One of the difficulties in implementing a selective breeding programme in aquacultural stocks is maintaining pedigree information. Progeny from each family must be reared together until they are large enough to be tagged. This needs, of course, huge facilities and intensive labor, and is likely to introduce environmental effects common to full-sib groups which are confounded with the genetic effects (Herbinger et al., 1995).

Genetic variability in a selective breeding programme may be lost due to poor management that loses pedigree and increases inbreeding, caused by mating between related individuals. Inbreeding also results in a decrease in genetic variability, which limits the potential for genetic gain from artificial selection. There are some methods for pedigree management, of which using PIT tags for individual identification is the most popular. Once reliable pedigree information is available, mating can be arranged with control over pedigree to minimize inbreeding.

Estimates of relatedness and genetic variability based on DNA genotyping offer aquacultural breeding programmes a method of avoiding inbreeding and maintaining genetic variation in the absence of other pedigree information. Some DNA markers,

particularly microsatellite markers, are useful for the purpose of discrimination of unrelated from related individuals in a population.

The pedigree information in selective breeding programmes is not only necessary for estimation of genetic merit of breeding candidates but also for genetic management of broodstock populations, that is effectiveness to minimize the deleterious effects of inbreeding associated with mating closely relative individuals and to avoid the loss of genetic variation (Crow and Kimura, 1970; Bulmer, 1971; Fimland, 1979; Falconer and Mackay, 1996). Parental selection affects genetic parameters that, in general, decrease the additive variance and increase the additive genetic mean for the selected traits. The gene frequencies change in the direction of fixing favourable alleles towards a stable equilibrium between alleles. Without pedigree information, the estimation of genetic variance is less accurate and we are unable to assess genetic variability within the broodstock population. Selection of breeding candidates without pedigree information is also less accurate.

3.1.1.2. Effective microsatellite markers for parentage assignment

The results of DNA profiling from microsatellite markers for aquaculture species can allow the tracing of individuals to family groups even from different progeny groups that have been reared communally after hatching. Parentage analysis using molecular genetic markers was first applied successfully in aquaculture on Atlantic salmon (Doyle and Herbinger, 1994; Wright and Bentzen, 1995). The efficiency of this approach has been assessed in communally reared rainbow trout, where 91% of fish were traced to one or two parental couples in a complete factorial cross of 10 sires and 10 dams, using 4 microsatellite loci (Herbinger et al., 1995). Applying 4 microsatellite

loci could match at least 99.5% of 792 offspring to one set of parents when 100 males and 100 females were mated to produce 12 full-sib families in Atlantic salmon (O'Reilly et al., 1998). A valuable advantage of molecular genetic analyses for DNA profiles lies in non-destructive sampling methods. This technique is now routinely used for some aspects of full-scale breeding programmes in several freshwater and marine fish species. Perez-Enriquez et al. (1999) used 5 microsatellite loci could assign 73.5% of 200 offspring from a population of 250 potential pairs of red sea bream, with 91 spawners contributed to produce the sampled offspring. In addition, 95.3% successful assignment of 550 offspring to a single parental pair from a complete factorial cross of 24 sires and 10 dams was reported using 8 microsatellite loci in the selective breeding programme of common carp (Vandeputte et al., 2004).

3.1.1.3. Parental statistical analysis

The two major methods for parental allocation are exclusion and likelihood-based approaches (Jones and Ardren, 2003). The exclusion approach is based on the principle of Mendelian genotypic incompatibilities between potential parents and offspring to filter out false parents and parental pairs. Where more than one set of non-excluded parents remain, likelihood approaches may be applied to select the most probable parents and parental pair (Meager and Thompson, 1986; Sancristobal and Chevalet, 1997). In comparison, there are some advantages and disadvantages of using either method. The exclusion method is conceptually simple and transparent but is particularly sensitive to typing errors and allele mutations. This method can provide a locus set with a highly significant assignment (>99%) and low error rates (less than 4%). The increasing of assignment accuracy is possible by using software that can

accommodate occasional mismatched alleles (Vandeputte et al., 2006). Likelihood computations allow for a less rigid approach to parental assignment, which often results in more apparent assignments from less genotypic data. The algorithms applied usually incorporate a means for dealing with some degree of transmission error and missing data. However, the relationships among the mathematical models implemented, the level of error set by the user for running the allocation and the sensitivity of the assignment are more difficult to understand, and extra care is needed when interpreting the results.

3.1.2. Aims of the study

Microsatellite markers have been developed for common carp (e.g. Crooijmans et al., 1997). The aim of the present study was to apply microsatellite markers to develop multiplex PCRs to explore the efficiency of parentage assignment of communally rearing progeny to their families and parents in the common carp selective breeding programme being carried out at the Research Institute for Aquaculture No.1, Vietnam.

3.2. Materials and methods

3.2.1. Sampling for DNA analysis

Fin clips of all G_0 parents were collected for microsatellite marker analysis to examine polymorphism and utility for parentage assignment. In addition, tissue samples of twenty progeny in each of fifteen known parental pairs were analysed to develop and standardize a protocol for tracing progeny to parents.

During mating to produce the G_1 and G_2 generations, the selected PIT-tagged male and female brooders were sampled for later allocation of progeny to parents. After nursing, early communal rearing fish were randomly fin clipped, sampling together with PIT tagging and growth performance measurement. The number of collected samples averaged about thirteen individuals per family for pedigree analysis (see details in Chapter 2).

3.2.2. DNA extraction

A variety of DNA extraction methods have been developed and applied elsewhere to produce high quality and quantity of extracted DNA. However, the requirement of DNA quality and quantity depends on the study purposes as well as technical application. Amplification of DNA for microsatellite analysis does not need DNA that is very pure, in large amounts or of high molecular weight as template. The requirement of this study was to be able to analyse a large number of samples in a short time, so simplicity of extraction method was more important than the production of very high quality and quantity of DNA. Two methods were adapted and optimized for DNA extraction using 96 well plates, which can later be used easily for PCR analysis.

3.2.2.1. DNA extraction using Dyna-beads

Dynabeads® are uniform superparamagnetic monodisperse polymer particles which were designed to adsorb DNA molecules to their surface. This DNA extraction technique was used successfully in Atlantic cod ethanol-preserved tissue samples including blood, fertilised eggs and larvae (Delghandi et al., 2003; Herlin et al., 2007). The following protocol was adapted and applied for carp fin clip samples.

About 25 mg fin clip tissue was digested in 100 µl digestion solution containing 4 µl of proteinase K (10 mg/ml) and 96 µl of Dynabeads at 55°C for approximately 4 hours or until absolute digestion by visual observation. The DNA/Dynabeads® complex was then washed twice using the buffer provided in the extraction kit. Finally, the DNA was separated from the magnetic beads by adding 10 µl of 0.1× TE buffer (1 mM Tris, 0.01 mM EDTA, pH 8.0) and incubating at 60°C for 15 minutes. The DNA extracted by this technique was only used for single PCRs, since all attempts at multiplex PCR were unsuccessful due to its requirement of higher DNA quantity.

3.2.2.2. DNA extraction using REAL kit

The REAL kit includes three solutions, a cell lysis solution, a protein precipitation solution and a DNA resuspension solution. This DNA extraction kit was specifically designed for the extraction of high quality genomic DNA from a wide variety of tissue and fluid samples. It was successfully tested on salmon and tilapia in the Institute of Aquaculture (IoA) molecular biology laboratory. The following protocol was adapted from the manufacturer's instructions to perform extractions in 0.2 ml PCR 96 well plates.

For one sample of DNA extraction, about 25 mg of carp fin clip was incubated until digestion was complete (by visual observation) after 5-6 hours at 55°C in 3 µl of proteinase K (10 mg/ml) and 57 µl of cell lysis solution. Then 30 µl protein precipitate solution was added to precipitate protein residues and the samples were kept on ice for 10 minutes before centrifuging at 4100 rpm for 15 minutes. About 40 µl of the supernatant, which contained the DNA, was transferred to a new PCR plate in which each well contained 60 µl of pure isopropanol. The DNA pellets were precipitated by centrifuging at 4100 rpm for 10 minutes. The DNA was washed two times in 70% ethanol. Finally, the DNA pellet was resuspended in 50-60 µl of 0.1× TE buffer (1 mM Tris, 0.01 mM EDTA, pH 8.0).

3.2.2.3. Measurement of DNA quality and quantity

3.2.2.3.1. Agarose gel electrophoresis

This is a primary technique to test for the quality (molecular weight) and quantity of DNA as well as PCR products. To prepare 1.5% agarose gel for fast running and examination on a mini gel tray, 0.45 g of agarose powder was boiled in 30 ml of 1× SB buffer (1 mM Sodium hydroxide, using Boric acid adjust to pH 8.5). 0.5 µl of 10 µg/ml Ethidium bromide was added into the gel liquid. Once the gel was approximately 60^0C it was poured in a casting tray and a comb inserted at one end of the gel tray. For each sample, 3 µl of DNA or PCR products was mixed with 6 µl of 3× Bromophenol blue dye (1× Bromophenol blue dye = 25 mg/ml Ficoll 400, 83 µg/ml Bromophenol blue, 83 µg/ml Xylene cyanol FF) and loaded into one well. One well was loaded with 1 µl of the DNA ladder (Phi X 174, 100 µg/ml) mixed with 4 µl of 6× Bromophenol blue dye, as a DNA size standard to assess the sample DNA or PCR products. The electrophoresis was run for 10 minutes in 1× SB buffer at 2.5

volts/cm. The DNA or PCR products could be visualized under UV light. Figure 3.1 shows a sample result of testing for PCR products on agarose gel.

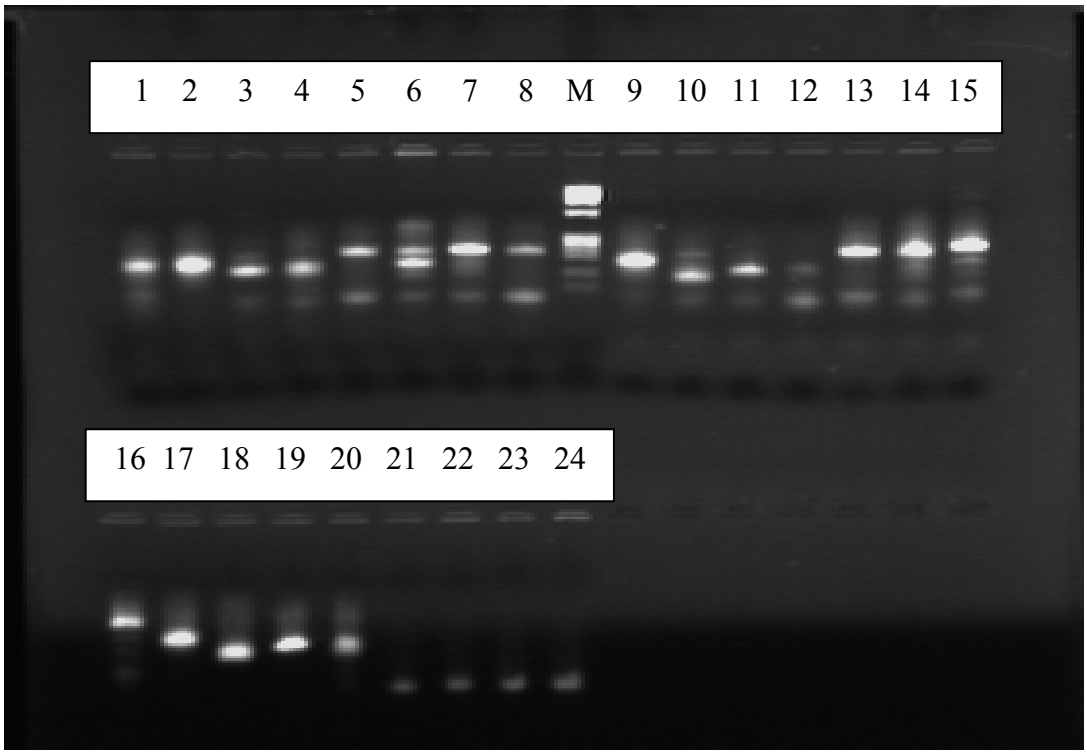

Figure 3.1. Result of testing PCR products for single locus on agarose gel (Lanes 1-24 are PCR products; M is 100bp DNA ladder; Some non-specific products are in lane 6, 10, 14 and 15; No products are in lanes 21-24).

3.2.2.3.2. DNA quantification

The total extracted genomic DNA could be measured more exactly for quality (purity) and quantity using a nanodrop ND-1000 spectrophotometer (Labtech International, UK). The nanodrop ND-1000 is a full spectrum (220-750 nm) spectrophotometer which operates by measuring the concentration of nucleic acids in 1 µl samples. The measurement of DNA purity is estimated based on the ratio of sample absorbances at 260 nm and 280 nm. If the ratio ranges from 1.8-2.0 this means that the DNA product has good quality (high purity) while a lower ratio could be due to protein residues or other contaminants.

3.2.3. Microsatellite loci and PCR optimization

3.2.3.1. Choosing available microsatellite loci

A series of microsatellite markers were isolated earlier from common carp genomic DNA (Crooijmans et al., 1997; Aliah et al., 1999; Yue et al., 2004; Tong et al., 2005). These published markers showed high levels of polymorphism in many studies on genetic diversity and variability in common carp (Desvignes et al., 2001; Kohlmann et al., 2003; Kohlmann et al., 2005; Lehoczky et al., 2005). In particular, the markers developed by Crooijmans et al. (1997) were applied in several studies including parentage assignment analysis (Vandeputte et al., 2004; Kocour et al., 2007). A set of ten microsatellite markers were initially chosen to test for amplification, level of polymorphism and reliability of parentage assignment in the breeding programme population. The forward primers were fluorescently labelled for detection of PCR products on an automated fragment analyser. As a result, parentage assignment of the common carp in this study was performed using seven of these microsatellite loci, which gave highly specific and clean products without severe stutter alleles and with good levels of polymorphism. The detailed description of the reliable microsatellite loci used in this study is presented in Table 3.1.

Table 3.1. Seven polymorphic microsatellite loci used in the present study (from Crooijmans et al., 1997).

Microsatellite name	Primer sequences (5' to 3')	Allele size (bp)	Annealing temperature
MFW4	F: TCCAAGTCAGTTTAATCACCG R: GGGAAGCGTTGACAACAAGC	102-166	55^0C
MFW7	F: TACTTTGCTCAGGACGGATGC R: ATCACCTGCACATGGCCACTC	181-285	55^0C
MFW9	F: GATCTGCAAGCATATCTGTCG R: ATCTGAACCTGCAGCTCCTC	79-194	55^0C
MFW11	F: GCATTTGCCTTGATGGTTGTG R: TCGTCTGGTTTAGAGTGCTGC	132-240	55^0C
MFW18	F: GTCCCTGGTAGTGAGTGAGT R: GCGTTGACTTGTTTTATACTAG	86-297	55^0C
MFW20	F: CAGTGAGACGATTACCTTGG R: GTGAGCAGCCCACATTGAAC	125-252	55^0C
MFW26	F: CCCTGAGATAGAAACCACTG R: CACCATGCTTGGATGCAAAAG	88-165	55^0C

3.2.3.2. Single PCRs

Polymerase chain reaction (PCR) amplifications were modified from the published protocols (Crooijmans et al., 1997). The PCR conditions were tested in unique conditions for each locus with different annealing temperatures, $MgCl_2$ concentrations, Betain concentrations, other conditions and thermal cycling conditions, to inform the later optimization of multiplex PCRs. PCR products were firstly examined on agarose gels before running fragment analysis on a sequencer

(CEQTM 8800 Genetic Analysis System), in order to check for the success of specific products. As a result, the optimal conditions for single PCRs were conducted in a 15 µl reaction volume which consisted of 30 ng of DNA template, 20 pmol each of forward and reverse primers, 100 µM each of dGTP, dTTP, dATP and dCTP, 1.5 mM $MgCl_2$, 1.5 M Betain, 1× reaction buffer IV (75mM Tris- HCl), ddH_2O and 0.4 units *Taq* polymerase (AB Gene) using a Biometra Gradient PCR machine. PCR cycles began by hot start with an initial denaturation step of 95 ^{0}C for 5 minutes, followed by 30 cycles of 30 s denaturation at 95 ^{0}C, 30 s annealing at 55 ^{0}C and 1 min 30 s elongation at 72 ^{0}C; a final extension step at 72 ^{0}C for 25 minutes was performed to ensure complete addition of adenine to the PCR products (Smith et al., 1995).

3.2.3.3. Multiplex PCRs

Two multiplexed sets, of three microsatellite loci (triplex) PCR and four microsatellite loci (tetraplex) PCR respectively, were optimized as shown in Table 3.2. The results of the single PCR optimizations (see details in section 3.2.3.2) facilitated the development of multiplex PCRs. The loci chosen for coamplification in multiplex PCR reactions relied on the allele size ranges and dye colours. For both the triplex and tetraplex PCRs, amplification needed to be optimized step by step, including reagent compositions (dNTPs concentration, *Taq* polymerase concentration and primer concentrations) and PCR conditions (number of cycles, time for denaturation, time for annealing and time for elongation). Finally, the two multiplex PCRs were best performed in 15µl reaction volume containing 60 ng of DNA template, 150 µM each of dGTP, dTTP, dATP and dCTP, 1.5 mM $MgCl_2$, 1.5 M Betain, 1× reaction buffer IV (75mM Tris- HCl), ddH_2O, 1 unit *Taq* polymerase (AB Gene) and primers (specific concentration are given in Table 3.2). The PCR amplification programme

was initial denaturation at 92 ^{0}C for 5 minutes, followed by 35 cycles of 40 s denaturation at 92 ^{0}C, 50 s annealing at 55 ^{0}C and 1 min 45 s elongation at 72 ^{0}C, with a final extension at 72 ^{0}C for 25 minutes.

Table 3.2. Two sets of multiplex PCRs for parentage analysis in common carp.

	Loci name	Allele size range	Dye colour	Concentration of each primer
Multiplex 1	MFW4	102-166	Blue	0.10 pm/µl
	MFW9	79-194	Black	0.25 pm/µl
	MFW11	132-240	Green	0.14 pm/µl
Multiplex 2	MFW7	181-285	Black	0.16 pm/µl
	MFW18	86-297	Blue	0.10 pm/µl
	MFW20	125-252	Green	0.22 pm/µl
	MFW26	88-165	Black	0.14 pm/µl

3.2.4. Genotyping and parentage assignment

3.2.4.1. Fragment analysis on Beckman-Coulter 8800

Genotyping was performed automatically on the CEQ 8800 genetic analysis system which is a capillary electrophoresis system using a laser detector sensitive for four fluorescent dyes, namely red, blue, green and black colours. Therefore, primers running in the sequencer were added with dye terminators for one of three colours except red colour, used for labelling the size standard. Genotyping was carried out using 0.2 ml 96 well-plates, each well containing 2 µl of undiluted PCR products, 28 µl of formamide solution, 0.25 µl of labelled size standard (60 bp - 400 bp) and a drop of mineral oil, running on the fragment analysis programme. The CEQ 8800 data

analysis software was used for initial calling of allele sizes of the amplified DNA fragments.

3.2.4.2. Allele scoring

Gel data were analysed using GenescanTM Analysis Software V3.2.1 (Applied Biosystems) and fragments were sized using the Local Southern method. Following the installation of a gel matrix, the matrix compensates for some fluorescent emission in the detection ranges of other dyes being detected in the wavelengths of each specific dye. The lanes on the gel images were tracked, cross-checked and extracted using automated procedures of the Genescan collection software. The size standard in each lane was aligned to standardise the size calling between lanes. Extracted lanes containing fragment size data for each sample were exported into Genotyper™ Analysis software V3.21 (Applied Biosystems). Genotyper is a software application that enables the analysis and interpretation of nucleic acid fragment size and quantifies data by converting it into user defined results.

Analytical parameters included the selection of the default advanced algorithm for allele peak detection and the cubic spline method for calling sizes. Bin sizes and allelic thresholds were both customised using advanced options in Genemapper. The microsatellite repeat units in the markers used in this study were all poly CA so the bin size was set to ± 1.45 base pairs of the actual allele size.

3.2.4.3. Allele polymorphism

In order to reduce the cost of fluorescent labelled primers, M13 tailed primers were initially tested for polymorphism, however this did not allow successful scoring of

alleles. The reasons for failure of score alleles were inconsistent PCR products and too many bands to be able to score the real alleles.

Fluorescent dye-labeled primers were ordered and run under the same PCR conditions and programmes outlined in section 3.2.3.2. Seven of the ten amplified loci gave specific products and were polymorphic among thirty samples from the G_0 generation. Attempts were made to optimize the conditions for the other three non-amplifying loci but they still displayed weak products or alleles could not be identified. The PCR products were firstly examined by electrophoresis (see in section 3.2.2.3.1) on agarose gel and then by fragment analysis on sequencer CEQ8800 (see in section 3.2.4.1) to accurately detect polymorphism.

The concept of allelic polymorphism is synonymous to the number of alleles (n) encountered at a single locus. Allelic frequencies (F) were calculated, for a given fish population, using the parentage analysis programme VITASSIGN (Vandeputte et al., 2006).

If “A”, “B” and “C” stand for the three different alleles encountered at a particular locus and if “F(AA)”, “F(AB)”, “F(AC)”, “F(BB)”, “F(BC)”and “F(CC)” represent the genotype frequencies for each possible allelic combination, then the frequency of allele “A” is:

$$F(A) = F(AA) + 0.5F(AB) + 0.5F(AC)$$

with $F(AA) + F(AB) + F(AC) + F(BB) + F(BC) + F(CC) = 1$

3.2.4.4. Parentage assignment

3.2.4.4.1. Simulation for parentage assignment

FAP (Family Assignment Programme) version 3.0 was developed for parentage assignment (Taggart, 2007). This programme operates by exclusion principles for two tasks. Firstly, it predicts the resolving power of specific parental genotypic data sets for unambiguously discriminating among families / groups of families. Secondly, it assigns all possible parental combinations to progeny. Both analyses performed by FAP assume a closed population meaning that all individuals are the progeny of known parental combinations for which full genotypic data is available. Another assumption of the programme is that the nuclear loci employed in the analyses are independently inherited in simple Mendelian fashion. FAP was only applied for simulation analyses in this study.

3.2.4.4.2. Vitassign software

VITASSIGN is a software for parental assignment developed by Vandeputte et al. (2006). The programme also allocates offspring to pairs of parents using the exclusion principle. In general, the functions offered by VITASSIGN are very similar to the ones provided by FAP. In assignment analyses, VITASSIGN can take into account allelic mismatches in the analysis and in cases of "multi-match" outcomes provides a list of the matching families. When allocation mismatches occur in more than one allele, VITASSIGN can identify the problematic locus/loci. In comparison to FAP, VITASSIGN has two other functions: (1) it can generate a mating matrix based on the allocation results and provide a summary of allele frequencies for each analysed locus; (2) it can be used to run simulations of allocation based on the genotypes of the

putative parents. The programme first generates a given number of offspring genotypes based on the declared matings matrix and the parents' genotypes. Next, the genotyped offspring are traced by the programme and the rate of single-matches is calculated.

3.2.4.4.3. Errors in parentage assignment

Some errors can arise from microsatellite genotyping. When the template DNA is of low quantity and/or quality, PCR amplification can become unreliable, particularly for multiplex PCR run on 96 well plates. A common problem is the failure of one allele to amplify that leads to heterozygotes appearing to carry only one allele. In other cases, even if specific products are amplified, one allele can be missed because of very asymmetric amplification (normally the larger allele is amplified much less that the smaller one). The presence of stutter bands generated by slippage of *Taq* polymerase during PCR can make it difficult to score alleles reliably. To solve these problems, the REAL kit (which produced larger quantity and better quality of DNA) was used instead of the Dynabead method. In addition, PCR conditions and programmes were maintained and performed using the same equipment, consumables and reagents. More importantly, reference samples were also used in every run to confirm base-pair additions, up or down bias in the size-calling between gels and manual allele scoring was used to check the automated calling. *Taq* polymerase is known to add an adenine nucleotide to the 3' end of PCR products which often results in the production of an additional band, one base-pair higher than the actual allele (Brownstein et al., 1996). Reference samples for each gel and a long final PCR extension step were used to overcome this problem.

3.2.4.5. Estimation of effective population size (N_e) and inbreeding (ΔF)

Effective breeding number is one of the most important parameters in the management of a population, since this gives an indication about the genetic stability of the population because N_e is inversely related to both inbreeding and genetic drift. To estimate the effective breeding population size (N_e) from a single day of spawning, an assumption of unequal individual contributions was made. N_e was calculated from the results of the parentage analyses and pedigreed mating as follows:

$$N_e = 4(N - 2) / [(K_s + V_s/K_s) + (K_d + V_d/K_d) - 2] \quad \text{(Chevassus, 1989)}$$

Where: N_e is effective breeding size; N is the offspring sample size; K_s and K_d are the mean number of offspring per sire and per dam; and V_s and V_d are the variances in family size for sires and dams, respectively.

The estimation of N_e is to assess inbreeding (ΔF) because it is inversely related to the level of loss of genetic diversity and the rate of increase in inbreeding in a finite population (Falconer and mackay, 1996). The equation to estimate inbreeding was:

$$\Delta F = \frac{1}{2N_e} \quad \text{(Falconer and Mackay, 1996)}$$

3.3. Results

The possibility for parentage assignment in this population of common carp was initially examined on fifteen known full-sib families with twenty individuals per pair of parents. All parents in the G_1 and G_2 generations were successfully genotyped for simulation and assignment. Genotypic data of parents and offsprings were collected over all seven microsatellite loci through the two multiplex PCRs after running fragment analysis on the sequencer. However, some progeny were not fully genotyped at all seven loci due to poor DNA quality and low amplification of alleles in the multiplex PCR reaction.

3.3.1. The polymorphism of the seven microsatellite loci

The numbers of samples genotyped for the seven microsatellite loci in G_0, G_1 and G_2 generations were 167, 1327 and 1396 respectively (Table 3.3). The analysis of the seven loci showed high levels of polymorphism in the base and selected populations. There was an overlap of allele size ranges for some loci in the triplex and tetraplex PCRs, however they were labelled with different dyes. Although two loci in the tetraplex PCR were labelled by the same colour, they were combined into one multiplex PCR based on a lack of overlap in their known allele size ranges. Fragment analysis showed that locus MFW4 had the lowest number of alleles (10) while the highest number of alleles was 20, in locus MFW7. Allele size ranges were very large, for instance, from 79 to 194 bp in locus MFW9 and from 149 to 285 bp in locus MFW7. The number and wide range of allele sizes found in each locus were examined and analyzed repeatedly on the same samples of known progeny and their parents. The alleles appeared from moderate to high frequencies and could be applied efficiently to trace the progeny to parents (data not shown).

Table 3.3. Allele polymorphism and changes at seven microsatellite loci in G_0, G_1 and G_2 generations of common carp in the breeding programme.

Locus	G_0	G_1	G_2
MFW4			
n	167	1327	1396
A	10	10	10
A_e	7.8	7.82	7.88
A_r	102-166	102-166	102-166
P	>0.01	>0.01	>0.01
MFW7			
n	167	1327	1396
A	20	20	18
A_e	9.33	9.25	8.91
A_r	149-285	149-285	149-285
P	>0.01	>0.01	>0.01
MFW9			
n	167	1327	1396
A	15	14	14
A_e	7.84	7.56	7.92
A_r	79-194	83-194	83-178
P	<0.01	<0.01	<0.01
MFW11			
n	167	1327	1396
A	16	16	16
A_e	8.7	8.7	9.0
A_r	132-249	132-249	132-249
P	>0.01	>0.01	>0.01

MFW18			
n	167	1327	1396
A	16	15	11
A_e	8.42	8.3	7.18
A_r	86-297	86-297	134-208
P	>0.01	>0.01	>0.01
MFW20			
n	167	1327	1396
A	13	12	12
A_e	10.72	10.6	10.7
A_r	125-252	125-252	152-252
P	>0.01	>0.01	>0.01
MFW26			
n	167	1327	1396
A	17	17	14
A_e	12.6	12.4	11.6
A_r	88-165	88-165	88-159
P	>0.01	>0.01	<0.01
Total number of alleles	107	104	95
Mean number of alleles	15.3	14.9	13.6
Mean of effective number of alleles	9.34	9.26	9.03
% loss of alleles	0	1.6	10.2

G_0: Base population; G_1: First generation of selection; G_2: Second generation of selection; N: Sample size; A: Number of alleles; A_e: effective number of alleles; A_r: Allele size range (bp); P: Probability of excess of homozygotes.

Significant excesses of homozygotes ($P<0.01$) were observed at locus MFW9 for all three generations and at locus MFW26 in the G_2 generation only.

The seven microsatellite loci produced a total of 128 alleles in the base population and this declined in advancing generations. Likewise, the mean number of alleles per locus decreased from 15.3 in the G_0 to 14.9 in the G_1 and 13.6 in the G_2 generations. Some losses of genetic diversity were based on rare alleles that occurred mainly in G_2 generation due to less parents contributing to the progeny. An estimation of allelic diversity loss was 1.6% after first generation of selection and approximately 10.2% at the second generation compared to the base population.

3.3.2. Parentage assignment

3.3.2.1. FAP simulation

Full genotypic data were analyzed on seven microsatellite loci from 156 parents producing 135 full-sib families in the G_1 generation and 118 parents producing 101 full-sib families in the G_2 generation. The data sets were used to predict parentage assignment with the FAP 3.0a programme (assuming equal family representation). Table 3.4 shows the results of parentage prediction in the two selection generations. In this prediction, 99.1% of offspring would be unambiguously assigned to a single family in the G_1 generation. In addition, the proportion of each family that could be assigned ranged from 0.75 to 1.00. In the G_2 generation, a lower proportion (98.7% of offspring) was predicted to be unambiguously assigned to family and the proportion of progeny with distinctively identifiable genotypes for each family ranged from 0.73 to 1.00.

Table 3.4. Prediction of parentage assignment of G_1 and G_2 progenies to their parents.

Assignment result	Predicted assignment by FAP	
Generation	G_1	G_2
Number of parents	156	118
Number of families	135	101
Single match	99.1%	98.7%
Multiple match	0.9%	1.3%

3.3.2.2. Assignment results for the G_1 and G_2 generations

A summary of the actual assignment results is presented in Table 3.5. The average numbers of genotyped offspring per full-sib family in the first and second generations were approximately 9.8 and 13.8 respectively. The results showed that the assigned progeny represented 113 families in the G_1 generation and 99 families in the G_2 generation. There were no progeny matching to 22 of the expected families in the G_1 generation and 2 families in the G_2 generation. The potential reasons for the absence of these families could be poor survival rate, sampling error/sizes and genotyping errors.

There was not much difference in the assignment results between the two batches of spawning and growing fish between and within generations. Using data of 7 loci, 87.2% and 86.3% of progeny could be perfectly assigned to their parents in the G_1 and G_2 generations respectively. This was lower than the prediction, when FAP simulation showed single matches of 99.1% in the G_1 generation and 98.7% in the G_2 generation (Table 3.4). This could be explained at least in part by errors occurring during genotyping, including amplification, fragment analysis and scoring of alleles for all loci in the two multiplex PCRs.

Table 3.5. Efficiency of parentage assignment used seven microsatellite markers over two generations of selection.

	G_1 (2006)			G_2 (2007)		
	Batch No.1	Batch No.2	Overall	Batch No.1	Batch No.2	Overall
Number of families	66	69	135	59	42	101
Number of typed progeny	656	671	1327	810	586	1396
Single assignment with perfect match for 7 loci	89.5%	84.8%	87.2%	86.4%	86.1%	86.3%
Single assignment with one mismatch for 7 loci	93.6%	94.5%	94.0%	92.4%	93.2%	92.8%
Single assignment with two mismatches for 7 loci	96.4%	97.1%	96.8%	95.8%	96.5%	96.2%
Individual with two mismatches assigned to more than one family	2.1%	1.7%	1.9%	2.8%	2.5%	2.6%
Not assigned individual with two mismatches for 7 loci	1.5%	1.2%	1.3%	1.4%	1.0%	1.2%
Number of families without any assigned progeny by two mismatches for 7 loci	11	11	22	1	1	2
Number of families with assigned progeny by two mismatches for 7 loci	55	58	113	58	41	99

Analysis of assignment allowing one mismatch for seven loci could improve 6.8% in the first generation and 6.5% in the second generation. The mean of single assignment

with two mismatches for the studied loci were 96.8% and 96.2% for the G_1 and G_2 generations respectively. The percentage of individuals with perfect matches assigned to more than one family ranged from 1.7% to 2.8%, fairly close to the prediction. The proportion of offspring not assigned to any parental pair in the G_1 generation (1.3%) was similar to the G_2 generation (1.2%) when two mismatches were allowed for the seven loci.

3.3.2.3. Family structure in the G_1 and G_2 generations

Samples for parentage analysis were derived from two batches in each generation of selection which included selected and control families. In addition, reference families were produced and reared communally for parentage assignment in the G_2. Partial factorial mating was applied for selective family production while control and reference families followed single pair mating. Table 3.6 shows a summary of the results of analysis for family size and representation in the G_1 and G_2 generations. An equal number of male and female parents contributed to produce offspring in the G_1 generation but there was an unbalanced sex ratio of parents contributing to surviving families in the G_2 selected generation. Overall, the observed number of families after assignment was lower than the expected number of families. A higher number of families were missing in the first generation (22 families) than in the second generation (2 families) although efficiency of parentage assignment was similar between the two generations. These could be partially explained by less samples having been analysed and a higher number of families in the G_1 compared to G_2 generations. High variation of family size was found between families and populations.

Table 3.6. Family size and representation in the G_1 and G_2 generations, based on family assignment using microsatellite markers.

	G_1		G_2		
	Selected	Control	Selected	Control	Reference
Total number of sampled and analysed fish	1327		1396		
Expected number of families	107	28	77	16	8
Observed number of families	93	20	76	16	7
Number of contributing males	58	20	33	15	7
Number of contributing females	58	20	40	16	7
Number of assigned offspring	1098	186	965	317	59
Number of offspring per family	1-49	2-23	1-38	1-43	1-25
Average number of assigned offspring per family	11.8	9.3	12.7	19.8	8.4

3.3.2.4. Parental contributions to the family size

The contribution of individual dams and sires to the progeny is given in detail in Tables 3.7, 3.8, 3.9 and 3.10. Seventy-eight male and seventy-eight female parents contributed progeny to 113 assigned families in the first generation. In the second generation of selection, fifty-five males and sixty-three females contributed to 99 assigned families. A total of seven sires and six dams had no offspring among the assigned progeny in the G_1 generation, while all brooders had representative progeny in the G_2 generation. In general, the number of assigned offspring per full-sib family had a Poisson distribution with more than half of the families having less progeny than the mean number expected.

Table 3.7. Number of offspring assigned into each family in the partial factorial mating in the first batch of the G_1 generation.

		Dam																				Observed total per sire	Expected total per sire
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	
Sire	M1	27																					27	9.9
	M2		11																				11	19.9
	M3		15	4																			19	19.9
	M4			2	3																		5	19.9
	M5				10																		10	19.9
	M6					2	13																15	19.9
	M7						7																7	19.9
	M8							4	12														16	19.9
	M9								4														4	19.9
	M10									1													1	19.9
	M11											3											3	19.9
	M12											17											17	19.9
	M13												1	6									7	19.9
	M14													5	1								6	19.9
	M15														12	2							14	19.9
	M16															2	13						15	19.9
	M17																8						8	19.9
	M18																	7	5				12	19.9
	M19																		3	3			6	19.9
	M20																				10		10	19.9
	M21																				22	10	32	19.9
Observed total per dam		27	26	6	13	2	20	4	16	1	0	20	1	11	13	4	21	7	8	3	32	10		
Expected total per dam		9.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	9.9		

Number of offspring assigned into each family in the partial factorial mating in the first batch of the G_1 generation (continued).

		Dam																				Observed total per sire	Expected total per sire
		F21	F22	F23	F24	F25	F26	F27	F28	F29	F30	F31	F32	F33	F34	F35	F36	F37	F38	F39	F40		
Sire	M1										17											17	9.9
	M22	13	26																			39	19.9
	M23		20																			20	19.9
	M24			4	18																	22	19.9
	M25				37	9																46	19.9
	M26					12																12	9.9
	M27						26															26	9.9
	M28						47															47	9.9
	M29							14														14	9.9
	M30								11													11	9.9
	M31									26												26	9.9
	M32										9											9	9.9
	M33											2										2	9.9
	M34												6									6	9.9
	M35													12								12	9.9
	M36														14							14	9.9
	M37																					0	9.9
	M38																12					12	9.9
	M39																	17				17	9.9
	M40																		21			21	9.9
	M41																					0	9.9
	M42																				14	14	9.9
Observed total per dam		13	46	4	55	21	73	14	11	26	26	2	6	12	14	0	12	17	21	0	14		
Expected total per dam		9.9	19.9	19.9	19.9	19.9	19.9	9.9	9.9	9.9	19.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9		

Table 3.8. Number of offspring assigned into each family in the partial factorial mating in the second batch of the G_1 generation.

		Dam																			Observed total per sire	Expected total per sire
		F41	F42	F43	F44	F45	F46	F47	F48	F49	F50	F51	F52	F53	F54	F55	F56	F57	F58	F59		
Sire	M1	15																			15	9.7
	M2	12	1																		13	19.4
	M3		1	5																	6	19.4
	M4			26	11																37	19.4
	M5				9																9	19.4
	M6						4														4	19.4
	M7						10	4													14	19.4
	M8							7	1												8	19.4
	M9								11	3											14	19.4
	M10										12										12	19.4
	M43										20	19									39	19.4
	M44											4	10								14	19.4
	M45												18	19							37	19.4
	M46													12	1						13	19.4
	M47														2	49					51	19.4
	M48															10	24				34	19.4
	M49																18	15			33	19.4
	M50																	16	4		20	19.4
	M51																		5	8	13	19.4
	M52																			6	6	9.7
Observed total per dam		27	2	31	20	0	14	11	12	3	32	23	28	31	3	59	42	31	9	14		
Expected total per dam		19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4		

Number of offspring assigned into each family in the partial factorial mating in the second batch of the G_1 generation (continued).

		Dam																		Observed total per sire	Expected total per sire
		F60	F61	F62	F63	F64	F65	F66	F67	F68	F69	F70	F71	F72	F73	F74	F75	F76	F77	F78	
Sire	M53	17																			17	9.7
	M54		21																		21	19.4
	M55		15	15																	30	19.4
	M56			22																	22	19.4
	M57					18															18	19.4
	M58					22															22	19.4
	M59																				0	9.7
	M60							6													6	9.7
	M61																				0	9.7
	M62								8												8	9.7
	M63									3											3	9.7
	M64										15										15	9.7
	M65											10									10	9.7
	M66												2								2	9.7
	M67													7							7	9.7
	M68														5						5	9.7
	M69																				0	9.7
	M70																9				9	9.7
	M71																	3			3	9.7
	M72																		13		13	9.7
	M73																				0	9.7
	M74																			23	23	9.7
	M75															2					2	9.7
	M76																1				1	9.7
	M77																	15			15	9.7
	M78																		7		7	9.7
Observed total per dam		17	36	37	0	40	0	6	8	3	15	10	2	7	5	2	10	18	20	23		
Expected total per dam		19.4	19.4	19.4	19.4	19.4	19.4	19.4	9.7	9.7	9.7	9.7	9.7	9.7	19.4	19.4	19.4	19.4	19.4	19.4		

Table 3.9. Number of offspring assigned into each family in the partial factorial mating in the first batch of the G_2 generation.

		Dam																			
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	Observed total per sire	Expected total per sire
Sire	M1	11																			11	13.7
	M2		20																		20	13.7
	M3		12	36																	48	27.5
	M4			15	18																33	27.5
	M5				15	14															29	27.5
	M6					17	9														26	27.5
	M7						23	6													29	27.5
	M8							13	14												27	27.5
	M9								8	22											30	27.5
	M10									23	16										39	27.5
	M11										32	8									40	27.5
	M12											3	2								5	27.5
	M13												7	1							8	27.5
	M14														18						18	27.5
	M15														4	5					9	27.5
	M16															4	24				28	27.5
	M17																12	38			50	27.5
	M18																	21	16		37	27.5
	M19																		2	6	8	27.5
Observed total per dam		11	32	51	33	31	32	19	22	45	48	11	9	1	22	9	36	59	18	6		
Expected total per dam		13.7	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	13.7		

Number of offspring assigned into each family in the partial factorial mating in the first batch of the G_2 generation (continued).

		Dam																			Observed total per sire	Expected total per sire
		F19	F20	F21	F22	F23	F24	F25	F26	F27	F28	F29	F30	F31	F32	F33	F34	F35	F36	F37		
Sire	M20	23	9																		32	27.5
	M21		3	2																	5	27.5
	M22			6	4																10	27.5
	M23				2	23															25	27.5
	M24					8															8	13.7
	M25						12														12	13.7
	M26							22													22	13.7
	M27								4												4	13.7
	M28									6											6	13.7
	M29										12										12	13.7
	M30											24									24	13.7
	M31												8								8	13.7
	M32													14							14	13.7
	M33														8						8	13.7
	M34															10					10	13.7
	M35																19				19	13.7
	M36																	17			17	13.7
	M37																		35		35	13.7
	M38																			10	10	13.7
Observed total per dam		23	12	8	6	31	12	22	4	6	12	24	8	14	8	10	19	17	35	10		
Expected total per dam		13.7	27.5	27.5	27.5	27.5	13.7	13.7	13.7	13.7	13.7	13.7	13.7	13.7	13.7	13.7	13.7	13.7	13.7	13.7		

Table 3.10. Number of offspring assigned into each family in the partial factorial mating in the second batch of the G_2 generation.

		Dam													Observed total per sire	Expected total per sire
		F38	F39	F40	F41	F42	F43	F44	F45	F46	F47	F48	F49	F50		
Sire	M1	15													15	14.0
	M2	12	12												24	27.9
	M3		17	5											22	27.9
	M4			5	24										29	27.9
	M5				31	17									48	27.9
	M6					20	20								40	27.9
	M7						7	18							25	27.9
	M8							6	6						12	27.9
	M9								24	34					58	27.9
	M10									7	6				13	27.9
	M39										2	2			4	27.9
	M40												21		21	27.9
	M41												24	5	29	27.9
Observed total per dam		27	29	10	55	37	27	24	30	41	8	2	45	5		
Expected total per dam		27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9		

Number of offspring assigned into each family in the partial factorial mating in the second batch of the G_2 generation (continued).

		Dam														Observed total per sire	Expected total per sire
		F50	F51	F52	F53	F54	F55	F56	F57	F58	F59	F60	F61	F62	F63		
Sire	M42	1	2													3	27.9
	M43		4	13												17	27.9
	M44			4	1											5	27.9
	M45					14										14	14.0
	M46					25										25	14.0
	M47						22									22	14.0
	M48							43								43	14.0
	M49								31							31	14.0
	M50									3						3	14.0
	M51										32					32	14.0
	M52											16				16	14.0
	M53												8			8	14.0
	M54													6		6	14.0
	M55														1	1	14.0
Observed total per dam		1	6	17	1	39	22	43	31	3	32	16	8	6	1		
Expected total per dam		14.0	27.9	27.9	14.0	27.9	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0		

In the G_1 generation, there was approximately 31.9% full-sib family of the assigned families which had from 1 to 5 assigned progeny. The main proportion of full-sib family (estimated 43.4%) had from 6 to 15 assigned progeny. The other percentage (about 24.7% of full-sib family) of assigned family had more than 15 offspring.

In the G_2 generation, the proportion of full-sib family had from 1 to 5 assigned progeny was approximately 24.2%. There was 37.4% and 29.3% assigned full-sib family had from 6 to 15 offspring and 16 to 25 offspring respectively. About 9.1% full-sib family had more than 25 assigned progeny.

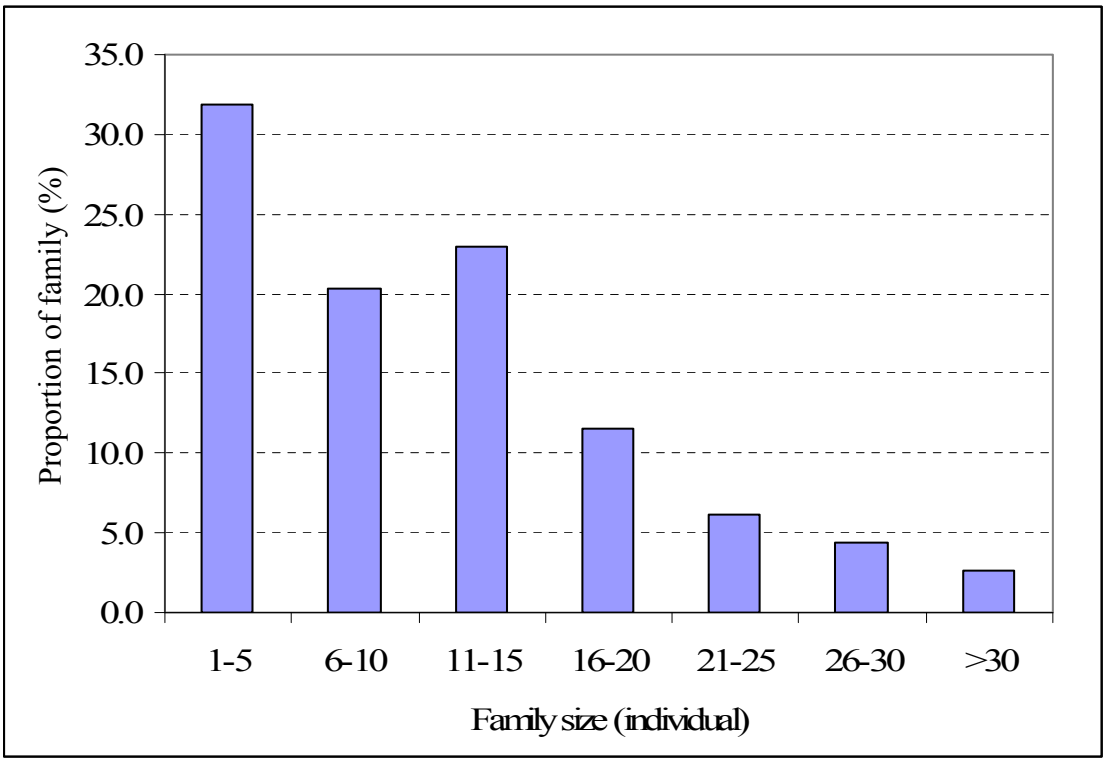

Figure 3.1. Frequency distribution of the number of progeny per full-sib family in the G_1 generation.

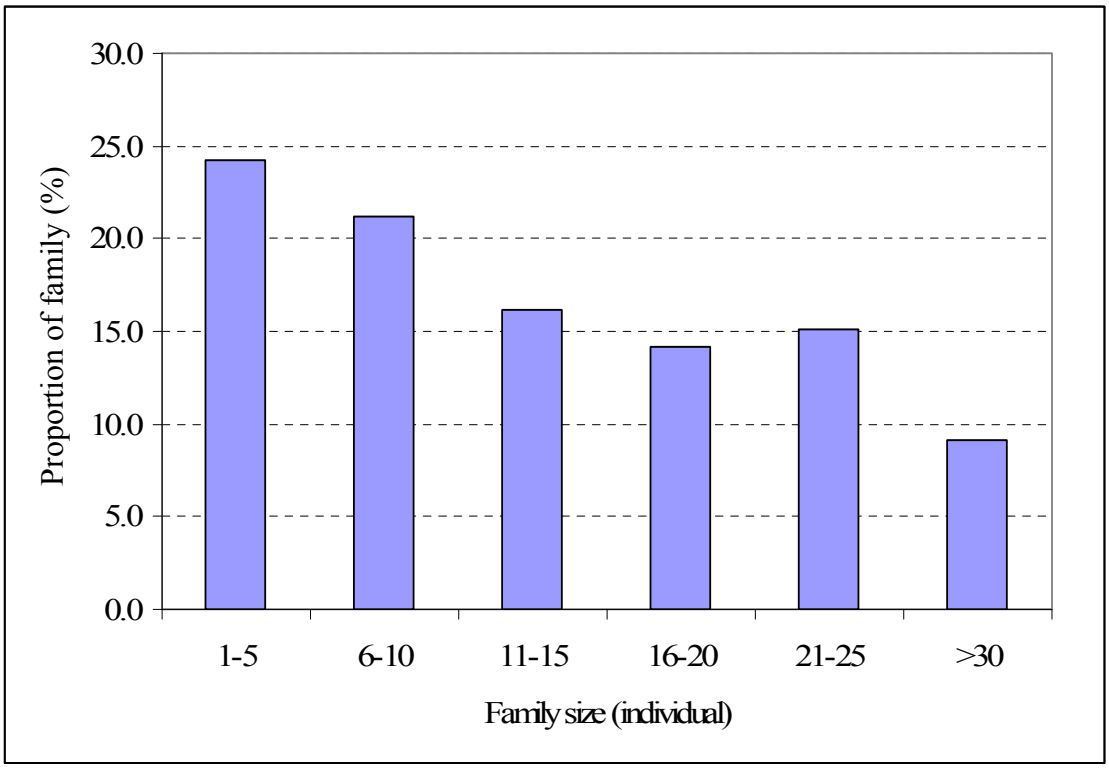

Figure 3.2. Frequency distribution of the number of progeny per full-sib family in the G_2 generation.

In the first generation of selection, the calculated number of progeny assigned to sires (total from crosses made using each sire) ranged from zero (seven sires) to fifty nine and for dams it varied from zero (six dams) to seventy three. Of these, thirty one sires contributed one to thirteen individuals in each family (from 0.1% to 1.0% of the total assigned offspring), 28 males contributed 14 to 29 progeny (from 1.1% to 2.2% of the total assigned offspring), seven sires produced 30 to 40 (from 2.3% to 3.1% of the total assigned offspring) and five sires had more than 40 progeny (from 3.2% to 4.6% of the total assigned offspring). A similar trend was seen for the dams, where a high number of females (33) contributed few progeny, from one to 13 (estimated from 0.1% to 1% of the total assigned offspring), 26 females produced from 14 to 29 individuals (estimated from 1.1% to 2.2% of the total assigned offspring), 8 females produced 30 to 40 (estimated from 2.3% to 3.1% of the total assigned offspring) and

five females had more than 40 offspring per family (estimated over 3.1% of the total assigned offspring).

For the second generation of selection (G_2), parentage assignment showed that contribution of parents to number of offspring from total crosses was from one to eighty-eight for sires and from one to fifty-nine for dams. Twenty four sires had from 3 (0.1% of the total assigned offspring) to 14 (1% of the total assigned offspring) offspring, 14 males had from 15 (1.1% of the total assigned offspring) to 29 offspring (2.2% of the total assigned offspring), six males had 30 (2.3% of the total assigned offspring) to 39 (2.9% of the total assigned offspring), and eleven sires had more than 40 progeny (over 3.0% of the total assigned offspring). Similarly for the dams, 27 females had 1 (contributed 0.1% of the total assigned offspring) to 14 (contributed 1% of the total assigned offspring) offspring, 28 females had 16 (contributed 1.1% of the total assigned offspring) to 39 (contributed 2.9% of the total assigned offspring), and eight females had more than 40 offspring (contributed over 3.0% of total assigned offspring).

In summary, there were very few mothers and fathers without any progeny, and this only occurred in the G_1 generation. About one third of both dams and sires had from one to ten offspring per family. The remaining parents had larger family sizes and contributed majority of the offspring in the assigned population.

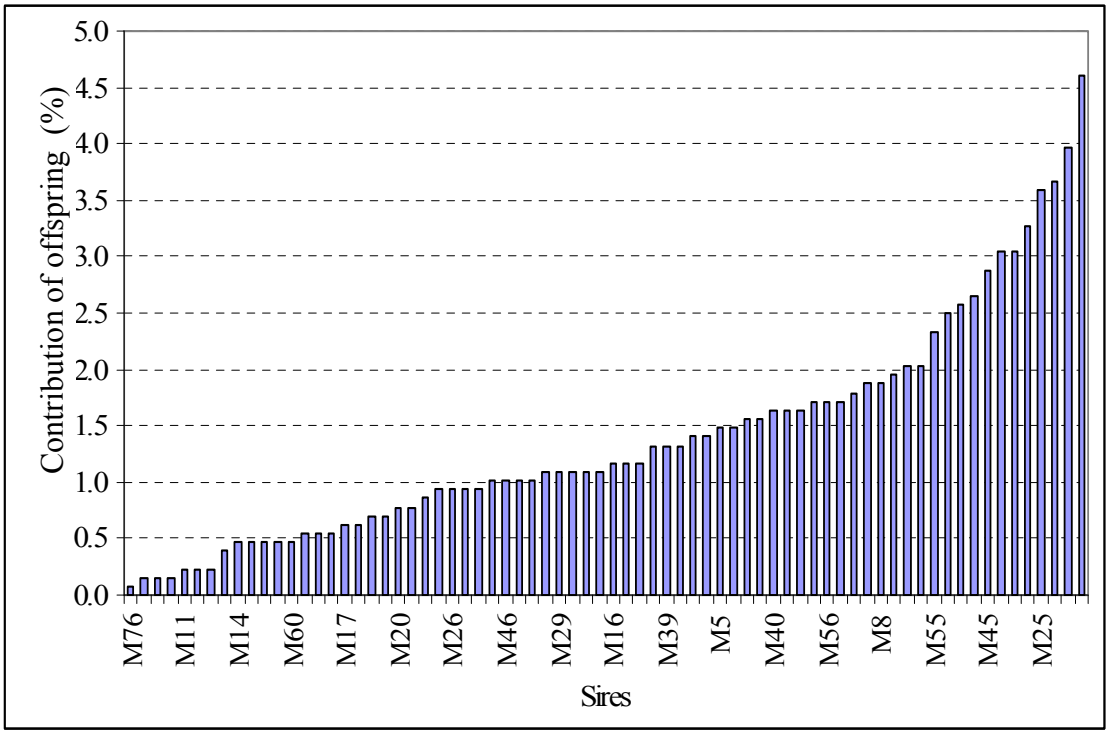

Figure 3.3. Percentage of offspring sired by males in the G_1 generation of common carp breeding programme.

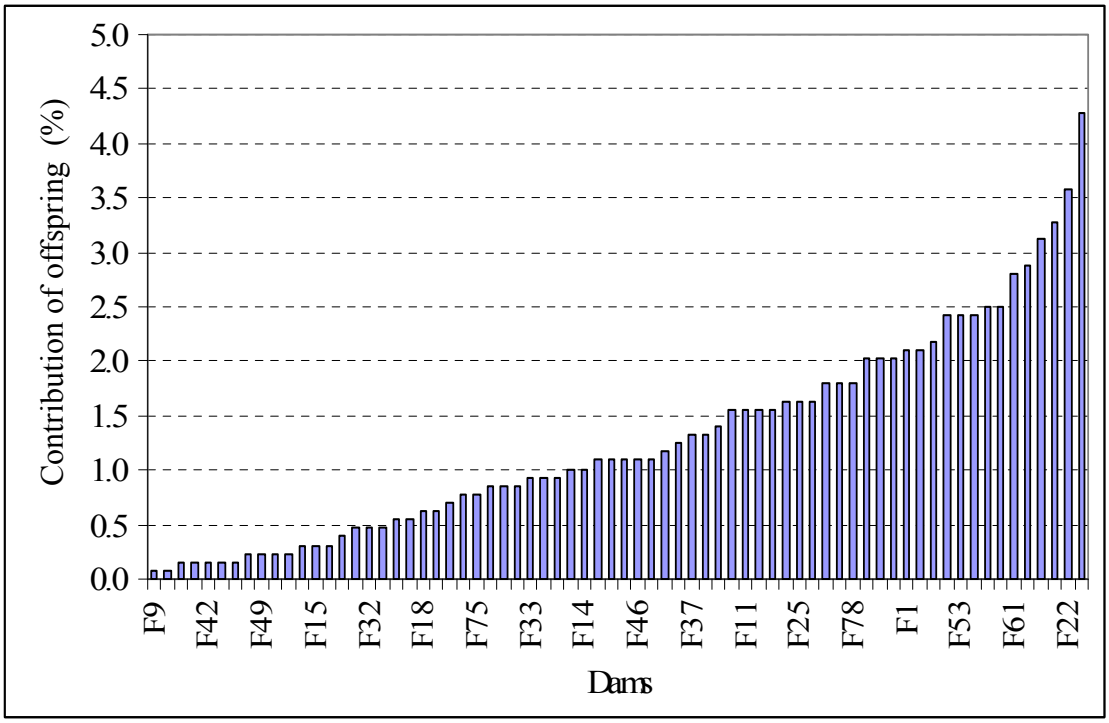

Figure 3.4. Dam contributions to the assigned progeny in the G_1 generation of common carp breeding programme.

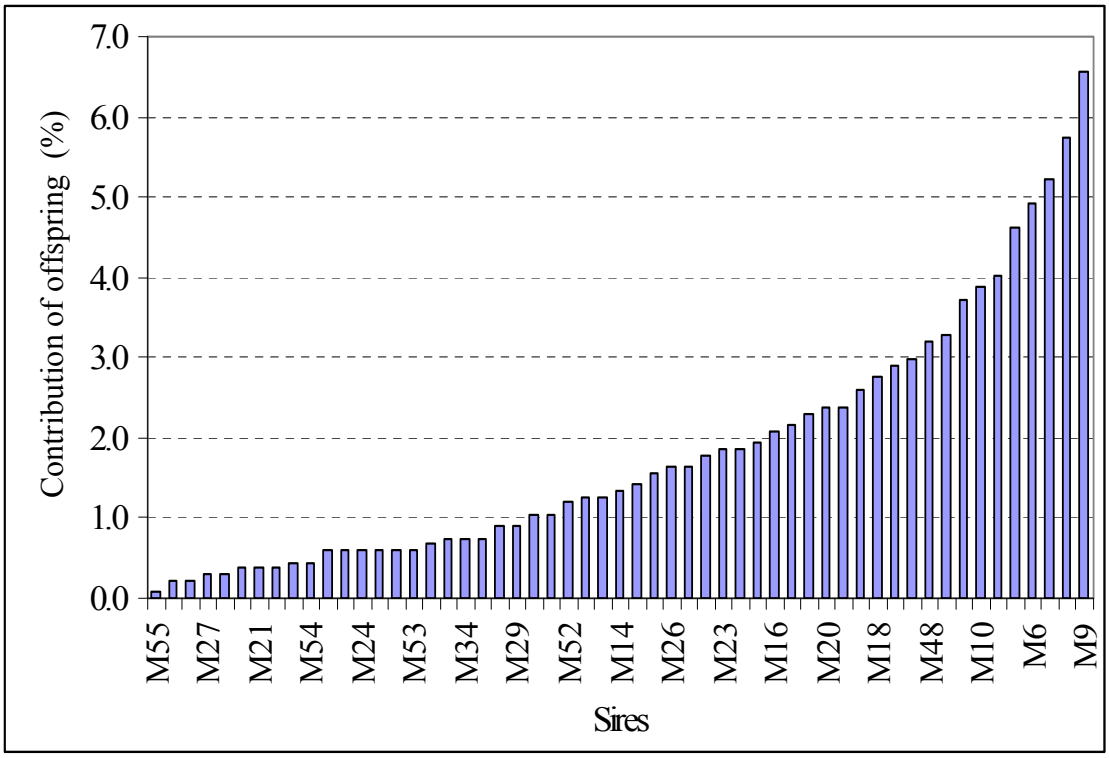

Figure 3.5. Percentage of offspring sired by males in the G_2 generation of common carp breeding programme.

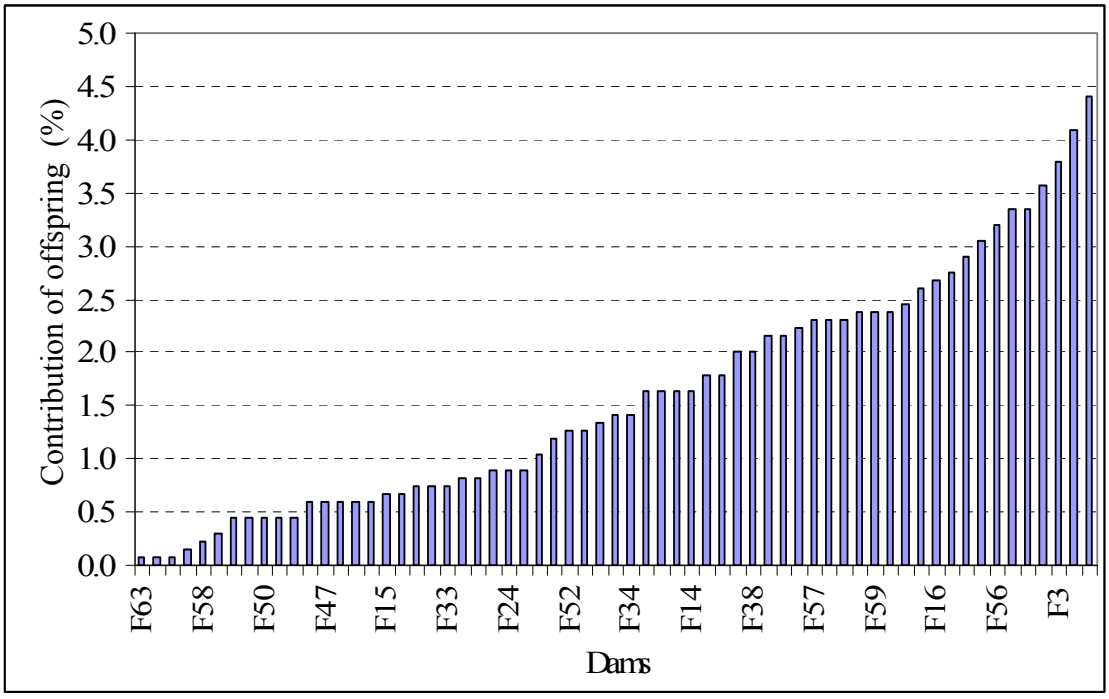

Figure 3.6. Dam contributions to the assigned progeny in the G_2 generation of common carp breeding programme.

3.3.3. Effective population size and inbreeding

The effective breeding population size was calculated for the combined batches of communally reared fish in the G_1 and G_2 generations of the common carp selection programme. The estimated effective population size (N_e) for selected population base on the family sizes derived from parentage assignment was 90 in the G_1 generation and 62 in the G_2 generation. The census population size (N) was 116 and 73 in G_1 and G_2 generations respectively. The observed N_e/N ratios were 0.78 in G_1 generation and 0.85 in G_2 generation. The inbreeding coefficient in the G_1 and G_2 generations were 0.5% and 0.8% respectively.

3.4. Discussion

Seven polymorphic microsatellite loci were used for genotyping and tracing communally reared offspring to their parents in the G_1 and G_2 generations of the common carp selective breeding programme. However, this study showed that there was some loss of genetic variation after two generations of selection, as judged by the loss of some rare alleles and reduction of effective number of alleles. The loss of rare alleles, sample size and genotyping errors resulted in lower efficiency of parentage assignment compared to that predicted.

3.4.1. Microsatellites polymorphism

The base population (G_0) in the current study was derived from different sources including inbred and outbreed common carp strains that originated from Hungary, Indonesia and Vietnam. So allele polymorphism for the seven loci was high and consistent with an earlier study by Gheyas (2006) on selective breeding of common carp involving pooling six different stocks and other studies on carp populations (Desvignes et al., 2001; Bártfai et al., 2003; Kohlmann et al., 2005). The mean number of alleles in this study was higher than reported by Vandeputte et al. (2004) and Lehoczky et al. (2005) because they analyzed fish originating from one strain of mirror carp.

The excess of homozygotes in the locus MFW9 for all three generations (G_0, G_1 and G_2) and in the locus MFW26 for generation G_2 only indicated the presence of null alleles in this study. The excess of homozygotes in locus MFW26 happened only in one generation and may have been caused by poor sample preservation and

transportation or technical issues. Decline of genetic variation levels between farmed and wild fish and between generations in aquaculture implementation as well as in selective breeding programme have been studied. In the current study, the percentage of allele losses were 1.6% and 10.2% compared to the base population after the first and second generations of selection respectively, although high numbers of males and females provided genetic material into the next generation under pedigree management. The estimated losses were very different between the two generations because the second generation had fewer parents contributing and some possible genotyping errors (discussed later in section 3.4.2). However, it is lower than in other studies, for instance, 4.1% and 12.32% loss of alleles were observed in the first (produced from mass spawning of 50 pairs of broodfish) and second generations (produced from mass spawning of 38 pairs of broodfish) of mass selection programme of common carp reported by Gheyas (2006). Perez-Enriquez et al. (1999) compared the genetic variation of a hatchery reared stock of red sea bream used for stock enhancement with that of their broodstock and found that the number of alleles per locus was reduced in about 25% from the broodstock to the progeny. Koljonen et al. (2002) estimated an average of 4.7% of allele loss for each generation of selection in two generations in Atlantic salmon selection programme, while 35% to 62% allele reduction in several G_1 hatchery reared abalone population compared to the wild stock was observed by genotyping some microsatellite loci (Evans et al., 2004). High percentage of allele loss (26%) was found between original wild caught individuals and G_1 generation in Atlantic halibut in Canada (Jackson et al., 2003). Similar results were observed in other species such as Sea trout (Was and Wenne, 2002) and rainbow trout (Butler and Cross, 1996).

3.4.2. Efficiency of parentage assignment

Microsatellite markers have been extensively used for many applications, particularly for clarification of relationships between individuals. This study evaluated ten microsatellite loci described by Crooijmans et al. (1997) for traceability of common carp in a selective breeding programme. However, three loci could not be used because of a failure of amplification or poor results. The other seven loci, amplified in triplex (MFW4, MFW9, MFW11) and tetraplex (MFW7, MFW18, MFW20, MFW26) PCRs, were polymorphic and analyzed for parentage assignment of common carp in the partial factorial mating of this selective breeding programme. The mean number of alleles at the seven loci used for parentage analysis in the G_1 and G_2 generations were 14.9 and 13.6 respectively, with assigned parentage (allowing for up to two mismatches) of 96.8% of the G_1 generation and 96.2% of the G_2 generation. The results for number of loci, allele polymorphism and assignment efficiency are comparable to published studies on common carp and other fish species. Vandeputte et al. (2004) reported over 95% assignment (using VITASSIGN) in the total of 550 offspring from a 10 × 24 factorial cross using eight microsatellite loci in common carp, although the mean number of alleles was 7.75 (much lower than the present case). In the hatchery reared stock of red sea bream, Perez-Enriquez et al. (1999) using only five microsatellite markers and the mean number of alleles was 29.75 analyzed on Microsoft Excel™, could trace 73% progeny (in a total of 200 juveniles) to their parental pairs out of more than 7800 possible pairs (about 65 dams × 65 sires in one tank and about 60 dams × 60 sires in the other) from at least 91 out of 250 breeders actually produced. In addition, the analysis of 20% un-assigned progeny was the result of scoring mistakes. Fishback et al. (2002) used 14 multiplexed markers and

analyzed data in PROBMAX to assign more than 91% of offspring to single parental pairs in a 48 × 2 factorial cross in rainbow trout.

In the initial assignment analysis, the means of single assignment with perfect matches only for 7 loci were 87.2% in the G_1 generation and 86.3% in the G_2 generation, while the final assignments were higher by 9.6% in the G_1 and 9.9% in the G_2 with two mismatches allowed. In the other studies, O'Reilly et al. (1998) reported almost 14% incorrectly typing at more than one allele for 674 surveyed offspring in Atlantic salmon and it could be successfully obtained after rescoring, cleaning up the data set and allowing mismatches. In addition, approximately 20% improvement of allocation to parent pairs was achieved after extensive correction of the dataset in Atlantic cod (Herlin et al., 2007). Typing errors in the present case appeared to be much lower than in the Atlantic cod example and moderate compared to the study in Atlantic salmon. Although control samples were added for each genotyping run, some types and rate of scoring error such as technical causes (non-amplification or present of amplification artefacts), heterozygotes for adjacent alleles and null alleles were observed among the seven microsatellite loci, particularly when many samples were analyzed in two multiplex PCRs of three and four loci.

Furthermore, genotyping errors and mutation are known as very common sources of errors in parentage assignment. They contributed at a rate of about 2% when using microsatellite markers in a range of species (Bonin et al., 2004; Castro et al., 2004; Castro et al., 2006; Hoffman and Amos, 2005). In the current study, means of 1.2% progeny in the G_2 and 1.3% progeny in the G_1 were not assigned to any pair of parents. In addition, a higher proportion of individuals with assignment to more than one family was found in the G_2 than those in the G_1. The excess of homozygotes due

to technical causes (non-amplification or present of amplification artefacts) in the two multiplex PCRs were considerably unable to distinguish and interpret the real alleles. The appearance of null alleles related to homozygotes were observed majority in locus MFW9 for all the G_0, G_1 and G_2 generations and in locus MFW26 for G_2 generation. These affected to the result of assignment where single assignment with one to two mismatches were much higher than perfect matches. There were no progeny giving new alleles that were not found in the parents, indicating that mutation at the seven microsatellite loci was probably not a serious problem.

3.4.3. Parental contribution to the family size

There were strong effects of both sire and dam on survival rate of animal as well as fish and shellfish like in seabass (Garcia de Leon et al., 1998). The most likely reason for maternal effects is differences in egg size and egg quality (Gjedrem, 1992). The effect of sires on survival was weak and observed just in case of very limited number of sires involving family production while the effect of dams was very high in rainbow trout (Herbinger et al., 1995). Vandeputte et al. (2004) reported a pattern of differential survival observed due to small effects of sires but large effects of dams and the contribution of maternal effects on survival tended to decrease from birth to one year old in trout. In the current study, a large number of female and male common carp were stripped to collect eggs and sperm for fertilization and production of families then separately incubated in jars until absolute yolk digestion of larvae. An equal number of exogenous feeding larvae were taken from each family by volumetric method for communal rearing in ponds. Although each generation had two batches and the interval time between them was only seven days, the batches were stocked and raised in different ponds. The present study pooled exogenous feeding larvae, and

so avoided pooling eggs of variable quality with unknown fertilization and hatching rates of eggs, as reported by Herbinger et al. (1995), Vandeputte et al. (2004) and Garicia de Leon et al. (1998). Therefore, it reduced as far as possible common effects on family size and survival caused by maternal and environmental effects.

Three females (F45, F63, F65) produced no offspring (either full- or half-sib), a total of 6 full-sib families in the first generation. Furthermore, none of the ten males participated in both spawning sets produced families which was not assigned any progeny in the first set but gave offspring in the second set. In this study, partial factorial mating allowed one male to be mated to two females and one female mated to two males. Some females (F5, F9, F12, F20, F49, F74) crossed to one male (M5, M9, M12, M21, M10, M69) and another male (M6, M10, M13, M20, M9, M75) produced zero to low number of offspring per family in the G_1 generation (see details in Table 3.7, 3.8, 3.9, 3.10) that indicates a strong female effect on survival to harvest. This was similar to females F13, F48, F53 in the G_2, which when crossed to males M14, M40, M45 produced no offspring but when crossed to other males (M13, M39 and M44) gave one or two offspring only. This shows that survival of families of common carp was strongly affected by the dam even though communal rearing started at the exogenous feeding larvae stage. Our results are in accordance with those of Vandeputte et al. (2004) on common carp.

There were higher number of families without any assigned offspring in the G_1 (22 families) than in the G_2 (2 families). Looking at the number of family and sample sizes, the G_1 generation possessed 34 families more than the G_2 generation, while sample sizes were similar, 1327 versus 1396. In addition, the fin clip samples for

parentage analysis were randomly collected from total seine netting of fish in the ponds. Therefore, family size of assigned offspring depends mainly on sample size and maternal effects. This result is consistent with recent studies in Atlantic salmon (Doyle and Herbinger, 1994; O'Reilly et al., 1998) and in rainbow trout (Herbinger et al., 1995; Fishback et al., 2002).

3.4.4. Effective population size (N_e) and inbreeding (ΔF)

The effective population size for small populations, as represented by the effective number of breeders, is essential information for the estimation of inbreeding (Gall, 1987). The classical calculation of N_e assumes random family samples and equally family sizes (Tave, 1993). However, in practice N_e depends strongly on mating systems because these determine variation of male mating success. The selective breeding programme studied here followed a partial factorial mating scheme and ten males used to produce families of the first batch were re-used for the second batch. The number of sires and dams in selection lines was the same in the G_1 generation (58 males and 58 females) but unbalanced (33 males and 40 females) due to limitation egg volume of spawned females in the G_2 generation. The variable full-sib family size, number of family and unequaly sex ratios had major impacts on the effective population size (N_e) that reduced the N_e to less than the census population size (N) as were observed from the N_e/N values in the G_1 generation (0.78) and G_2 generation (0.85). The reduction of effective population size may increase the rate of inbreeding. In our study, the G_1 and G_2 generations were produced from 116 and 73 unrelated breeders respectively which were higher than estimated N_e of 90 breeders for the G_1 and 62 breeders for the G_2.

The inbreeding coefficients were low in the two generations (0.5% in the first generation and 0.8% in the second generation). It is known that many breeding programmes run on the assumption that the effects of inbreeding through sib matings for growth traits selection can lead to inbreeding coefficients of more than 10% (Myers et al., 2001). An increase in the rate of inbreeding of >1% (corresponding to an effective population size of 50) per generation should be avoided in order to maintain fitness in a breed (FAO, 1998). The current study avoided matings between close relatives and increased the number of breeding parents in each generation so that the rates of inbreeding were lower than 1%.

3.5. Conclusions

The results demonstrated that the seven microsatellite loci used showed high polymorphism and satisfactory parentage assignment in the studied population of common carp. These molecular markers were used to establish the pedigree of fish communally reared from the early larvae stage. The estimation of effective population size (N_e) based on molecular assignment of offspring to families is useful information to conduct effective breeding programme. These results suggested that the genetic improvement programme of common carp at RIA 1, Vietnam can be conducted efficiently when based on this molecular method. The early communal rearing, even at hatching stage, does not need intensive labour for management or huge facilities for separate family rearing such as tanks and hapas. It also reduces common environmental effects including environmental and maternal effects in the selection programme. However, some errors were observed in the genotyping analysis, of which null alleles and scoring errors affected assignment results.

Chapter 4. Genetic and Phenotypic Analyses of the Base Population

4.1. Introduction

4.1.1. Quantitative genetic selection in hatcheries

4.1.1.1. No planned selection

Even without planned selection, unintentional selection can change the gene pool of captive fish populations by eliminating potentially valuable alleles for disease resistance and growth, thus causing negative impact on future selective breeding programmes. Unintentional selection can occur at any stage in a hatchery. There is a general phenomenon that fish populations with narrow genetic bases often result in hatcheries where the best broodstock are selected from a limited gene pool i.e. the fish that are able to live and reproduce under hatchery conditions. The fish that are unable to survive and reproduce in hatcheries may be the ones that perform best in the wild. An example of unintentional selection that apparently eliminated the potential for increased growth rate was found in a common carp breeding programme in Israel (Moav and Wohlfarth, 1976). The absence of an additive genetic effect for increased growth rate in the population was thought to be due to the practice of spawning the largest fish without pedigree record to obtain more eggs per female. If the largest fish were selected over several generations, this might not result in faster growing fish thereafter since the number of broodstock used was small so relatives of fish will soon start mating with each other and inbreeding starts accumulating. Eknath and Doyle (1985) indicated that the standard practice of spawning the largest catla and rohu in Indian hatcheries reduced productivity when the largest females were chosen as future brood fish on the basis that they were likely to be more fecund and faster growing.

However, if the broodstock were derived from several different spawnings, the larger fish are likely to be the ones produced early in the breeding season; therefore, they unintentionally selected for faster growing and early maturing fish. Additionally, the effective population sizes of the stocks were low, with high expected rates of inbreeding, up to 4.4% per generation (Basavaraju et al., 2004). Another example of unintentional selection was the way in which many commercial channel catfish fingerling producers obtained brood stock. Brood fish commonly came from brood fish growout ponds that contained large fish. These fish were available in quantity and at relatively low prices. The culturist had the impression that the fish might be good brood stock because they were large, appealing fish when compared to the rest of the pond population. However, most growout ponds in the lower Mississippi River valley were operated for years without draining, and after several cycles of harvesting and restocking, it was impossible to know the age, origin, or history of the individual fish in the pond. The larger fish were usually older fish that evaded capture rather than fast-growing fish. When these fish were chosen as brood stock, the culturist could well be unintentionally selecting for slow-growing fish (Tucker and Robinson, 1990).

Unintentional selection in general could result in losses of some potentially valuable alleles; however in some cases it could be beneficial in fish production. The domestication of food fish populations has resulted in better performance in some cases, shown in trials at farm level compared to the wild stocks. For instance, hatchery strains of channel catfish (not subjected to deliberate selection for growth) grow faster than wild strains when they are stocked at commercial rate and fed artificial diets (Dunham and Smitherman, 1984). Dunham and Smitherman (1983) also reported that the domestication process has increased growth rate by 2-6% per generation in channel catfish.

4.1.1.2. Directional selection

Directional selection aims at improving productivity by changing the (genetic) mean of the population. A systematic genetic improvement programme includes several steps from setting up very clearly defined breeding goals to development of selection strategies. The effect of directional selection for heritable traits is a change in gene frequency at the loci affecting traits in the next generation. The average phenotypic value of progenies of selected parents is increased in constant environmental conditions (Gjedrem and Thodesen, 2005). The population's phenotypic mean for quantitative traits could be either increased or decreased depending on the desire in terms of productivity and profits. Breeding goals in conjunction with well designed plans are essential requirements for a successful programme of directional selection. Plans are the key point to achieve the goals, including a set of instructions that outline the methods of phenotype measurements and selection.

4.1.2. Synthetic populations for selection

It is possible to increase the growth rate of common carp for farm culture by carrying out a genetic improvement programme. The two most commonly used and immediately applicable methods of genetic improvement are crossbreeding and selection. Before a genetic improvement programme can be implemented, however, it is necessary to estimate a number of genetic parameters through direct research. The amount of genetic improvement achieved by crossbreeding is often expressed as the level of heterosis for a trait of interest, mainly resulting from the dominance effects of alleles. The extent of heterosis may differ depending on the strains or lines being crossed and the direction of cross. Theoretically, it may be that the most inbred lines give the best heterosis, but the inbreeding may have reduced the parental line means.

The rate of genetic improvement in a selection programme is a function of the selection intensity, the phenotypic variance of the trait and the heritability of the trait. Heritability is the proportion of phenotypic variance due to additive genetic differences among individuals. Furthermore, genetic improvement in one trait may also have indirect effects on other traits, and these are dependent upon the genetic correlations among traits of interest.

4.1.2.1. Crossbreeding

Crossbreeding is mating between breeds, populations, strains or inbred lines. When lines are inbred without selection the mean of all their crosses is expected to be equal to the mean of the outbred population from which they were derived (Lynch and Walsh, 1998). Therefore inbreeding followed by crossing cannot produce any improvement - there must be selection at some stage if any improvement is to be made. So crossbreeding is considered as a supplement to a programme for additive genetic improvement. A number of selective breeding programmes have been conducted to change length, weight, time of spawning, viability, disease resistance, meristics and sex ratio (Gjedrem, 2005). Some of these programmes incorporated crossbreeding in addition to selection. Kirpichnikov et al. (1974) described a selection programme for increasing weight gain in the Ropsha strain of common carp, combining individual and family selection for weight gain, crossbreeding and progeny testing.

4.1.2.2. Heterosis

The superiority or inferiority of hybrids is measured as heterosis (Tave, 1993). Fjalestad (2005) described two methods generally used to estimate heterosis, namely:

(1) to compare crossbred progenies with the average of both parental lines and/or strains; or (2) to compare the crossbred progenies with the average of the best parental line or strain. If parents originated from different gene pools, crossbreds have increased heterozygosity and therefore a higher heterosis is expected. The extent of the heterosis level of a studied trait depends on the genetic distance between the parent populations.

Heterosis is generally assumed to be controlled by dominance effects. If this is so, heterosis of F_2 hybrids should be a half of that of the F_1 hybrids. In case of heterosis controlled by dominance effects, F_1 hybrids will be the best. It is likely that heterosis is also influenced by effects of additive genetics, maternal genetics, maternal heterosis and epitasis, implying that F_2 or other types of hybrids can be better than F_1 hybrids. Consequently, the production of F_2, backcross hybrids or other types of hybrids could produce outstanding fish for grow-out. Jayaprakas et al. (1988) compared growth of two strains of *Oreochromis niloticus* and their F_1, F_2 and backcross hybrids and found that heterosis of the F_2 and backcross hybrids was greater than that of the F_1 hybrids.

Tave et al. (1990) indicated that maternal heterosis was the reason why the F_2 and backcross hybrids were better. Maternal heterosis is produced when crossbred mothers are spawned. Maternal heterosis does not refer to increased egg production or other traits expressed by the mother. Those traits are part of heterosis for the F_1 hybrids. Maternal heterosis is expressed in the progeny of F_1 hybrid mothers as well as in the F_2 and the backcross hybrids. Even though dominance effects in the F_2 hybrids were only half as great as those in the F_1 hybrids, maternal heterosis is

expressed in the F_2 and backcross hybrids, and thus they grow faster than the F_1 hybrids (Tave et al., 1990).

In summary, relative gains to be achieved from crossbreeding and selection depend on the magnitude of additive and non-additive variation for the trait or traits of interest. If non-additive variance is large, substantial gains can be made by crossbreeding. Furthermore, once the various genetic parameters that contribute to heterosis are known, they can be used to predict the result of other hybrid mating.

4.1.2.3. Forming a base population

It is very important to start with a broad genetic variation when developing a breeding programme for aquaculture species. There is some research demonstrating that mass selection for improved growth rate in experimental populations of carp and tilapia failed because of narrow genetic material in the base population (Moav and Wohlfarth, 1973, 1976; Hulata et al., 1986; Huang and Liao, 1990). Even if some response was observed for downward selection, Moav and Wolfarth (1976) suggested that genetic bottlenecks and high levels of inbreeding in such closed, experimental populations might have reduced the genetic variation significantly. The synthetic populations are expected to accumulate more heterozygosity than the parental strains and they should show some heterosis gain. The heterosis may be reduced by inbreeding subsequent to a reduction in the synthetic population size (Fjalestad, 2005).

High genetic variability in the base population may be obtained by creating a synthetic population (Skjervold, 1982). Synthetic populations are produced from a

variety of parental populations, breeds, stocks or lines that combine the genetic material of the parental populations. A base population should combine characteristics of the subpopulations. Bondari (1983) created a synthetic base population for channel catfish by crossing six different cultured stocks and obtained significant response to selection for growth rate. The Norwegian breeding programme for Atlantic salmon was initiated by collecting and testing breeding candidates from 41 wild river strains (Gunnes and Gjedrem, 1978). The base for the synthetic tilapia population used in the breeding programme Genetically Improved Farm Tilapia (GIFT) was an 8×8 diallel cross between eight tilapia strains (Eknath et al., 1993). Six stocks were crossed to create a base population of rohu in India (Reddy et al., 2002). Selecting the best individuals across populations should form the founder stock of a synthetic population. Some minimum level of representation from each of the tested populations may be demanded, to ensure the genetic variability of the synthetic population (Bentsen, 1990).

4.1.3. Aims of the study

A series of single pair mating was conducted to form the base population from six lines of common carp. Separate family nursing in hapas and physical tagging for communal grow-out in earthen pond were applied for the experimental fish. This study aimed at analysis and assessment for heterosis, additive and non-additive genetic effects, heritability, phenotypic and genetic correlations of this synthetic base population for the future breeding programme for growth related traits in common carp.

4.2. Materials and methods

4.2.1. Synthetic population

4.2.1.1. The founder populations and their genetic variation

The founder population was formed from six lines of common carp. Pedigree information was recorded with the aid of PIT tagging (see details in Chapter 2). Broodfish taken from the previous selection programmes had been kept in the live gene pool conservation programme at the National Broodstocks Center, Research Institute for Aquaculture No.1, Vietnam. Their exact age was unknown but their weight was between 1.5 kg and 3.0 kg. Samples of six founder carp lines (parents of G_0 generation) were collected and genotyped for seven microsatellite loci (see details in section 3.2 of Chapter 3).

4.2.1.2. Spawning

The techniques of spawning induction, mating and family incubation were described in Chapter 2. After two doses of hormone injection, an equal number of eggs defined by volume measurement were collected from individual females and fertilized to sperm of each male to produce full sib families. As a result, 101 families (see Table 2.3 in Chapter 2) were mated and incubated separately in 10 liters jars. During artificial incubation, the water temperature dropped to around 18^0C and the eggs took 7 to 9 days to hatch. Due to the long time of incubation in the low temperature water, diseases especially fungi occurred and thus the hatching rate of some families was relatively low. Nevertheless, 2,000 individuals were obtained from each family for rearing in fine mesh hapas.

4.2.1.3. Family rearing procedures and code wire tagging (CWT)

Within three days after hatching, the larvae completed yolk absorption. Swim-up fry were fed with egg yolk for two days before they were transferred to hapas for rearing. The larvae were gathered and approximately 2,000 individuals per family were transferred to nurse in $1m^3$ (1m×1m×1m) fine net hapas for 30 days. The larvae in hapas were daily fed 1.0 kg soybean powder per 100,000 larvae in the first week and increased to 2.0 kg in the second and third weeks. After four weeks of nursing in fine mesh hapas, fry were transferred to raise in $5m^3$ (2.5m×2m×1m) plastic net hapas followed by standardizing at equal number of 120 fish per family. At this stage, fish were provided pellet food containing 25% crude protein at the rate of 10% body weight per day for 60 days.

Fingerlings were marked with coded wire tags (CWT) in different positions to allow communal rearing of five families in each section of earthen pond. One hundred individuals in each family were reared at a stocking density of 2.5 fish per m^2 in blocks of $200m^2$ separated by plastic net from the $4000m^2$ pond (200m length × 20m wide) with 1.5m depth. The fingerlings were fed daily with pellet food (25% crude protein) at 7% of their body weight per day. This rearing period was 120 days.

4.2.1.4. PIT tagging and fish raising

Individuals in each family with CWT were screened by positional detector and thirty five fish, randomly chosen from each family, were PIT tagged when their weight reached an average of 150 g per fish. There were 86 families available for PIT tagging. The PIT tag was injected in the dorsal muscle of each fish, close to the head,

since the application of intraperitoneal injection for carp was not introduced to RIA 1 until the G_2 generations produced. All the tagged fish were communally reared in a 4000m^2 grow-out pond of 1.5m depth at a stocking density of almost 0.8 fish per m^2. The feeding regime, applied daily, was at the rate of 5% body weight with the ration containing 25% crude protein. Feeding was adjusted monthly based on the sampled fish weight measurement. Water parameters were monitored and kept in good condition for fish growth.

4.2.1.5. Harvesting and data collection

In early March 2004, an unknown disease outbreak in several Northern provinces of Vietnam caused high mortality in the population. After six months of communal rearing in the grow-out pond, the fish were harvested completely. All fish were scanned and the tag numbers were recorded. There was about 35% loss in each of the 86 stocking families. The number of dead fish ranged from 5 to 12 per family. Body weight and length were individually measured on fish.

4.2.2. Statistical analysis

4.2.2.1. Genetic variation analysis

The total number of alleles were counted for all markers within each line using GENETIX version 4.02 (Belkhir et al., 1998). Observed heterozygosity (H_o) and expected heterozygosity (H_e) values were calculated for each line. The estimation of within population fixation of alleles (F_{IS}) were obtained by using GENETIX 4.02.

4.2.2.2. General analysis

A preliminary analysis using general linear model (GLM) was firstly used to investigate systematic non-genetic effects on body traits. All analyses were carried out in SAS procedures (SAS Inc, 2002). Data were analyzed to determine significance of all possible fixed effects. The GLM tested the effects of cross, sex (possible genetic effects) and age of fish on the harvest data. The two-factor interactions were also investigated, and were removed from the model if they failed to show significant ($P > 0.05$) effects on the traits of interest.

Secondly, a mixed model also developed and applied on the same data set. Pair-wise comparisons were generated using the PDIFF option of the Least Squares Means statement of PROC MIXED in all analyses (Littell et al., 1996). The model consisted of cross, sex and age as fixed effects. Family was considered as a random effect. The mixed model was written as follows:

$$Y_{ijkl} = \mu + CROSS_i + SEX_j + \beta AGE + F_k + e_{ijkl} \qquad \text{(Model 1)}$$

Where,

Y_{ijkl} is an observation of the individual l

μ is the overall mean

$CROSS_i$ is the fixed effect of cross ($i = 1 \ldots 19$)

SEX_j is the fixed effect of sex ($j = 1, 2$)

AGE is the covariable effect of age (age is accounted for days from hatch to measurement)

F_k is the random effect of family k^{th} nested to cross

e_{ijkl} is the residual error

4.2.2.3. Estimation of phenotypic and genetic parameters

Preliminary analysis result showed that the interaction between crosses and sex was not significant for the traits, and hence this random effect was not included in the final model. Age differences at stocking (date of hatch) were expected to result in size differences at the start of the grow-out stage and possibly at harvest so the age at stocking was included as a co-variable in the model. Rearing full-sib families in separate hapas and rearing groups of tagged families in partitioned ponds were intended to reduce systematic and non-genetic hapa effects which may be confounded with common environmental effect. Therefore, the common environmental effect included an effect due to the separate rearing of the full-sib families until tagging (hapa and partitioned pond effects), dominance genetic effect common to full-sibs and the maternal effect. A mixed model fitting individual and common environmental effect as random terms together with the significant fixed effects was as follows:

$$Y_{ijkln} = \mu + CROSS_i + SEX_j + \beta AGE + I_k + C_l + e_{ijkln} \qquad \text{(Model 2)}$$

Where,

Y_{ijkln} is an observation of the individual *n*

μ is the overall mean

$CROSS_i$ is the fixed effect of cross ($i = 1 \ldots 19$)

SEX_j is the fixed effect of sex ($j = 1, 2$)

AGE is the covariable effect of age

I_k is the random additive genetic effect of individual n^{th}

C_l is the random common environmental effect/hapas

e_{ijkln} is the residual error

- Heritability: Variance components for growth trait were estimated from a univariate model. Phenotypic variance (σ_P^2) calculation was based on additive genetic variance of individual (σ_A^2), variance common to full-sibs (σ_C^2) and residual error variance (σ_e^2), as $\sigma_P^2 = \sigma_A^2 + \sigma_C^2 + \sigma_e^2$. Then the heritability was calculated from individual components as $h^2 = \frac{\sigma_A^2}{\sigma_P^2}$. The common environmental effect was calculated as $c^2 = \frac{\sigma_C^2}{\sigma_P^2}$.

- Genetic and phenotypic correlations between weight and length were calculated as the covariance divided by the product of the standard deviations of traits: $r_g = \frac{\sigma_{12}}{\sqrt{\sigma_1^2}\sqrt{\sigma_2^2}}$, where σ_{12} was the estimated additive genetic or phenotypic covariance between the two traits.

All computations were carried out on the ASREML software package (Gilmour et al., 2002). Variance and covariance components were estimated using restricted maximum likelihood. Convergence for log-likelihood of variance component estimation was considered satisfactory when two successive rounds of interaction changed by less than 0.1%. All known pedigree information was included in the analyses through a numerator relationship matrix.

Heritability and common environmental effects and correlation estimates were tested for significantly different from each other, or zero by using z-scores:

$$z = \frac{x_i - x_j}{\left(\sigma_i^2 + \sigma_j^2\right)^{0.5}}$$

Where, x_i and x_j are the estimates of heritability and common environmental effects, or genetic correlations for the two traits and σ_i and σ_j are their respective standard errors. Both x_j and σ_j were set to zero or one when test of an estimate was significantly different zero or one, respectively. The resulting z-scores were then tested against a large sample normal distribution.

4.3. Results

4.3.1. Descriptive statistics

Table 4.1 shows the means, standard deviations and coefficients of variation for all body measurements of the G_0 population. The coefficients of variation for weight were much greater than for length. The measured and analyzed traits were at final harvest. Harvest was carried out within a few days. In addition, all families were simultaneously spawned. Therefore, the coefficient of variation for age is low (only 1.86%).

Table 4.1. Sample size (N), mean, maximum, minimum, standard deviation (SD), coefficient of variation (CV) of raw data for weight, length and age in G_0 generation.

Variables	N	Mean	Minimum	Maximum	SD	CV
Weight (g)	1750	211.5	50	879.0	99.16	46.94
Length (cm)	1750	23.0	10	38.2	3.75	16.29
Age (day)	1750	382.2	365	401	7.12	1.86

4.3.2. Prediction of fixed effects

General linear model (GLM) analysis showed highly significant ($P<0.001$) effects of crosses, sex and age for all traits. The R^2 values estimated by GLM indicated that a large proportion of the variation in body traits was related to crosses, 21% for weight and 26% for length. The estimates of fixed effects for the model 1 are presented in Table 4.2. There was no interaction between crosses and sex for weight ($P=0.9432$) or length ($P=0.9725$). The mixed model gave the same results as the Model 1 for estimation of effects on weight and length at harvest (data not shown).

It is one of the assumptions of GLM estimates that the observations are uncorrelated while mixed model employs a more general covariance structure approach. So GLM provides more extensive results for the traditional univariate and multivariate approaches to repeated measures and mixed model offers a better result of both mean and variance-covariance. However, there is no difference of estimated fixed effects between the two models because the estimations were based on unbalanced data of one observation only.

Table 4.2. The general linear model (GLM Procedure: SAS, 2002) estimates for the fixed effects of cross, sex and age.

	Degrees of	Weight		Length	
Effects	freedom	F-value	Probability	F-value	Probability
Cross	18	13.2	<0.001	21.7	<0.001
Sex	1	49.1	<0.001	20.7	<0.001
Age	1	44.7	<0.001	21.0	<0.001

4.3.3. Population characteristics and genetic parameters

4.3.3.1. Genetic variation of the founder population

There were differences of sample numbers between the founder populations which were genotyped, depending on their contribution to the G_0 generation. These genotype data were taken from the primary test for parentage assignment of limited sample number. The total number of alleles for all seven markers varied from 16 in the Vietnamese 6^{th} generation carp to 36 in Hungarian scale carp (Table 4.3). The observed heterozygosity was not significantly different from expected heterozygosity in all carp populations. The lowest F_{IS} value was observed in the 2^{nd} generation of family selection carp line.

Table 4.3. Founder populations of common carp: sample numbers (N), total number of alleles (A), expected heterozygosity (H_e), observed heterozygosity (H_0) and within strain fixation index (F_{IS}), based on analysis of seven microsatellite loci.

Carp lines of founder population	N	A	H_e	H_0	F_{IS}
2th generation of family selection	20	32	0.80	0.66	0.07**
Hungarian 6th generation	16	35	0.83	0.76	0.11
Hungarian scale carp	14	36	0.82	0.79	0.20
Indonesian 6th generation	12	22	0.77	0.63	0.21
Indonesian yellow carp	8	20	0.72	0.69	0.21
Vietnamese 6th generation	6	16	0.66	0.54	0.18

*** significant (P<0.01).*

4.3.3.2. Growth performance of G_0 generation

The least squares means of body weight and body length by crosses at harvest according to the Model 2 are shown in Table 4.4. High variation in number of family between crosses were due to initial intention that better growth carp lines could contribute more genetic material to the base population so this was relied on the ranks of their growth performance reported by Tuan et al. (2005). There was high variation of weight and length of the fish at final harvest for pure-breed and cross-breed. The measured data indicated no consistent contribution of genetic materials or lines for better growth performance in term of crosses. The growth performance of the crossbreds from lines B and C tended to be lower than that the mean of the pure breed lines, others intermediate (lines A, E, F) or higher (line D). The highest growth of fish was observed in a cross-breed (E×F), while another (A×B) presented the lowest weight (159.4g) and length (19.9cm). There was no representative family in one other crosses (A×E) due to poor survival rate.

Table 4.4. Least-squares means (±S.E.) of traits for crosses in the G_0 generation of common carp, according to the mixed model.

Cross	Number of families	Weight (g)	Length (cm)
A×A	8	161.3 ± 9.56	19.4 ± 0.33
A×B	9	159.4 ± 7.93	19.9 ± 0.28
A×C	7	193.8 ± 7.36	22.9 ± 0.27
A×D	5	211.5 ± 8.94	23.7 ± 0.32
A×F	3	279.3 ± 8.30	25.3 ± 0.30
B×B	8	212.7 ± 7.42	22.8 ± 0.27
B×C	7	210.8 ± 6.12	23.6 ± 0.22
B×D	4	220.8 ± 8.35	23.5 ± 0.30
B×E	1	194.3 ± 12.58	22.4 ± 0.46
B×F	2	162.0 ± 17.25	21.0 ± 0.63
C×C	6	224.9 ± 7.12	24.0 ± 0.26
C×D	4	254.2 ± 11.06	24.9 ± 0.40
C×E	4	197.7 ± 14.96	23.2 ± 0.54
C×F	1	165.1 ± 19.23	22.6 ± 0.70
D×D	5	178.9 ± 10.35	22.5 ± 0.38
D×E	3	187.9 ± 12.82	22.3 ± 0.57
D×F	3	216.1 ± 15.65	24.1 ± 0.57
E×E	3	206.0 ± 13.81	22.4 ± 0.50
E×F	3	285.7 ± 13.10	25.9 ± 0.47

[*] *A=Family selection carp; B=Hungarian mass selection carp; C=Hungarian scaled carp; D=Indonesian mass selection carp; E= Indonesian yellow carp; F=Vietnamese mass selection carp.*

4.3.3.3. Heterosis and sex

The percent heterosis measures the non-additive genetic effects relative to the additive genetic effect. Mean percent heterosis for weight and length for all crosses were -4.1% and 1% respectively.

The least square means of weight at harvest for males (191.0g) and females (221.9g) across crosses analyzed by the Model 2 were highly significantly different ($P<0.01$). A similar trend was observed for length between males and females (Table 4.5).

Table 4.5. Least-squares means (±S.E.) of traits by sex obtained from the mixed model.

Sex	Female	Male
Weight (g)	$221.9^{a} \pm 3.16$	$191.0^{b} \pm 3.74$
Length (cm)	$23.4^{a} \pm 0.11$	$22.7^{b} \pm 0.14$

**Means with different superscript letters in the same line are statistically different ($P<0.01$).*

4.3.3.4. Contribution of genetic materials to the base population

The founder genetic material contribution to the synthetic base population measured by the proportion of ancestors of the individuals in the base population from each line is presented in Figure 4.1. The calculation of genetic contribution of the founder population based on the genetic principle that each father or mother delivers 50% genetic material to their progeny. The percentage of successful brooders in each line to the total contributed brooders was their genetic contribution. The different initial proportion of ancestors' contribution was based on their growth performance in a previous study that will be discussed later in this chapter. The greatest contribution of

genetic material was from Hungarian scaled carp, family selection carp and Hungarian mass selection carp which ranged from 22.9% to 23.9%, followed by the Indonesian mass selection carp at about 13.8%. The proportion of ancestors of Vietnamese mass selection carp and Indonesian yellow carp were 7.7% and 8.2% respectively.

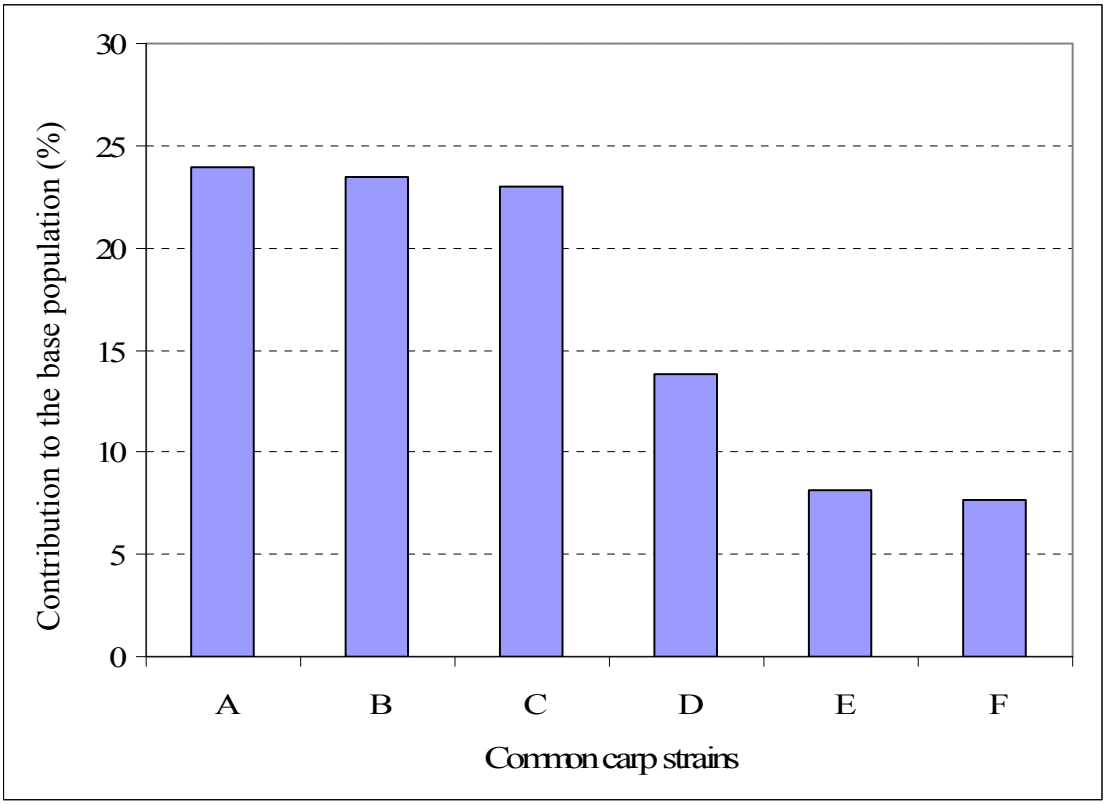

Figure 4.1. Contribution of genetic materials of the founder lines in the synthetic base population of common carp in the selective breeding programme.

A=Family selection carp; B=Hungarian mass selection carp; C=Hungarian scaled carp; D=Indonesian mass selection carp; E= Indonesian yellow carp; F=Vietnamese mass selection carp.

4.3.3.5. Heritability estimates

Heritability estimates for body weight and body length at harvest are given in Table 4.6. All the estimates of heritability were significantly different from zero ($P<0.001$)

and rather high for weight (0.63) and length (0.68). Furthermore, high standard errors of heritability were observed in this study. The common full-sib effects (c^2) accounted for small proportions of total variance for weight (4%) and length (9%).

Table 4.6. Estimated additive variance (σ_A^2), common full-sib variance (σ_C^2) residual variance (σ_e^2), heritability (h^2 ± S.E.), common full-sib effects (c^2 ± S.E.) for weight and length from mixed model fitting individual as random effects in the G_0 generation.

Traits	σ_A^2	σ_C^2	σ_e^2	h^2	c^2
Weight	5766	340	3052	0.63 ± 0.11	0.04 ± 0.05
Length	9.15	1.22	3.04	0.68 ± 0.15	0.09 ± 0.08

4.3.4. Genetic and phenotypic correlations between traits

The genetic and phenotypic correlations between weight and length at harvest were calculated. The correlations were positive with similar values, both close to 0.91. Furthermore, relatively low standard errors were observed for weight (0.007) and length (0.034).

4.4. Discussion

Creating the base population is one of the most important steps to establish a selective breeding programme. Most experiments demonstrated large genetic variation between strains of carp. Vandeputte (2001) summarized that there were large differences in growth rate between pure strains of common carp, with the best line in one experiment being often 50-100% larger than the smallest ones. The variation of top cross progenies were in the same range and heterosis was between 20% and 30% of the parental mean. Variations between strains and heterosis seem to be very large for survival and disease resistance but much smaller for flesh yield and performance traits. Bialowas (1991) and Wohlfarth (1993) suggested that heterosis may affect the performance of this species; however, it seems unlikely that they would be the main genetic determinants of performance in common carp, as large differences also exist between pure strains. Vandeputte (2001) suggested that high genetic variation observed between strains for the quantitative traits of interest is one very good indicator for possible additive genetic variance within some strains of the studied species. Since the heterosis estimates were generally low in the present study, thus it seems possible to create a synthetic strain with high genetic variability, which should be good material to start a selective breeding programme.

In the current study, the single pair mating design was applied to form the base population from six founder stocks of pure breed and selected lines. Three lines originated from the sixth generation of a previous mass selection programme in common carp. The initial materials used to form these mass selection lines were three hybrid stocks derived from crossing among the Vietnamese white carp, the Hungarian scale carp and the Indonesian yellow carp. After six generations of mass selection, the growth rate of selected fish had increased by 33 % compared to the base population (Thien, 1996). The other founder stock was from between family selection which was

implemented over two generations, using the sixth generation of common carp as the initial materials, and achieved 7 % faster growth than the base population (Dan et al., 2000). The last two pure-breed lines were Hungarian scaled carp and Indonesian yellow carp. Hence, the founder stocks in the present selective breeding programme were of diverse genetic origin and exhibited differences in growth performance between pure-breed and cross-breed progenies as shown in Table 4.4. The initial intention was that each founder stock should contribute to the base population according to their performance rankings in a previous study by Tuan et al. (2005) (Figure 4.2.).

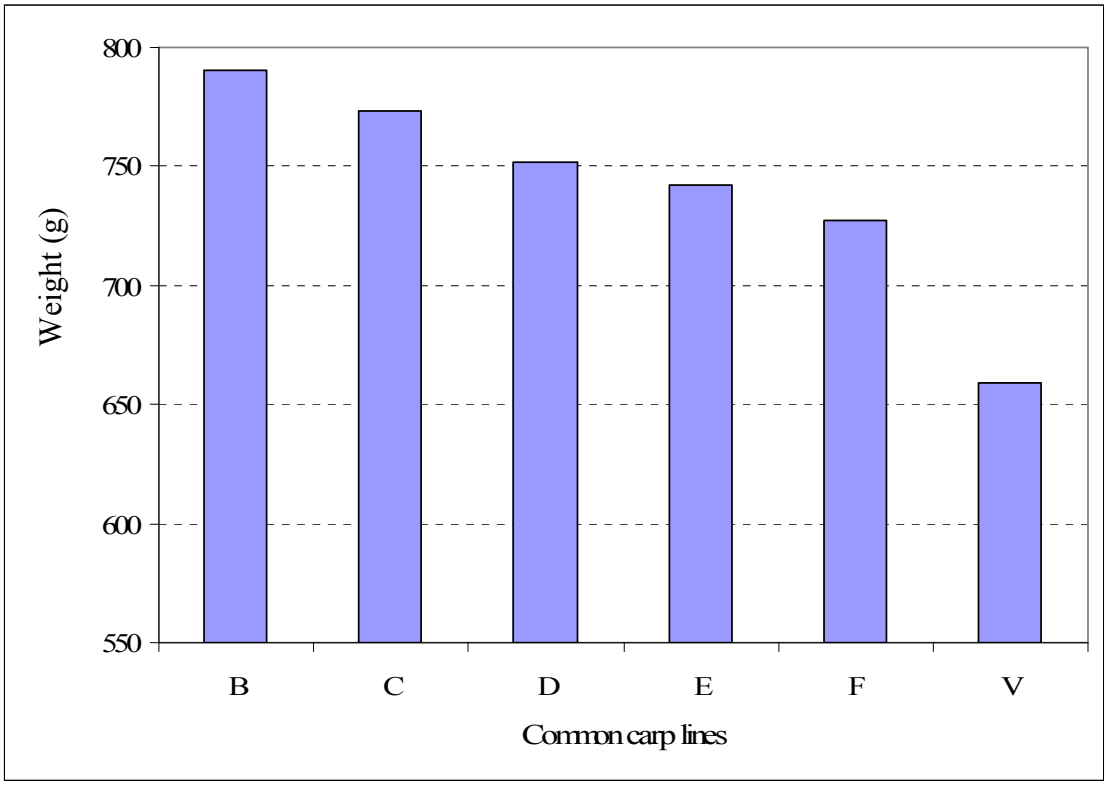

Figure 4.2. Growth performance of six common carp lines raised in polyculture systems for ten months (Line A-Family selection carp was not assessed in this research) (from Tuan et al., 2005).

B=Hungarian mass selection carp; C=Hungarian scaled carp; D=Indonesian mass selection carp; E=Indonesian yellow carp; F=Vietnamese mass selection carp; V: Vietnamese white carp.

Although the performance of the between-family selection carp had not been assessed relative to the others, this line was still proposed to make the highest contribution to the base population because they showed a positive response to selection (based on the genetic material of the mass selection programme).

However, the present data showed that line A had the highest contribution but the lowest mean weight; line E had the second lowest contribution but the third highest weight. The unexpected and poorest growth performance of line A (second generation of family selection carp) may be due to inbreeding depression since the first selection generation was selected from the five best families only (Dan et al., 2000) and there was no available data for the number of families among the selected fish from the second generation of selection. In the current study, the greatest contribution to the base population came from high ranking crosses. In addition, the best performing individuals of low ranking crosses were also included and represented in the base population. Therefore, the objective was that the selection should pass on desired or neutral allelic variation to later generations. The neutral variation may later become non-neutral when the breeding goal or farming environment is changed or expanded. This procedure of maintaining a broad ancestry in the synthetic population can only be evaluated after some generations of selection. There were also high numbers of male and female ancestors represented in the base population. Therefore, the broad genetic origin was planned to help ensure long-term response to selection. Some failure to achieve response to selection in common carp and other aquaculture species has often been attributed to an initial narrow genetic variance (Moav and Wohlfarth, 1976; Hulata et al., 1986; Huang and Liao, 1990). High levels of heterozygosity were observed in most of the founder carp lines. Varying inbreeding coefficients (F_{IS}) were calculated, which were significantly different from zero in the 2nd generation of family

selection carp line. The decrease of heterozygosity in the 2nd generation of family selection carp could originate from the low number of families in the population and consequent accumulation of inbreeding and small effective population size. The synthetic common carp base population in the current study showed large genetic variation in growth performance at harvest as demonstrated by coefficient of variation of 46.94% for weight and 16.29% for length and the heritability estimates, ranging from 0.63 to 0.68. However, the heritability estimates are likely to be confounded with common environmental effects because the mixed model estimates were based on data of a single generation where families were produced from single pair mating.

The estimates of heritability for growth traits at harvest in the current study were higher than most of the previous studies, however it fell within the range of published estimations of heritability values in common carp (Table 4.7). However, none of these was estimated from a first generation synthetic base population. Our values in this generation were higher than in the subsequent generations for the same age so it is possible that heterosis as well as the mating/rearing design with full-sib families only had inflated the heritability estimate. The estimated values may be biased upward by dominance or maternal non-genetic effects because they are based on the full-sib component of variance. Otherwise, the present estimates are calculated from high number of sires (101) that reflect large genetic variance among the breeders. Also, the high heritability estimate is likely confounded by effects common to full-sibs, such as environmental effects due to separate rearing of the families in hapas and CWT rearing of five families in each group until tagging, maternal effects and components of non-additive genetic effects common to full-sibs. In the present study, families were produced within a short period of time, thus the variation of age was low (CV=1.86). In addition, larvae in each family were separately stocked and managed in

the same condition in hapas for a short period of time (60 days) before applying positional marking by CWT and communally rearing five families in one section of a pond, separated by plastic netting. All marked fry from families were stocked and grown in the same pond until fingerling size for PIT tagging and subsequent communal rearing. These practices should reduce the variance of full-sib effects due to early separate family rearing. Therefore, the high heritability obtained in the present base population should give good prospects for genetic improvement of growth performance.

Table 4.7. Heritability (h^2) estimates for weight and length in common carp (S.E. is standard error).

Age	h^2_{weight} (± S.E.)	h^2_{length} (± S.E.)	Reference
1 summer	0.34 ± 0.05	0.37 ± 0.05	Nenashev (1966)
2 summer	0.51 ± 0.08	0.55 ± 0.08	Nenashev (1966)
Fingerlings	0.21	0.21	Nenashev (1969)
Harvest	<0.01	-	Moav and Wohlfarth (1976)
Harvest	0.20 - 0.29	-	Thien (1993)
13 months	0.58	0.5	Bongers et al. (1997)
110 days	0.09	0.11	Tanck et al. (2001)
8 weeks	0.33 ± 0.08	0.33 ± 0.07	Vandeputte et al. (2004)
8 months	0.25 ± 0.02	0.35 ± 0.04	Wang et al. (2006)
20 months	0.30 ± 0.03	0.27 ± 0.03	Wang et al. (2006)
3 seasons	0.70 ± 0.08	0.69 ± 0.1	Kocour et al. (2007)

The high economic value of growth rate in aquaculture species makes it a desirable trait to improve. The breeding objective in a genetic improvement programme for growth performance is usually size at harvest under conditions similar to commercial

aquaculture. Therefore, selection decisions choosing fish as candidates for future broodstock are also made on the basis of size at harvest. The estimates of c^2 effects in the present study were low and ranged from 0.04 for weight to 0.09 for length, although c^2 was still significant at harvest time and had an effect on the estimate of the additive genetic variance. This result is in agreement with estimates for growth related traits which ranged from 0.06 in Atlantic salmon (Rye and Mao, 1998), 0.08 in chinook salmon (Winkelman and Peterson, 1994) to 0.09 in rainbow trout (Elvingson and Johansson, 1993).

The present study showed very high genetic and phenotypic correlations between weight and length. The high additive genetic correlation between weight and length together with the high direct heritability indicates that either weight or length could serve as an indirect selection criterion for growth performance of common carp. However, one study also showed high heritability for body shape, which could mean that correlated changes in body shape should be monitored (Ankorion et al., 1992).

4.5. Conclusions

The heritability estimates for harvest weight and length of the synthetic common carp base population were relatively high compared to other reports for common carp. The high estimates of heritability are likely to be confounded with common environmental effects although the practices and calculated model were expected to reduce the common environment variance. The high estimated values of heritability suggest that rapid gains could be achieved through selective breeding for growth rate in common carp. Also, the high genetic and phenotypic correlations indicate that phenotypic selection for weight of common carp would result in associated genetic changes for length.

Chapter 5. Selective Breeding of Common Carp Using Early Communal Rearing

5.1. Introduction

5.1.1. Parentage assignment for selection

Large numbers of families will increase the accuracy of the evaluation of the genetic components of progeny performance traits. Microsatellite marker-based family assignment can be used to allow communal rearing from fry stages, overcoming many of the problems of separate rearing until fish are large enough to be tagged. This can be used to improve the accuracy of estimation of heritability and relative performance of different families during selection.

Communal rearing of common carp in ponds and cages has been proven to be a more valuable and efficient method for performance testing of numerous family groups of fish compared to separate rearing (Wohlfarth and Moav, 1991). Similar results have been demonstrated in channel catfish (Dunham et al., 1982) and tilapia (McGinty 1987). Although competition among families could potentially occur in communal rearing conditions, consistent ranking of phenotypic trait means in both separate and communal rearing experiments were observed in these studies on carp, catfish and tilapia. Other evidence for the advantage of mixed family rearing was reported by Jacobs et al. (1999) who reared several strains of striped bass in two intensive culture facilities, with one facility utilizing separate rearing and the other utilizing communal rearing. Culture conditions were slightly different between the two facilities, however, the rank order of growth performance did not differ among strains between the two facilities. It is therefore revealed that communal rearing of striped bass might produce

results consistent with separate rearing. Communal rearing techniques have been applied to assess performance of catfish (Dunham et al., 1982; Bosworth et al., 1998), various stocks of carp (Wohlfarth and Moav, 1991), coho salmon (Hershberger et al., 1990), African catfish (Volckaert and Hellemans, 1999), rainbow trout (Iwamoto et al., 1986; Herbinger et al., 1995), European sea bass (Garcia de Leon et al., 1998), Atlantic salmon (O'Reilly et al., 1998; Obedzinski and Letcher, 2004) and brown trout (Glover et al., 2004). Application of parentage assignment for selective breeding of communal rearing common carp was first studied by Vandeputte et al. (2004) who mixed individuals from different families at the fry stage. Although the results from communal rearing for common carp are expected to show higher heritability values, lower common environmental source of variances and better relation to normal culture practices, comparisons between communal early rearing and separate early rearing should be carried out to provide valuable information for development of a selective breeding programme for this species.

5.1.2. Estimation for parental selection

In the communal rearing experiments for growth performance traits, families of larvae of common carp in this study were mixed and stocked in ponds only a few days after hatching. For the family selection, when communally reared fish were big enough for tagging using PIT tags, they were traced to ascertain their parents and relatives by using highly variable microsatellite markers (as described in Chapter 3). Parents and their progeny were genotyped so the parentage of each communally reared offspring can be identified. This technique has been successfully applied in a number of communal rearing strategies for aquaculture species such as seabass, halibut, salmon and shrimp (Herbinger et al., 1995; Garcia de Leon et al., 1998; O'Reilly et al., 1998).

There are some advantages and disadvantages of communal rearing and separate family rearing techniques in a selective breeding programme. The major problem of separate family rearing of common carp is highly variable mortality, with high mortality in some families often occurring in early life stages, during the first 5 days after hatching. This causes high variation in growth performance of individuals in different families in later. However, early larval survival may not be greatly influenced by the additive genetic variation that can be exploited by selective breeding (Falconer and Mackay, 1996).

Maternal effects (dam effects) associated generally with egg size and egg quality have been found in many species such as rainbow trout (Springate et al., 1984; Herbinger et al., 1995; Nagler et al., 2000), chinook salmon (Heath et al., 1999), cod (Gjerde et al., 2004) and striped bass (Houde, 1987; Monteleone and Houde, 1990). Paternal effects (sire effects) on family survival in early stage were not observed in African catfish (Volckaert and Hellemans, 1999) or from larvae to one year old rainbow trout (Herbinger et al., 1995). However, differences in survival among families of 40 days old European seabass were strongly influenced by both the sire and dam (Saillant et al., 2001).

In addition, growth performance in early life stages may have genetic effects and might function as a predictor of the future performance of individuals or families of fish. Size variation of common carp from fingerling to adult stages are related to genetic merit and most likely based on additive genetic variation. The contribution of genetic effects in determining early growth in length and body weight of communally reared common carp has been reported by Vandeputte et al. (2004) and heritabilities were estimated for these traits, ranging from 0.3 to 0.4. In the communal stocking

culture, environmental conditions should be equal for all families, thus providing an advantage for investigating genetic influence on performance of individuals and families. So, communal rearing from hatching may be a better solution for selective breeding, although variable survival is likely to occur, and may result in the loss of certain families, or too few individuals from some families to allow assessment and selection for some traits. The pedigree record from the molecular markers will allow optimization of the selection of best progeny or families which enables the achievement of maximum genetic gain in the next generation. In addition, molecular pedigree information also allows control of inbreeding (e.g. avoiding mating between close relatives, estimation of effective population size in selection programme).

In summary, the success of parentage assignment in common carp using microsatellite markers allows communal rearing of all families from the very early larval stage. This simplifies key steps in the selective breeding programme since the early communal rearing of all family can alleviate confounding effects caused by environmental factors on phenotypic and genetic components.

5.1.3. Aims of the study

Multiplex PCRs were developed for genotyping and analysis to efficiently resolve the pedigree of communally reared larvae in the G_1 and G_2 generations of common carp, as presented in Chapter 3. This study aimed to estimate genetic and phenotypic parameters and correlations for selection of growth-related traits using the results of microsatellite parentage assignment that is expected to reduce common environmental effects, and therefore improve the efficiency of the selective breeding programme in common carp.

5.2. Materials and methods

5.2.1. Pedigree profiling

The founder population (G_0) was formed from six lines of common carp. Pedigree information was recorded by physical PIT tagging (see details in Chapter 2). A partial factorial mating design was applied for production of selection families while control families were all full-sib families in the G_1 and G_2 generations. In addition, reference families were used in the G_2, from the P33 strain of improved carp introduced from HAKI, Hungary. Parents of the G_2 generation were selected from the CER G_1 generation as a result of parentage assignment using microsatellite markers and quantitative genetic analysis.

The total number of full-sib families was 135 and 101 in the G_1 and G_2 generations, respectively. The G_1 generation had 107 selection and 28 control families, while the G_2 generation had 77 selection families, 16 control families and 8 reference families. At the time of sperm and egg collection for fertilization, fin tissue of physically tagged parents was sampled and preserved for molecular genetic analysis. Mated families were incubated separately in jars until hatching and yolk digestion of larvae. An estimated equal number of exogenous feeding larvae were taken and stocked communally in ponds. Fish were raised in two different ponds according to each batch of spawning which were about 7 days apart. The same procedure was practiced in both the G_1 and G_2 generations.

After approximately four months of communal rearing, the fish in ponds were completely harvested (see more in Chapter 2). Fin clipping together with PIT tagging were applied to 1327 fish in the G_1 generation and 1396 fish in the G_2 generation

(randomly collected) and these fish were restocked in different ponds by batches. The results of parentage analysis using microsatellite markers (Chapter 3) showed that means of 96.8% offspring in the G_1 and 96.2% offspring in the G_2 were unambiguously assigned to 113 families and 99 families of known parents, respectively. The assigned offspring had full pedigree information, on which further data collection and analysis for selection of growth performance was based.

5.2.2. Data of growth traits

5.2.2.1. G_1 generation

The number of male and female brooders contributing to the 113 matched families was 78 and 78 respectively. The total of 1284 assigned individuals included 632 fish in the first batch and 652 fish in the second batch, of which 1098 individuals were allocated to selection families and 186 individuals to control families. Growth performance data consisting of standard length and live weight and were collected three times: at about 4 months old (at this time, the fish from parallel experiment of separate early rearing could reach suitable size for PIT tagging), 7 months old (this time is start of winter season) and final harvest at 12 months old (preferred marketable size). The selection decision was carried out based on the data at last measurement (age of 12 months). In addition, data for pre-dorsal height of the fish was also measured one time at final harvest. The data set of G_1 was combined with that of G_0, G_2 for quantitative genetic analysis.

5.2.2.2. G_2 generation

Parents of the G_2 generation included 63 females and 55 males, which contributed to 99 assigned full-sib families in a partial factorial mating design. There were 1342

traced offspring, including 776 fish in the first batch and 566 fish in the second batch. The numbers of matched offspring in selection, control and reference families were 965, 317 and 59 individuals, respectively. Growth performance data were collected for standard length, live weight and body height. They were measured three times at 3 months old (time of PIT tagging and fin clip sampling), 6 months old and 10 months old (final harvest) (see Chapter 2 for further details).

5.2.3. Statistical analysis

The full pedigree information from G_0, G_1 and G_2 were recorded and genetically linked. The quantitative genetic analyses were carried out on the data collected over two selection generations (G_1 and G_2) in connection to the G_0 generation.

5.2.3.1. General analysis

Firstly, general linear model (GLM) was applied to investigate environmental effects, using the GLM procedure in SAS (Littell et al., 1996). Data were analyzed to determine which fixed effects have significant influence on the data. The GLM tested for the effects of generation, line, sex, pond environment and age of fish on final harvest data. All two-factor interactions were examined and were removed from the model if they failed to show significant ($P > 0.05$) effects on the traits of interest.

Secondly, a mixed model was developed and used to analyze the whole data sets to estimate the fixed effects and initial values of variance components. Pair-wise comparisons were generated using the PDIFF option of the Least Squares Means statement of PROC MIXED in all analyses (Littell et al., 1996). The model consisted generation, line, sex and environment as fixed effects, and age at harvest as a linear

covariate. Sire, dam and the interaction between sire and dam were considered as random effects. The mixed model was written as follows:

$$Y_{ijklnpq} = \mu + GEN_i + LINE_j + SEX_k + PND_l + \beta AGE + S_n + D_p + I_{np} + e_{ijklnpq} \quad \text{(Model 1)}$$

Where,

$Y_{ijklnpq}$ is an observation of the individual q

μ is the overall mean

GEN_i is the fixed effect of generation (i = 1, 2, 3)

$LINE_j$ is the fixed effect of line (j = 1, 2)

SEX_k is the fixed effects of sex (k = 1, 2)

PND_l is the fixed effect of pond (l = 1, 2)

AGE is the covariable effect of age

S_n is the random effect of sire n^{th}

D_p is the random effect of dam p^{th}

I_{np} is the interaction between n^{th} sire and p^{th} dam

$e_{ijklnpq}$ is the residual error

5.2.3.2. Estimation of phenotypic and genetic parameters

A complete pedigree of the experimental fish from G_0 onwards was available and was used in analysis for phenotypic and genetic parameters. The variance components obtained with Model 1 were used as starting values. Primary analysis result showed that the interaction between sire and dam was not significant for traits, and hence this random effect was not included in the final model. The models fitted in the greatest log likelihood value included the fixed effects of generation (G_1 and G_2), line

(selection and control), sex (male and female), environment (pond) and covariate of age (first, second, third time of measurements).

In Model 2A, animal and dam (the non-genetic component including maternal, dominance and environment) were fitted as random effects. This model (animal model) used pedigree information to partition the observed phenotypic variance of a trait into various genetic and environmental components, hence it enabled the estimation of variance components, from which phenotypic and genetic parameters were calculated more accurately. The animal model (Model 2A) also supported the estimation of breeding values for all fish and made selection decisions in the selection and control lines and estimated the genetic trend.

$$Y_{ijklnpq} = \mu + GEN_i + LINE_j + SEX_k + PND_l + \beta AGE + I_n + D_p + e_{ijklnpq} \qquad \text{(Model 2A)}$$

Where,

$Y_{ijklnpq}$ is an observation of the individual q

μ is the overall mean

GEN_i is the fixed effect of generation (i = 1, 2, 3)

$LINE_j$ is the fixed effect of line (j = 1, 2)

SEX_k is the fixed effects of sex (k = 1, 2)

PND_l is the fixed effect of pond (batch) (l = 1, 2)

AGE is the covariable effect of age

I_n is the random additive genetic effect of individual n^{th}

D_p is the random effect of dam including the maternal effect and the effect of common environment (environmental conditions such as temperature, water quality between ponds and years).

$e_{ijklnpq}$ is the random residual effect associated with individual $ijklnpq$

The model terms were changed for single generation analysis. In such case, generation effect was excluded from the model. The D_p is the effect of dam and effect of common environment (environmental differences between ponds).

Heritability estimates under Model 2A: Variance components for growth traits were estimated from a univariate model. Although dam was fitted as a random effect, it actually accounted for any common environmental effect on the progeny. So the dam component (σ_D^2), in this case, is a combination of the maternal effect and the common environment effect (means $\sigma_D^2 = \sigma_{D+E}^2$, referred to as σ_C^2 in later use). Phenotypic variance (σ_P^2) calculation was based on additive genetic variance of individual (σ_A^2), maternal and common environmental variance (σ_C^2) and residual variance (σ_e^2) as $\sigma_P^2 = \sigma_A^2 + \sigma_C^2 + \sigma_e^2$. Then the heritability using individual variance component was calculated as $h^2 = \frac{\sigma_A^2}{\sigma_P^2}$. The maternal and common environmental effect was calculated as $c^2 = \frac{\sigma_C^2}{\sigma_P^2}$.

Genetic and phenotypic correlations between all traits were calculated as the covariance divided by the product of the standard deviations of traits: $r_g = \frac{\sigma_{12}}{\sqrt{\sigma_1^2}\sqrt{\sigma_2^2}}$,

where σ_{12} was the estimated additive genetic or phenotypic covariance between the two traits.

In addition, a sire model (Model 2B) was used to estimate variance components for heritability calculation in order to compare to results from the animal model (Model 2A). Heritability estimated from a sire model may be slightly less accurate both due to lower accuracy (particularly in case of few progeny per sire) and potential bias, because there is no correction for differences between dams. The sire model analysis ignores the dam information and assumes that all dams are from the same

homogenous population all with the same expected mean. However, the sire model may cause overestimation in the present study because partial factorial mating was applied in selection line and the selection line produced by mating one sire with two dams and possibly four dams when ten males from the first batch were used again to mate with females from the second batch (see Chapter 2 for further details). Similar to the Model 2A, a sire model fitting sire and dam as random effects (Model 2B) was used to estimate genetic parameters and written as follows:

$$Y_{ijklnpq} = \mu + GEN_i + LINE_j + SEX_k + PND_l + \beta AGE + S_n + D_p + e_{ijklnpq} \qquad \text{(Model 2B)}$$

Where,

S_n is the random additive genetic effect of individual with sire n^{th}

D_p is the effect of the dam plus common environmental/full-sib effects (environmental conditions such as temperature, water quality between ponds and years), and a quarter of non-additive genetic effects

The other factors of Model 2B are the same as in Model 2A

The model terms were changed for single generation analysis. In such case, generation effect was excluded from the model. The D_p was the effect of dam and effect of common environment (environmental differences between ponds).

Heritability estimates under Model 2B: Variance components for growth traits were estimated from a univariate model. Phenotypic variance (σ_P^2) calculation was based on additive genetic variance of sire (σ_S^2), maternal and common environmental variance (σ_C^2) and residual variance (σ_e^2) as $\sigma_P^2 = \sigma_S^2 + \sigma_C^2 + \sigma_e^2$. Then the

heritability using the sire variance component was calculated as $h^2 = \frac{4\sigma_S^2}{\sigma_P^2}$. The maternal and common environmental effect was calculated as $c^2 = \frac{\sigma_C^2}{\sigma_P^2}$.

All computations for the Model 2A and Model 2B were carried out using the ASREML software package (Gilmour et al., 2002). Variance and covariance components were estimated using restricted maximum likelihood. Convergence for log-likelihood of variance component estimation was considered satisfactory when two successive rounds of interaction changed by less than 0.1%. All known pedigree information was included in the analyses through a relationship matrix.

Heritability and common environmental effects and correlation estimates were tested for significantly different from each other, or zero by using z-scores:

$$z = \frac{x_i - x_j}{\left(\sigma_i^2 + \sigma_j^2\right)^{0.5}}$$

Where, x_i and x_j are the estimates of heritability and common environmental effects, or genetic correlations for the two traits and σ_i and σ_j are their respective standard errors. Both x_j and σ_j were set to zero or one when test of an estimate was significantly different zero or one, respectively. The resulting z-scores were then tested against a large sample normal distribution.

5.2.3.3. Response to selection

The least squares means estimated by the mixed model (Model 1) were used to calculate response to selection between lines over generations for growth-related traits. Mean genetic selection response was estimated as the difference of the least square means between successive generations. Responses to selection for selective

breeding line to control line were also calculated in similar manner to those by generation. In addition, the selection differential between generations and lines were calculated from the univariate estimated breeding values.

5.2.3.4. Estimates of realized heritability, selection differential and selection intensity

The realized heritability (h_r^2) is a value to quantify the degree to which change in a trait in a population can be achieved by selection. For each of the selected traits, response to selection was estimated as difference between the observed mean of the selected progeny group and the observed mean of the control progeny group. The observed selection differential was estimated as the different between the mean of the selected parents and the mean of the total number of individuals measured prior to selection. Realized heritability was calculated as the ratio of the response to selection and the observed selection differential (Falconer and Mackay, 1996). The response to selection and the observed selection differential was obtained from mixed model analysis.

Selection intensity (i) which is equal to the selection differential expressed in term of standard deviation by the following equation:

$$i = \frac{S}{\sigma_P}$$

Where,

i is the selection intensity

S is the selection differential

σ_P is the standard deviation of fish population before selection

5.3. Results

5.3.1. Test for randomly sampling data

When choosing a limited number of fingerling for PIT tagging from communally reared fish in the G_1 and G_2 generations it is possible that this choice was not random and that fish of a particular size were preferentially chosen, which could have confounding effects on the outcome of communal rearing. To test this possibility, the average weight of fingerlings from each family was plotted against the number of fish in that family. If there is a relationship between family and fish size at this stage, and the procedure for choosing fingerlings preferentially selected fish of a particular size, then there would be a relationship between the numbers of fish selected in that family and the average weight of that family. For example, if larger fingerlings tended to be chosen then there would be more fish from families with larger individuals at this experimental stage.

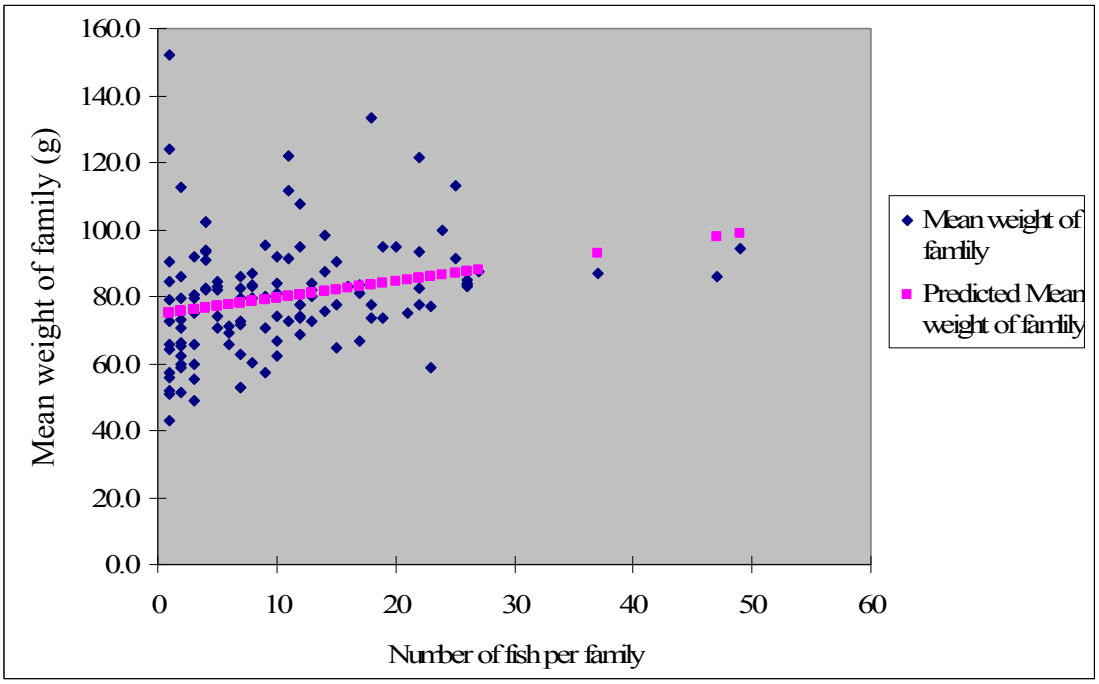

Figure 5.1. The relationship between mean weight of fingerlings (at PIT tagging) from a particular family and the number of fish in that family in the G_1 generation.

The plots (Figures 5.1 and 5.2) demonstrate that there is no correlation (P=0.257 in the G_1 and P=0.108 in the G_2) between average size of fingerlings in a particular family and the numbers of fish in that family, which validates the fingerling selection procedure in the G_1 and G_2 generations for CER set up.

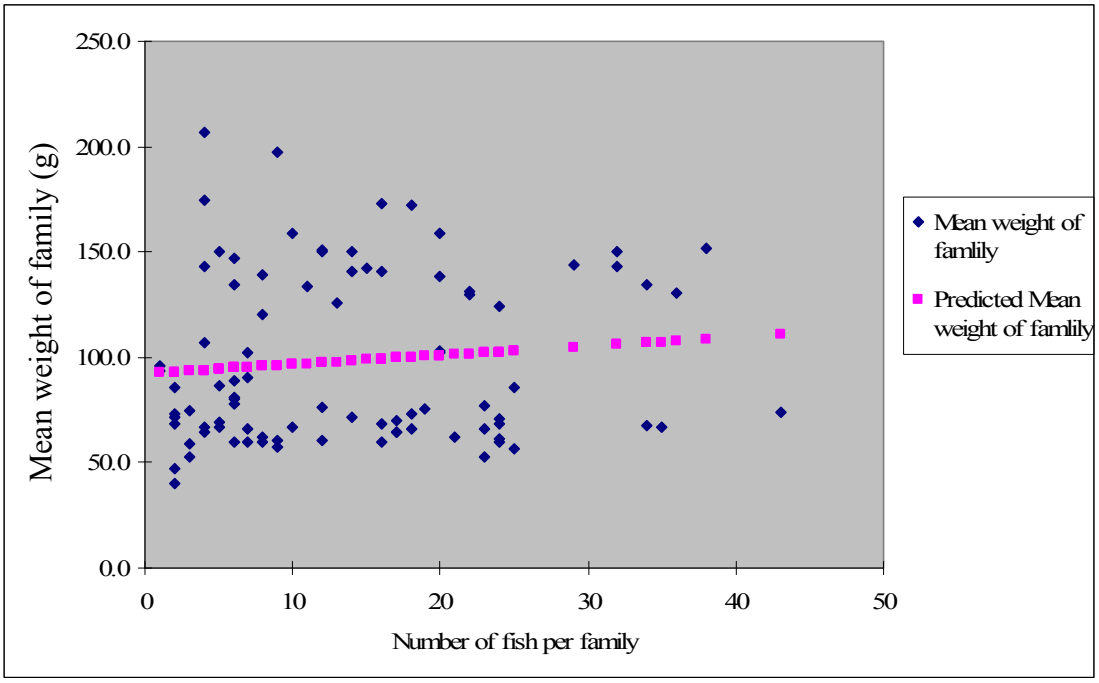

Figure 5.2. The relationship between mean weight of fingerlings (at PIT tagging) from a particular family and the number of fish in that family in the G_2 generation.

5.3.2. General summary data of selected and control fish

Means, standard deviations and coefficients of variation from raw data for all traits are presented in Table 5.1. The number of analyzed samples was unbalanced between times of data collection due to fish deaths and mis-reading of tag numbers. Coefficients of variation were particularly high for weight (46.9 - 56.4%), followed by age (9.2 – 25.0%) and length (14.6 - 18.6%). Body height was recorded only at the final harvest, showing medium variation (CV = 22.8%). Final average age of fish used in this analysis was calculated from age at respective harvests. Standard deviations increased with age and size.

Table 5.1. Sample size (N), mean, maximum, minimum, standard deviation (SD), coefficient of variation (CV, %) of raw data for weight, length, height and age over the G_1 and G_2 generations.

Traits*	Unit	N	Mean	Minimum	Maximum	SD	CV
Weight1	g	2555	91.40	24.0	353.0	45.5	49.8
Length1	cm	2555	17.58	11.6	27.2	2.7	15.2
Age1	days	2555	111.22	62	138	27.8	25.0
Weight2	g	2512	251.38	34.0	907.0	118.0	46.9
Length2	cm	2512	24.90	15.0	37.8	3.6	14.6
Age2	days	2512	192.12	157	204	17.7	9.2
Weight3	g	2391	775.57	137.0	2689.0	437.0	56.4
Length3	cm	2391	34.31	17.7	57.3	6.4	18.6
Height3	cm	2391	10.41	4.9	15.5	2.4	22.8
Age3	days	2391	355.91	290	399	42.5	11.9

** First, second and third measurements at three ages.*

High standard deviations for weight and length indicated that fish growth varied widely between individuals in the same population. Coefficient of variation depended on age, which was different between the two generations at the time of data collection because the weather changed environmental condition over the years. The spawning season of carp starts normally in spring (in early March) however it depends mainly

on temperature and broodstock management. If the water temperature is lower than 18 ^{0}C, brooders are unable to spawn and delays in spawning may result in different growing time to a specific size of progeny. In this breeding programme, changes in environmental conditions caused differences of rearing time to suitable size for PIT tagging and subsequent data collection points. In addition, rearing techniques differed between generations due to changing types of food and frequency of water exchange that changed following sources of pellet feed (supplier) and available water. Other reasons that may have had effects on variation of weight, length and height were genetic control of the fish and food competition. In the present study, age of measurement between two generations might have had a larger contribution to size variation. Therefore, the effect of age on growth performance should be included as a cofactor for satisfactorily comparison of the data sets.

5.3.3. Prediction of fixed effects

A general linear model (GLM) was used to investigate for significance of fixed effects on final harvest data. The model tested all possible effects including generation (G_0, G_1, G_2), lines (control, selection), sex (male, female), environment (culture pond conditions, two batches grown separately into two ponds in each selection generation) and age (first, second and third measurements) and their interactions. The main effects were statistically significant ($P<0.001$) for all the studied traits. Generation and environment had greater influence on variation in weight and length than other effects. The main variation in height was caused by sex (4.5%). The overall variation of the model (R^2) was 30.5%, 52.3% and 77% for weight, length and height at harvest, respectively, of which the total contribution of the fixed effects was 22.4% in weight, 32.8% in length and 17.0% in height. Therefore, higher variation due to residual errors was observed in height compare to weight and length (Table 5.2).

Table 5.2. The marginal contribution of fixed effects (generation, line, sex, environment and age) to the proportion of the variance explained by the general linear model (R^2) (GLM Procedure: SAS, 2002).

		Weight			Length			Height		
Effects	Degrees of freedom	F-value	Probability	R^2	F-value	Probability	R^2	F-value	Probability	R^2
Generation	2	73.9	<0.001	0.096	38.7	<0.001	0.075	17.9	<0.001	0.028
Line	1	13.7	<0.001	0.022	28.0	<0.001	0.056	18.5	<0.001	0.029
Sex	1	11.0	<0.001	0.019	26.7	<0.001	0.054	23.6	<0.001	0.045
Environment	1	28.1	<0.001	0.060	39.0	<0.001	0.079	22.5	<0.001	0.039
Age	1	16.5	<0.001	0.027	31.7	<0.001	0.064	18.3	<0.001	0.029

5.3.4. Phenotypic analysis

Least-squares means were calculated basing on the mixed model with sire, dam and sire × dam interaction as random effects; generation, line, sex, environment and age as fixed effects. This analysis was undertaken on traits of the two selection generations (G_1 and G_2).

5.3.4.1. Generation and line differences

Figures 5.3, 5.4 and 5.5 show least squares means of weight, length and height respectively, for selection, control and reference lines in the G_1 and G_2 generations. Least squares means for weight were all significantly different between selection and control lines in the two generations. The difference between selection and control lines was even shown at the first time of measurement, and increased with age. The weight of the selection line in the G_1 generation (83.9 g) was similar to the control line in the G_2 generation (78.4 g) at the first time of measurement but it was higher at the second and third time of measurements. In addition, the selection line in the G_2 generation (105.7 g) was heavier than selection line in the G_1 generation at the first time of observation but it was much lower at the two later measurements. So weight at later assessments in the G_1 generation was higher than the G_2 generation, because of differences in age at measurements and environment such as changes of weather, type of food, water exchange and pond sediments. The weight of the reference line was higher than that of the selection and control lines in the first two measurements but there was no significant difference between the reference and selection lines at the final harvest. So the selection line may show good performance at later stage because of better competition for food and environmental adaptation.

Low variation of length between selection and control lines was observed in each generation, however the least squares means of length in the selection line were significantly higher than in the control line. The highest different between these two lines was shown at the final harvest data. The length of the reference line was greater than the selection line at the first and second time of measurements but not significantly different at the third measurement.

There was no difference in height at final harvest between selection and control lines in the G_1 generation but there was a significant difference in the G_2 generation. The least squares mean of height in the reference line was similar to the selection line. However, this trait was only included as a reference trait.

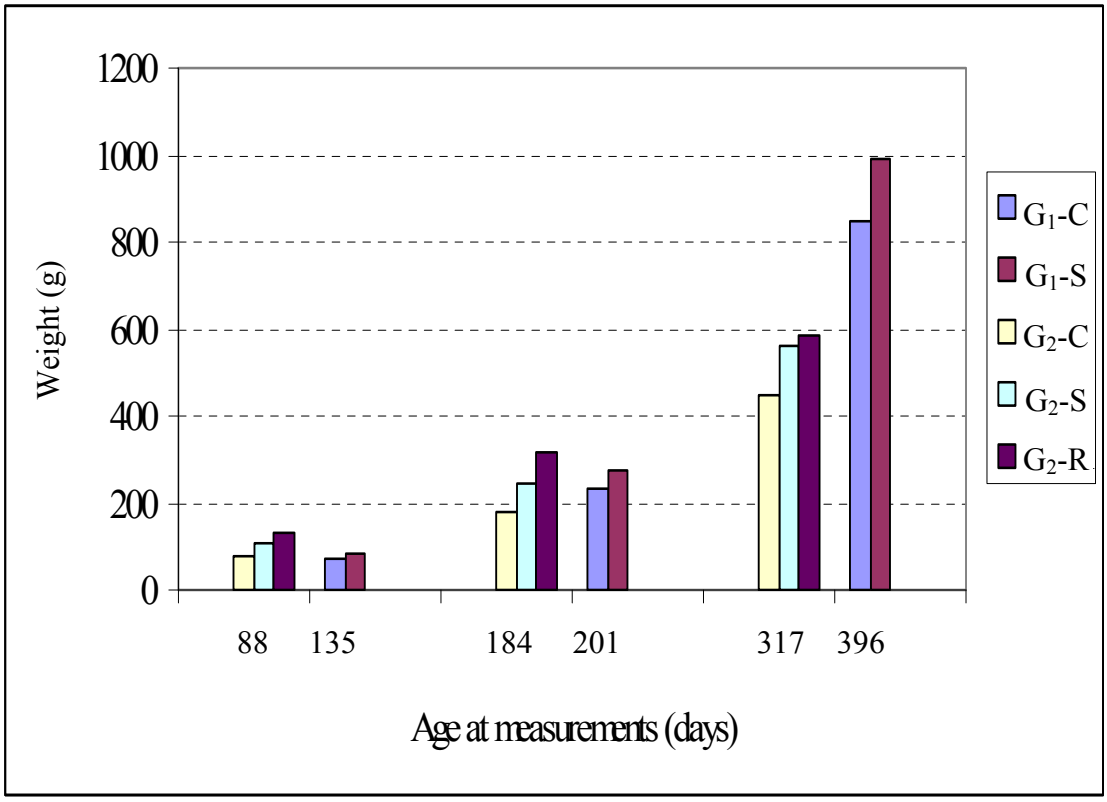

Figure 5.3. Least squares means of weight at different measurements for each generation (G_1, G_2) and line (C: Control, S: Selection, R: Reference).

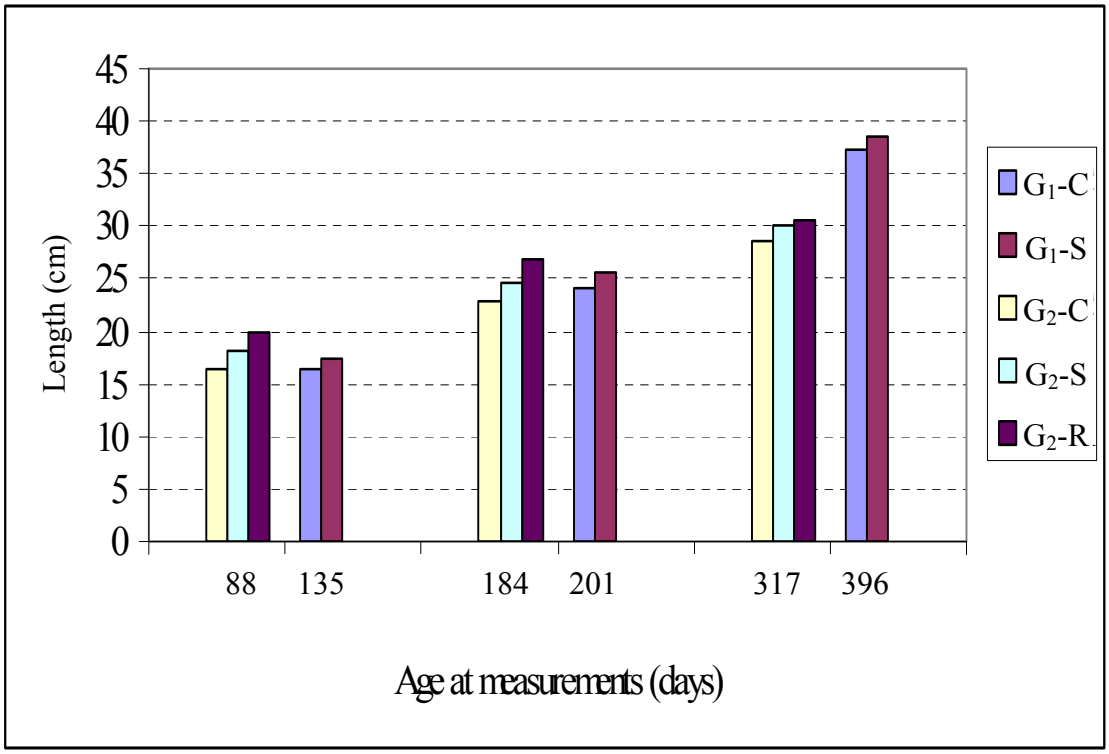

Figure 5.4. Least squares means of length at different measurements for each generation (G_1, G_2) and line (C: Control, S: Selection, R: Reference).

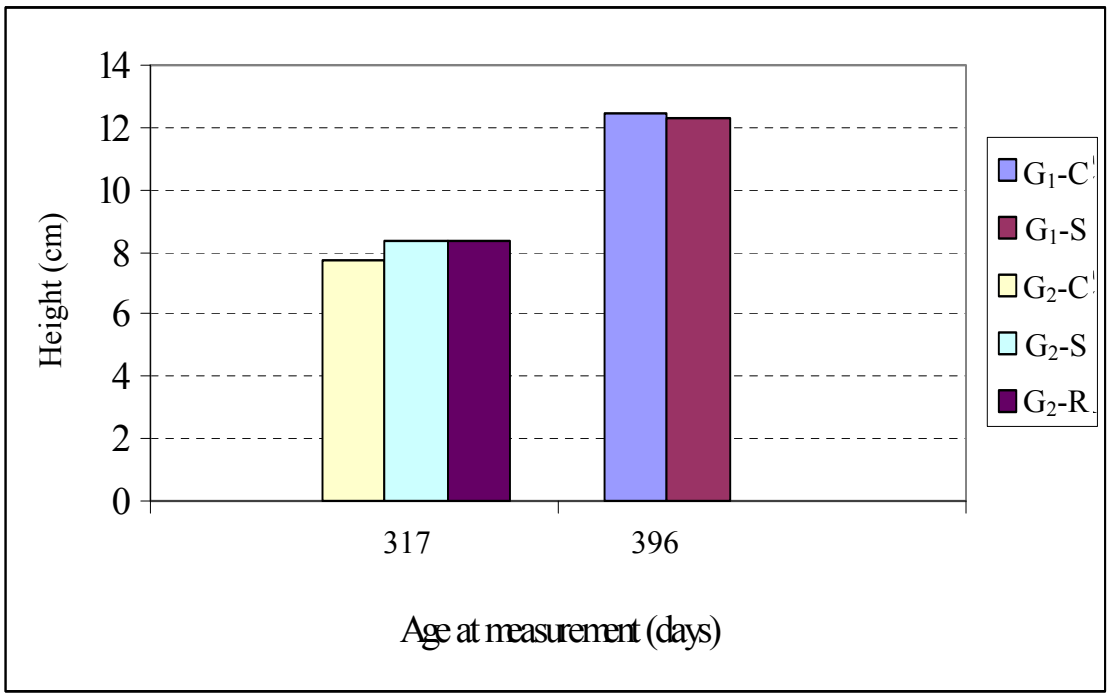

Figure 5.5. Least squares means of height at final harvest for each generation (G_1, G_2) and line (C: Control, S: Selection, R: Reference).

5.3.4.2. Sex differences

The least squares means of growth performance traits for females and males are presented in Table 5.3. Females grew faster than males for all the studied traits. Between sex differences were significant for all growth performance traits (weight, length and height), even at the earliest assessed stage of growth development ($P<0.05$). Females were greater than males from 5.81% to 7.38%, 1.68% to 2.78% and 1.64% for weight, length and height respectively. The differences between females and males increased with age.

Table 5.3. Least-squares means (±S.E.) of traits for females and males according to the mixed model for selected and control lines.

Traits	Sex		Proportion of differences (%)
	Female	Male	
Weight1 (g)	$87.07^{a} \pm 3.09$	$82.01^{b} \pm 3.15$	5.81
Length1 (cm)	$17.26^{a} \pm 0.18$	$16.97^{b} \pm 0.18$	1.68
Weight2 (g)	$241.14^{a} \pm 6.48$	$223.34^{b} \pm 6.68$	7.38
Length2 (cm)	$24.57^{a} \pm 0.19$	$24.07^{b} \pm 0.21$	2.04
Weight3 (g)	$757.62^{a} \pm 17.61$	$703.73^{b} \pm 18.47$	7.11
Length3 (cm)	$34.21^{a} \pm 0.23$	$33.26^{b} \pm 0.24$	2.78
Height3 (cm)	$10.\ 35^{a} \pm 0.06$	$10.18^{b} \pm 0.07$	1.64

**Means with different superscript letters in the same line are statistically different.*

5.3.5. Genetic parameters

5.3.5.1. Heritability estimates

Variance components and heritability (h^2) were estimated in the G_1 generation using Models 2A (Table 5.4) and 2B (Table 5.5). In the first generation, the maternal/common environmental variances were all close to zero for traits at different ages in both models. Heritability estimated by Model 2A was slightly higher than that in Model 2B for traits at different assessed ages except for weight and length at the final harvest.

Table 5.4. Estimated additive variance (σ_A^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h^2 ± S.E.) and common environmental effect (c^2 ± S.E.) for weight, length and height from the mixed models including individual and dam (Model 2A) as random effects in the G_1 generation.

Traits	σ_A^2	σ_C^2	σ_e^2	h^2	c^2
Weight1	290.53	<0.01	648.28	0.31 ± 0.08	<0.001
Length1	1.39	<0.01	2.66	0.34 ± 0.07	<0.001
Weight2	3303.59	<0.01	6928.62	0.32 ± 0.08	<0.001
Length2	2.92	<0.01	6.37	0.31 ± 0.08	<0.001
Weight3	37048.10	<0.01	120887.0	0.23± 0.06	<0.001
Length3	3.77	<0.01	14.85	0.20 ± 0.04	<0.001
Height3	0.023	<0.01	1.00	0.02 ± 0.01	<0.001

The present data was collected from progeny of mating in a partial factorial design so genetic assessment from sire component should be unbiased. The heritability was

almost constant at the first and second measurements however it reduced little at the last observation. The estimated heritabilities for weight and length were moderate at the final harvest. Estimated heritability for height was very low, from zero (Model 2B) to 0.02 (Model 2A). The standard errors of heritability estimated were all relatively low in the two models, ranging from 0.01 for height to 0.10 for weight.

Table 5.5. Estimated sire variance (σ_S^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h_S^2 ± S.E.) and common environmental effect (c^2 ± S.E.) for weight, length and height from the mixed models including sire and dam (Model 2B) as random effects in the G_1 generation.

Traits	σ_S^2	σ_C^2	σ_e^2	h_S^2	c^2
Weight1	73.42	<0.01	792.10	0.31 ± 0.090	<0.001
Length1	0.35	<0.01	3.36	0.34 ± 0.09	<0.001
Weight2	829.90	0.09	8574.60	0.32 ± 0.10	<0.001
Length2	0.74	<0.01	7.82	0.32 ± 0.10	<0.001
Weight3	9743.35	0.14	138524.0	0.25 ± 0.06	<0.001
Length3	1.00	<0.01	16.60	0.22 ± 0.04	<0.001
Height3	<0.01	<0.01	1.00	<0.001	<0.001

The variance components, heritability and common environmental effect in the G_2 generation were also estimated by Model 2A (Table 5.6) and Model 2B (Table 5.7). The estimated heritability was not constant and decreased in magnitude with age for all traits and heritability and common environmental effect in Model 2B were all higher than that in Model 2A. The common environmental variances were very low, close to zero at all measurements. The standard errors of estimates were very low for common environmental effect and for heritability. There was a reduction in

heritability for weight from 0.41 ± 0.09 at the first measurement to 0.24 ± 0.05 at the final harvest even though common environmental effect was almost zero at all ages estimated by Model 2A. A similar trend was observed for length. The lowest heritability was 0.12 ± 0.045 for height.

The common environmental effect reduced from 0.036 at first measurement to zero at the final harvest for weight, as estimated by Model 2B. Length and weight had high heritability at the first measurement but it decreased to moderate at the final harvest. The decreases of heritability and common environmental effect with advancing age may be because common environmental variance was due to maternal effect at early life stage.

Table 5.6. Estimated additive variance (σ_A^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h^2 ± S.E.) and common environmental effect (c^2 ± S.E.) for weight, length and height from the mixed models including individual and dam (Model 2A) as random effects in the G_2 generation.

Traits	σ_A^2	σ_C^2	σ_e^2	h^2	c^2
Weight1	441.03	1.36	630.10	0.41 ± 0.09	0.001 ± 0.00
Length1	1.80	<0.001	2.59	0.41 ± 0.09	<0.001
Weight2	2614.83	0.77	5525.61	0.32 ± 0.07	<0.001
Length2	2.35	<0.001	6.66	0.26 ± 0.05	<0.001
Weight3	22436.16	0.15	70383.46	0.24± 0.05	<0.001
Length3	4.48	<0.001	10.86	0.29 ± 0.05	<0.001
Height3	0.09	<0.001	0.65	0.12± 0.04	<0.001

Table 5.7. Estimated sire variance (σ_S^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h_S^2 ± S.E.) and common environmental effect (c^2 ± S.E.) for weight, length and height from the mixed models including sire and dam (Model 2B) as random effects in the G_2 generation.

Traits	σ_S^2	σ_C^2	σ_e^2	h_S^2	c^2
Weight1	114.54	38.20	804.73	0.43 ± 0.11	0.036 ± 0.004
Length1	0.48	0.12	3.31	0.44 ± 0.10	0.027 ± 0.005
Weight2	660.98	20.76	6801.34	0.32 ± 0.08	0.003 ± 0.001
Length2	0.58	0.23	7.55	0.26 ± 0.06	0.025 ± 0.002
Weight3	5470.29	0.01	81877.77	0.24 ± 0.06	<0.001
Length3	1.18	0.17	12.80	0.31 ± 0.09	0.011 ± 0.004
Height3	0.03	0.01	0.66	0.16 ± 0.05	0.013 ± 0.002

Heritability (h^2) was estimated for growth performance traits for both selection and control lines at three different ages over generations, using the two genetic models, as shown in Table 5.8 and Table 5.9. In the Model 2A estimates, low common environmental variance and high residual variance for the traits of interest were observed at all ages. The largest residual variance accounted for 71.7%, 74.4% and 92.1% of the total variation for weight, length and height at the final harvest, respectively. The proportion of common environmental variance to the phenotypic variance caused by maternal/environmental effects were highest at younger age (3.8% at PIT tagging) and reduced to 0.8% at the second measurement before increased to 3.4% at the final harvest for weight. However, c^2 for length reduced from the first (0.017 ± 0.06) to the final (0.01 ± 0.003) measurements. Heritability for weight and length also decreased in magnitude with age. Furthermore, heritability for weight and

length were all moderate (0.25) at the final harvest. Low heritability was observed for height (0.07).

Comparable estimated results were achieved by Model 2A which gave slightly higher levels of variation for common environmental effects to Model 2B. The heritability of weight, length and height were all lower in Model 2B compared to the Model 2A at the first and second measurements. However, heritability was very constant between two models for all traits at the final harvest. Otherwise, standard errors of heritability were higher than those estimated in the Model 2A. These should be explained by accuracy calculation of the models. The h^2 and c^2 for all growth related traits also decreased from the first to the last measurements.

Table 5.8. Estimated additive variance (σ_A^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h^2 ± S.E.) and common environmental effect (c^2 ± S.E.) for weight, length and height from the mixed models including individual and dam (Model 2A) as random effects over the G_0, G_1 and G_2 generations.

Traits	σ_A^2	σ_C^2	σ_e^2	h^2	c^2
Weight1	813.81	78.59	1188.49	0.39 ± 0.06	0.038 ± 0.006
Length1	3.43	0.15	5.19	0.39 ± 0.06	0.017 ± 0.006
Weight2	6214.60	140.72	12201.85	0.33 ± 0.05	0.008 ± 0.004
Length2	5.93	0.24	12.42	0.32 ± 0.06	0.013 ± 0.004
Weight3	6477.20	880.62	18607.30	0.25 ± 0.04	0.034 ± 0.002
Length3	8.36	0.35	25.38	0.25 ± 0.06	0.010 ± 0.003
Height3	0.14	0.01	1.75	0.07 ± 0.04	0.005 ± 0.004

Table 5.9. Estimated sire variance (σ_S^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h_S^2 ± S.E.) and common environmental effect (c^2 ± S.E.) for weight, length and height from the mixed models including sire and dam (Model 2B) as random effects over the G_0, G_1 and G_2 generations.

Traits	σ_S^2	σ_C^2	σ_e^2	h_S^2	c^2
Weight1	195.49	24.32	1851.44	0.34 ± 0.10	0.011 ± 0.006
Length1	0.82	0.13	7.08	0.37 ± 0.12	0.015 ± 0.006
Weight2	1755.91	178.50	17903.54	0.33 ± 0.09	0.008 ± 0.003
Length2	1.36	0.22	16.86	0.27 ± 0.08	0.011 ± 0.004
Weight3	17041.68	1246.81	233782.0	0.25 ± 0.07	0.005 ± 0.002
Length3	2.35	0.31	32.19	0.25 ± 0.08	0.008 ± 0.003
Height3	0.04	0.00	1.85	0.08 ± 0.04	0.001 ± 0.003

5.3.5.2. Genetic and phenotypic correlations between traits

Table 5.10 presents phenotypic and genetic correlations between traits of interest measured at three different periods. All the estimates of genetic and phenotypic correlations between traits were found to be positive, indicating that overall performance of the fish could be improved through selection for either weight, length or height. Genetic correlations were mostly greater than phenotypic correlations for pairs of traits as reported in common carp (Kocour et al., 2007), rainbow trout (Su et al., 1997, Kause et al., 2003), channel catfish (Walser, 1993), bighead carp (Kamilov et al., 1990) and tilapia (Rutten et al., 2005).

The genetic correlations were moderate to high, ranging from 0.37 to 0.99 but with relatively large standard errors (up to 0.239) for some pairs of traits. Genetic

correlations between measurements of the same parameter at different times (e.g. weight1, weigh2, weigh3) were larger than those between different traits. The genetic correlations between measurements of body length reached the upper limit (almost one). Low genetic correlations were observed between height and other traits, ranging from 0.37 ± 0.238 to 0.74 ± 0.149.

The phenotypic correlations ranged from 0.31 to 0.99, with small standard errors (0.001-0.026). Phenotypic correlations between traits of the first and second times of measurement were high but were moderate between those measurements and the final harvest. Higher phenotypic correlations were found between the same traits and between consecutive measurements. Phenotypic correlations of weight, length and height were all moderate for the final harvest data.

Table 5.10. Phenotypic (above diagonal) and genetic (below diagonal) correlations (±S.E.) between all traits.

	Weight1	Length1	Weight2	Length2	Weight3	Length3	Height3
Weight1		0.99 ± 0.001	0.90 ± 0.006	0.87 ± 0.008	0.58 ± 0.019	0.61 ± 0.021	0.53 ± 0.024
Length1	0.97 ± 0.000		0.60 ± 0.002	0.90 ± 0.006	0.58 ± 0.019	0.62 ± 0.019	0.49 ± 0.026
Weight2	0.95 ± 0.023	0.61 ± 0.000		0.93 ± 0.004	0.67 ± 0.014	0.67 ± 0.016	0.48 ± 0.023
Length2	0.94 ± 0.026	0.97 ± 0.016	0.85 ± 0.104		0.64 ± 0.015	0.73 ± 0.013	0.49 ± 0.022
Weight3	0.80 ± 0.100	0.87 ± 0.088	0.98 ± 0.016	0.85 ± 0.094		0.72 ± 0.011	0.31 ± 0.023
Length3	0.91 ± 0.083	0.99 ± 0.001	0.93 ± 0.086	0.99 ± 0.001	0.85 ± 0.087		0.56 ± 0.018
Height3	0.74 ± 0.149	0.73 ± 0.153	0.40 ± 0.232	0.59 ± 0.207	0.37 ± 0.238	0.63 ± 0.239	

5.3.6. Response to selection

Response to selection (realized increase) calculated as the difference in least squares means for body traits between the selection and control lines was estimated by Mixed Model (Table 5.11). In the Mixed model, selection resulted in significant response for body weight, from 15.0% to 21.4% per generation across measurements. The response to selection for length ranged from 4.0% to 7.2% per generation. When percent genetic gains were expressed as the difference between the lines, the magnitude of responses decreased with age of measurement. For each generation, correlated response in height was negative (but very close to zero) in the first generation then increased to 7.5% in the second generation.

Table 5.11. Response to selection (%) per generation estimated by the difference between least squares means (Mixed model) of selection and control lines.

Traits	G_1	G_2	Overall per generation
Weight1	15.2	25.9	21.4
Length1	5.7	9.2	7.2
Weight2	15.1	25.7	20.1
Length2	5.5	7.4	6.3
Weight3	14.2	19.6	15.0
Length3	3.3	4.8	4.0
Height3	-0.73	7.5	2.8

5.3.7. Realized heritability

The realized heritability (h_r^2) estimates based on response to selection and selection differential are presented in Table 5.12. The observed response to selection and selection differential relied on selection intensity of 10.9% in the G_1 and 12.4% in the G_2. High h_r^2 for weight and length were obtained in the G_1 generation, however h_r^2 for height at final harvest was negative (-0.16). The h_r^2 for weight and height increased in the G_2 generation (to 0.34 and 0.24 respectively) but declined to 0.24 for length. The high h_r^2 reflected the offspring of the selected parents differing from the original population.

Table 5.12. Selection intensity (i), selection differential (S), response to selection (R) and realized heritability (h_r^2) of weight, length and height at final harvest in the G_1 and G_2 generations.

Traits	G_1				G_2			
	i (SD)	S	R	h_r^2	i (SD)	S	R	h_r^2
Weight3 (g)	1.158	464.32	140.41	0.30	1.016	320.85	109.76	0.34
Length3 (cm)	0.377	4.17	1.28	0.31	1.336	6.16	1.45	0.24
Height3 (cm)	0.067	0.55	-0.09	-0.16	1.977	2.59	0.63	0.24

5.3.8. Estimated breeding values

The results of univariate estimated breeding values for the traits of interest for lines (selection and control) in each generation (G_1 and G_2) are presented in Table 5.13. Estimated breeding values (EBVs) were almost zero for all traits in the base population G_0 (data not shown). EBVs increased consistently in the G_1 and G_2

generations that were smallest at stocking and highest at the final harvest. Furthermore, the estimated breeding values for the control population were smaller than that for selection population. The selection differential could be calculated from EBVs therefore it reflects the expected response to selection. The selection differential in weight at the final harvest was 16.7 g in the G_1 and 14.7 g in the G_2 corresponding to an average of 15.7 g per generation.

Table 5.13. Univariate estimated breeding values (±S.E.) of traits for lines (control and selection) and generations (G_1 and G_2) relative to the G_0 generation.

	G_1		G_2	
Traits	Control	Selection	Control	Selection
Weight1 (g)	1.47 ± 0.67	1.72 ± 0.52	2.83 ± 0.67	3.78 ± 0.42
Length1 (cm)	0.27 ± 0.05	0.29 ± 0.03	0.32 ± 0.04	0.38 ± 0.02
Weight2 (g)	14.01 ± 0.43	16.94 ± 0.75	10.96 ± 0.93	15.12 ± 0.58
Length2 (cm)	0.66 ± 0.05	0.72 ± 0.03	0.54 ± 0.04	0.62 ± 0.02
Weight3 (g)	69.30 ± 2.05	86.00 ± 2.03	46.78 ± 1.40	61.53 ± 1.21
Length3 (cm)	1.95 ± 0.11	2.07 ± 0.04	1.64 ± 0.08	1.72 ± 0.03
Height3 (cm)	0.21 ± 0.00	0.20 ± 0.00	0.19 ± 0.00	0.20 ± 0.00

5.4. Discussion

The current analyses of growth-related traits data were performed on the common carp population whose pedigree information was resolved from parentage assignment using molecular markers (see Chapter 3). The establishment of the molecular pedigree information enabled the accurate estimation of phenotypic and genetic parameters for growth traits including weight, length and height of common carp under communal rearing. There were big differences between family sizes and the number of assigned offspring to each dam and sire, however REML and mixed model accounted for unbalanced data, thus the estimated genetic and phenotypic parameters are expected to be unbiased.

5.4.1. Models for analysis

Partial factorial mating was applied for the selective breeding line to produce full- and half-sib families. In each generation, ten males in the first batch of spawning were used again in the second batch to generate genetic connectedness in the full pedigree. Control and reference populations were produced by single pair mating (see Chapter 2 for further details). A limited number of exogenous feeding larvae of the selection, control and reference families from each spawning date were all communally stocked in the same pond. Using seven microsatellite markers analysis for pedigree establishment allowed the pooling of exogenous feeding larvae from all families for communal rearing. However, there were large differences in the number of progeny assigned to each full-sib family although the initial number of larvae from each family for communal rearing was equalized volumetrically. Growth performance was observed three times for weight and length but only one time, at harvest, for height in

each generation. The different ages of measurement within and between generations may contribute significant variation to phenotypic parameters of the combined data. The effects of environment including water temperature, pond environment, feed characteristics and husbandry were likely different between generations or/and within generations. So generation, sire, dam, line, environment, age, sex and possible interactions were all tested by PROC MIXED on SAS. Finally, the Model 1 was used to fit fixed and random effects and was then used for further analysis.

The animal model (Model 2A) used for analyses in the current study utilized all available data (full-sib and half-sib families in connection with molecular pedigree information) in each generation and across generations, so that calculation of additive genetic variance over generations could be obtained with minimal bias. The model enabled the separation of the additive genetic variance from other components such as the common environmental and non-additive genetic effects.

Furthermore, the analysis of sire model (Model 2B) was also applied to estimate variance components and compared to the animal model. The sire estimate is typically the preferred estimates for heritability since it is free from possible maternal and/or dominance variance. This is one of the most common methods of estimating heritability and genetic correlations, especially in case of using half-sib designs in which each male is mated to several females (Roff, 1997; Lynch and Walsh, 1998). However, genetic parameter estimates using the sire model may have lower accuracy (particularly in case of few progeny per sire) and potential bias so this analysis was used for comparison only in the present study.

5.4.2. Phenotypic variance

The overall analysis of the G_1 and G_2 generations showed large variation in body weight, standard length and height at the final harvest as evidenced by the coefficient of variation ranging from 18.6% for length and 56.4% for weight. The coefficient of variation, including the effects of measurement at different ages, was higher compared than that reported by Kocour et al. (2007) even though the mean weight was approximately one half. In addition, their study was based on the first generation of selection of Hungarian synthetic mirror carp. In comparison, the current study examined two selection generations originating from six stocks so the high coefficient of variation appears to be appropriate. When the data were analyzed separately for each environment (pond) within generations, the same figures of high variation were observed for all traits of interest. The trend of variation was consistent for traits (weight and length) but not for ages, as it was reduced at the second measurement before increasing to the highest level at the last measurement. Wang et al. (2006) also reported very high coefficient of variation of growth related traits in Oujiang color common carp and decrease with advancing age from 47.5% to 39.3% for body weight and 13.9% to 12.2% for total length at 8 months and 20 months of age respectively. Large coefficient of variation in body traits has also been reported for other aquatic species, e.g. 21% in rainbow trout (Gjerde and Schaeffer, 1989), 29% in Atlantic salmon (Rye and Refstie, 1995), 40% in coho salmon (Hershberger et al., 1990) and from 48% to 60% in tilapia (Ponzoni et al., 2005). The high coefficient of variation obtained can give a raw evidence of prospect that additive genetic variance may contribute large proportion of total variance components.

Generation and environment contributed more to the total variation than the effects of age, line and sex on weight and length. The appearance of high variation between generations may be the result of good selection progress that moves forward the mean of the selected population or/and it was likely incorporate with other factors such as variation of age at observation and environmental conditions, for instance weather change between generations. The strong effect of environment could be explained by different within and between generations included factors such as rearing conditions, water temperature, feed characteristics and husbandry. The availability of natural feed and bottom sediment in pond are examples where competition of fish for food and other habitat may have occurred especially since stocking density was also relatively high in ponds. All of those effects can be partially approved by looking at the different means between lines and generations in Figures 5.3, 5.4 and 5.5. For example in Figure 5.3, the weight of the control and selection lines in the G_2 generation was similar to the control line and even lower than the selection line in the G_1 generation at tagging, however both the selection and control lines in the G_1 generation were significantly heavier than that in the G_2 generation at the second and last measurements. The differences of fish age at the time of measurement between ponds and generations were a covariable factor that might cause high variability in growth performance traits, as demonstrated in tilapia (Ponzoni et al., 2005; Maluwa et al., 2006), channel catfish (Rezk et al., 2003) and rainbow trout (Fishback et al., 2002). Sex had a higher proportional effect on variation in height (4.5%) because the observed phenotype of males is generally slimmer than females. Growth of common carp depends on sex, that is, females showed bigger size than males, from 5.8% to 7.0% for weight, 1.7% to 2.8% for length and 1.6% for height (Table 5.3). In other research, common carp could be 7% to 8% heavier at market size (1kg) in Israel by

rearing all-female populations (Cherfas et al., 1996). Kocour et al. (2007) obtained from 4.8% to 6.8% better growth of three years old all-female population compared to mixed sex.

5.4.3. Genetic parameters

5.4.3.1. Heritability estimates

In the present study, heritability values estimated from the animal model (Model 2A) for growth traits were moderate and generally decreased from the first to last measurements in each generation. A similar trend of declining heritability was also observed in combined analysis of the two selection generations. A decrease of heritability with increasing age was also reported in some other studies. In common carp, Wang et al. (2006) carried out separate family rearing of 30 full-sib families (10 males × 30 females) and heritability, estimated from the sire component, reduced from 0.35 at 8 months old to 0.27 at 20 months old for standard length. Winkelman and Peterson (1994) produced 48 full-sib families per strain in Chinook salmon and each family was reared in two sites. Within each strain, the mixed model estimated heritability for weight and length at first winter season were not constant between sites and measurements. However, neither of the above studies gave any reason for decreased heritability with increased age. The study by Crandell and Gall (1993) in rainbow trout could propose a reasonable answer for reduction of heritability. They communally stocked eighteen fin clipped fish from each of 54 families (3 males × 1 females) in one tank. Heritabilities estimated by mixed model at 159 and 180 days weight were 0.53 and 0.50 respectively, and decreased to 0.36 at 278 days. The last heritability estimation occurred just prior to maturation at one year old. For weight from 355 to 544 days, heritabilities were moderate and ranged from 0.26 to 0.41. The

lowest estimated of 0.26 occurred at 398 days, after all males had reached maturity. So the heritability estimated decreased in magnitude before and after one year sexual maturity. Pre and post-maturity estimates may have been smaller due to uncorrectable effects associated with sexual maturity. In our case, common carp can mature and spawn after one year old so the second measurement just before winter season at age of about 7 months was at the start of gonad development and the third measurement at almost 12 months old was beginning of spawning season, corresponding to sexual maturity. Therefore, our low heritability at the last measurement might be associated with sexual maturity. Because the last measurement of each generation was taken when fish were showing strong development of gonad for spawning that may have affected the genetic parameters.

The estimated heritability values of common carp were 0.39 ± 0.06 at the first measurement and 0.25 ± 0.04 at the final harvest for both weight and length, which are similar to the findings of Vandeputte et al. (2004) who analyzed parentage assignment of 550 communal early rearing offspring from a full factorial cross of 10 dams to 24 sires common carp. This heritability estimated by animal model was 0.33 for body weight and 0.33 for total length at eight weeks of age. However, they are much smaller than those observed in the study by Kocour et al. (2007) who also estimated genetic parameters based on molecular pedigree and found very high heritability for standard length (0.69), body weight (0.7) and body height (0.32) after three rearing seasons (mean weight about 1549 g and 35.2 cm for standard length). Wang et al. (2006) obtained heritabilities from 0.2 to 0.35 for 8 months old and from 0.14 to 0.3 for 20 months old fish observed in five growth-related traits of separate early rearing. All of the above studies did not account for any common environmental

effect since the communal early rearing assumed no common environmental effect occurred. The moderate to high heritability for growth-related traits in our study demonstrated the potential for this selective breeding programme in common carp for growth performance.

The mating design and early communal rearing were expected to reduce the common environmental/full-sib effects and result in better additive genetic estimates on growth performance in the present study. The overall obtained maternal and/or common environmental effects (c^2) were very low and close to zero. The existence of c^2 in the combined data analysis was thus likely due to different environment between generations and perhaps the genetic by environment interaction that were uncorrectable effects in the analyzed model.

The environment and genotype by environment interaction were considered as potentially serious effects that could bias estimation between generations. This caused significantly higher dominance variance than additive variance for growth-related traits at 8 months and 20 months of age in common carp (Wang et al., 2006). They reported that the proportion of dam variance in the total phenotypic variance was from 0.29 to 0.56 while it was 0.00 to 0.61 for additive genetic variance. However, they did not calculate for other common environmental effect than dominance and residual variances. Some environmental factors that can cause differences between generations are pond conditions (water quality, bottom sediment), weather (water temperature), feed characteristics (feed quality, quantity and type of supplier). Genotype by environment interaction for growth-related traits has been found to be significant in common carp (Wang and Li, 2007) and rainbow trout (Fishback et al., 2002; Kause et al., 2003).

The common environmental/full-sib variance has been known as a challenging effect on family-base selection for aquaculture species, for instance environmental differences between full-sib and half-sib families had significant effects on growth measurements in Chinook salmon (Winkelman and Peterson, 1994). However, by application of early communal rearing and molecular parentage assignment, no common environmental effect was accounted in recent publications on selection in common carp (Kocour et al., 2007; Vandeputte et al., 2004, 2008) and rainbow trout (Fishback et al., 2002). Maternal genetic effects were also not estimated separately even though they might be present in our study. In fish species, maternal genetic effects caused by egg size and egg quality could be important particularly in early growth stage of carp (Hulata et al., 1976; Vandeputte et al., 2002).

5.4.3.2. Genetic and phenotypic correlations

The genetic and phenotypic correlations were all positive and moderate to high in this study. Slightly lower genetic and phenotypic correlations were obtained between weight and length (0.85 for genetic correlation and 0.72 for phenotypic correlation) at the final harvest. The correlations between body height and other traits were moderate to high, consistent with the study of Wang et al. (2006). Kocour et al. (2007) reported negative genetic (-0.14) and phenotypic (-0.17) correlation between height and length, however this study was on European carp stocks, which show wide variation in body shape. Genetic and phenotypic correlations between weight and length of Hungarian synthetic mirror carp reported by Vandeputte et al. (2004) were very high at 0.98 and 0.97 at the age of eight weeks of communal early rearing application, respectively. High correlations between body weight and body length of common carp were also observed in other studies, e.g. by Wang et al. (2006) (0.95 for genetic correlation and

0.8 for phenotypic correlation) and Kocour et al. (2007) (0.97 for genetic correlation and 0.92 for phenotypic correlation used molecular pedigree method). Our results indicate that selection of increased body weight would result in greater body length in common carp. In addition, genetic improvement of weight and length could slowly change height of fish. This result is consistent with other studies in rainbow trout (Kause et al., 2003), tilapia (Nguyen et al., 2007) and common carp (Kocour et al., 2007)

The greater genetic correlations of weight (0.98 ± 0.016) and length (0.99 ± 0.001) between the third and second measurements could allow selection to based on the age of second observation since at this age of measurement weight and length have higher heritability and this might reduce stress on selected broodfish for spawning.

5.4.4. Response to selection and estimated breeding values

- By Mixed model:

Two generations of selection produced significant response for growth performance. Direct response to selection for body weight ranged from 14.2% to 19.6% at the final harvest in each generation (Table 5.11). The rates of response to selection in each generation in our study are similar to findings of 14% in Atlantic salmon (Gjerde, 1986), 13% in rainbow trout (Gjerde, 1986), 10% in coho salmon (Hershberger et al., 1990), 7% to 10% in channel catfish (Rezk et al., 2003) and 10% to 20% in tilapia (Gjedrem and Thodesen, 2005).

- By univariate breeding value (calculation based on differences of breeding values between lines in each generation in Table 5.13, data not shown):

Correlated responses were also achieved for body length and height. The expected response was high for length and height. This is consistent with the theoretical predictions in tilapia by Nguyen et al. (2007). By using selection index theory, Nguyen et al. (2007) found that selection for greater harvest weight will slowly result in relatively longer and thinner fish due to greater response in length than width and depth. In the present study, the results of genetic progress per line calculated by the estimated breeding values were higher than that accounted for phenotypic observation by mixed model. The observed response to selection was 19.6%, 4.8% and 7.5% for weight, length and height respectively at the final harvest in the G_2. They were expected to improve in the estimated breeding values between selected and control lines in the G_2 from 46.78 g to 61.53 g for weight, 1.64 cm to 1.72 cm for length and 0.19 cm to 0.20 cm for height. These results and moderate heritability of traits at different measurements suggest that a rapid rate of genetic improvement is feasible in the selective breeding programme.

5.5. Conclusions

This study demonstrated the application and effectiveness of molecular parentage assignment as a tool in genetic selection in common carp. The estimates of heritability for body traits were moderate to high. The similar heritabilities were obtained for weight and length at different time of measurements. Substantial selection responses in growth-related traits were achieved from the analysis of a known molecular pedigree utilizing all available information. It is concluded that direct selection on body weight appears to be correlated positive selection on length and height, to improve overall growth performance of common carp.

Chapter 6. Selective Breeding of Common Carp Using Separate Early Rearing

6.1. Introduction

6.1.1. Additive genetic effect

Selective breeding programmes for improvement of growth traits in common carp have been practised in several countries. In programmes where full pedigree is maintained, this has generally been achieved by separate early rearing of families and physical tagging. Based on pedigree information, genetic parameters can be estimated using various statistical models for analyses. The commonly reported genetic parameters include heritabilities for direct and maternal effects, and genetic, phenotypic and environmental correlations among traits.

Heritability estimates for growth traits in aquaculture species are abundant in the literature. The estimations of heritability are based on additive genetic effects, however it is generally confounded with maternal and common environment effects under separate early rearing, even though attempts were made to separate these from additive genetic effects. Since additive genetic variance is shared and confounded with common environmental variance, the heritability values estimated have often been lower than that under communal early rearing. The heritability estimates reported for growth performance of carp were generally not very high, ranging from less than 0.01 (Moav and Wohlfarth, 1976) to 0.58 (Bongers et al., 1997) based on separate early rearing and from 0.33 (Vandeputte et al., 2004) to 0.70 (Kocour et al., 2007) by communal early rearing methods.

Almost all estimated genetic and phenotypic correlations between body weight and body length were found to be positive, indicating that selection for body weight should indirectly increase body length as a correlated response. The estimates of

phenotypic and genetic correlation between weight and length of common carp were high in the studies of Vandeputte et al. (2004), Wang et al. (2006) and Kocour et al. (2007). However, the genetic and phenotypic correlations between height and weight, and height and length varied from low to moderate. Wang et al. (2006) and Kocour et al. (2007) reported negative correlations among these traits.

6.1.2. Effects other than additive genetics

Reported systematic factors other than additive genetics that may affect growth performance of common carp are maturity of sire and dam, sex of fish, age from hatch to measurement, breeding groups (lines), environmental conditions and their interactions. These factors need to be taken into account in statistical models to adjust the data before selection decisions are made for growth traits.

6.1.2.1. Common environment

The common environmental effects are mostly due to the combined differences in survival rate, hapa conditions, management and nutrition, and natural/climate effects. Research on common carp reported that common environment has highly significant effects on growth traits (Wang and Li, 2007). Environmental differences among generations of selection also increase bias in estimation of genetic parameters (Wang et al., 2006). The common environmental effects that could be confounded with genetic values are also found in other species, such as sea bass (Vandeputte et al., 2001).

6.1.2.2. Maternal

The phenotypic expression of growth performance in progeny, particularly in early stages of life, may be influenced by the genotype and phenotype of the dam. The contribution of a dam to the growth of its progeny is through direct maternal genetic

effects in the oocyte and thus egg quality. In addition, the ability of the dam to provide a suitable environment for the expression of such traits in her progeny is caused by both genetic and environmental factors. Similar to other genetic effects of an individual, the maternal genetic component can be divided into additive, dominance and epistatic effects. The environmental effects may be partitioned into permanent and temporary environmental components.

In contrast to mammals, maternal effects are less important for growth traits of aquaculture species. The maternal effects were only found in some fish species and mainly expressed at early life stages. So estimates that include maternal effects decrease rapidly from birth to later ages. Estimation of maternal effects and the corresponding genetic parameters has always been considered inherently problematic. Difficulties arise because direct and maternal effects are generally confounded. Moreover, the expression of maternal effects is also sex-limited and occurs relatively late in the life of the female (Willham, 1980). The restricted maximum likelihood (REML) method fitting an animal model has enabled the estimation of (co)-variance components due to maternal effects as well as other additional random effects (Meyer et al., 1989), although the estimates are sometimes biased and that there are high sampling correlations among parameters.

6.1.2.3. Sex

The sex of fish has a highly significant effect on size of individuals by harvest stage in many species. In common carp, males have a slimmer body and are smaller than females. Size difference by sex normally occurs at later stages of life because of its stimulation by hormonal factors. Sex differences have been shown to increase as growth rate increases, indicating that the females are more responsive to their environment (Hopkins, 1977). Kocour et al. (2007) reported that sex had a significant effect on growth of common carp in Europe because more males than females

matured in the third spring measurement. Similar results were found in the three summers old common carp by Kocour et al. (2005) in a study comparing all-female and mixed sex populations. However, this advantage of females disappeared at four summer old carp due to female maturation leading to an increase in their gonadosomatic index (Kocour et al., 2005). Common carp are mature at one year old and females are larger than males at this age in Vietnam (Thien, 1996), indicating the importance of the environment in determining age at maturation in this species.

6.1.2.4. Others

Interactions between environmental effects should be tested in statistical analyses of selective breeding programmes. Significant interactions causing variation in growth performance of fish were reported on survival rate or stocking density, time of spawning, nutrition and feeding management, pond or hapas conditions. The interaction effects demonstrated that the non-genetic factors can be dependent upon each other.

In some cases, dam age has a significant effect on both survival rate and growth performance of their progeny. However, it is not considered for analysis in this research since all dams involved in family production were at the same age.

6.1.3. Aims of the study

The objectives of this study were to estimate phenotypic and genetic parameters over two generations of selection for growth-related traits of common carp whose individual families were reared separately in hapas until physical tagging (called "separate early rearing", SER). In addition, response to selection, genetic and phenotypic correlations and breeding values were estimated and assessed in the selective breeding programme.

6.2. Materials and methods

6.2.1. Family rearing

6.2.1.1. Base population (G_0)

The base population (parents of the G_1 population) was produced in spring of 2005, following an incomplete diallel mating design among 6 local common carp lines namely the 2nd generation of family selection, Hungarian 6th generation of mass selection, Hungarian scale carp, Indonesian yellow 6th generation of mass selection, Indonesian yellow carp, and Vietnamese 6th generation of mass selection (see Chapters 2 and 4 for further details). Each sire was mated to one dam and each dam mated to one sire generating only full-sib families.

6.2.1.2. G_1 and G_2 generations

The selected fish from separate family rearing of the G_0 generation were used to produce the G_1 generation. The separate early rearing fish with physical PIT tagging did not reach sexual maturity after one year. By contrast, communal early rearing fish were about four times bigger than SER fish, and both females and males were ready for spawning. The decision was therefore made to use the CER fish to produce the G_2 generation. More details of the production of the selective breeding population, control population and reference population in the G_1 and G_2 generations were presented in Chapter 2.

6.2.2. Separately Early Rearing monitoring data

After hatching, an equal number of about 2,000 exogenous feeding larvae were taken from each family by volumetric method to nurse separately in $1m^2$ (1m×1m×1m) fine

net hapas. The nursing period in fine net hapas was about 30 days. After this period, an equal number of 100 individuals in each family was taken at random by hand netting and transferred to a $5m^2$ (2.5m×2m×1m) plastic net hapa. Separate rearing of individual families was continued in the respective hapas until tagging size of about 5-10g. As soon as a family reached a suitable size for tagging, an equal number of randomly chosen fingerlings from each full-sib family were individually PIT tagged. All physically tagged fish of families in each generation were then stocked into the same earthen pond for communal grow-out. Fish pond management and data collection strategies were described in details in Chapter 2.

6.2.3. Selection procedure

Combined selection was implemented in this study. Brooders were selected based on EBVs, ranked separately for males and females. EBVs were estimated from an animal model (Model 2A below) at the final harvest data set (see Chapter 2 for further details).

6.2.4. Statistical analysis

6.2.4.1. General analysis

A preliminary analysis using general linear model (GLM) was firstly used to investigate systematic fixed effects on body traits. All analyses were carried out in SAS (SAS Inc, 2002). Data were analyzed to determine the significance of all possible fixed effects. The GLM tested the effects of line, sex and age of fish on the final harvest data. The two-factor interactions were also investigated, and were removed from the model if they failed to show significant ($P > 0.05$) effects on the traits of interest.

Secondly, the mixed model was developed and used to analyze the whole data sets to estimate the fixed effects and initial values of variance components. Pair-wise comparisons were generated using the PDIFF option of the Least Squares Means statement of PROC MIXED in all analyses (Littell et al., 1996). The model consisted of line and sex as fixed effects, and age at harvest as a linear covariate. Sire, dam and the interaction between sire and dam were considered as random effects. The mixed model is written as follows:

$$Y_{ijklm} = \mu + LINE_i + SEX_j + \beta AGE + S_k + D_l + I_{kl} + e_{ijklm} \quad \text{(Model 1)}$$

Where,

Y_{ijklm} is an observation of the individual *m*

μ is the overall mean

$Line_i$ is the fixed effect of line (i = 1, 2)

SEX_j is the fixed effects of sex (j = 1, 2)

AGE is the covariable effect of age

S_k is the random effect of sire k^{th}

D_l is the random effect of dam l^{th}

I_{kl} is the interaction between k^{th} sire and l^{th} dam

e_{ijklm} is the residual error

6.2.4.2. Estimation of phenotypic and genetic parameters

Data in each generation of the G_1 and G_2 were analysed separately for phenotypic and genetic parameters. The variance components obtained with Model 1 were used as starting values. Primary analysis result showed that the interaction between sire and

dam was not significant for traits, and hence this random effect was not included in the final model. The models fitted in the greatest log likelihood value included the fixed effects of line (selection and control), sex (male and female) and covariate of age (first, second, third time of measurements).

In Model 2A, animal and dam (the non-genetic component including maternal, dominance and environment/full-sib) were fitted as random effects. This model (animal model) used animal information to partition the observed phenotypic variance of a trait into various genetic and environmental components, hence it enabled the estimation of variance components, from which phenotypic and genetic parameters were calculated more accurately. The animal model (Model 2A) also supported the estimation of breeding values for all fish and could lead to selection decisions for the selection and control lines and estimation of the genetic trend.

$$Y_{ijklm} = \mu + LINE_i + SEX_j + \beta AGE + I_k + D_l + e_{ijklm} \quad \text{(Model 2A)}$$

Where,

Y_{ijklm} is an observation of the individual *m*

μ is the overall mean

$LINE_i$ is the fixed effect of line (i = 1, 2)

SEX_j is the fixed effects of sex (j = 1, 2)

AGE is the covariable effect of age

I_k is the random additive genetic effect of individual k^{th}

D_l is the random effect of dam including the maternal effect and the effect of common environment/full-sib family rearing.

e_{ijklm} is the random residual effect associated with individual *ijklm*

Heritability estimates under Model 2A: Variance components for growth traits were estimated from a univariate model. Although dam was fitted as a random effect, it actually accounted for any common environmental/full-sib effect on the progeny. So the dam component (σ_D^2), in this case, is a combination of the maternal effect and the common environmental/full-sib effect (means $\sigma_D^2 = \sigma_{D+E}^2$, referred to as σ_C^2 in later use). Phenotypic variance (σ_P^2) calculation was based on additive genetic variance of individual (σ_A^2), maternal and common environmental/full-sib variance (σ_C^2) and residual variance (σ_e^2) as $\sigma_P^2 = \sigma_A^2 + \sigma_C^2 + \sigma_e^2$. Then the heritability using individual variance component was calculated as $h^2 = \frac{\sigma_A^2}{\sigma_P^2}$. The maternal and common environmental/full-sib effect was calculated as $c^2 = \frac{\sigma_C^2}{\sigma_P^2}$.

Genetic and phenotypic correlations between all traits were calculated as the covariance divided by the product of the standard deviations of traits: $r_g = \frac{\sigma_{12}}{\sqrt{\sigma_1^2}\sqrt{\sigma_2^2}}$,

where σ_{12} was the estimated additive genetic or phenotypic covariance between the two traits.

In addition, a sire model (Model 2B) was used to estimate variance components for heritability calculation in order to compare to results from the animal model (Model 2A). Sire components estimated from a sire model may be slightly less accurate both due to lower accuracy (particularly in case of few progeny per sire) and potential bias, because there is no correction for differences between dams. The sire model analysis ignores the dam information and assumes that all dams are from the same homogenous population all with the same expected mean. However, the sire model may cause overestimate in the present study because partial factorial mating was

applied in selection line, produced by mating one sire with two dams and possibly four dams when ten males from the first batch were used again to mate with females from the second batch (see Chapter 2 for further details). Similar to the Model 2A, a sire model fitting sire and dam as random effects (Model 2B) was used to estimate genetic parameters and written as follows:

$$Y_{ijklm} = \mu + LINE_i + SEX_j + \beta AGE + S_k + D_l + e_{ijklm} \quad \text{(Model 2B)}$$

Where,

S_k is the random additive genetic effect of individual with sire k^{th}

D_l is the effect of the dam plus common environmental/full-sib effects including the effect of common environment (full-sib rearing), and a quarter of non-additive genetic effects

The other factors of Model 2B are the same as in Model 2A

Heritability estimates under Model 2B: Variance components for growth traits were estimated from a univariate model. Phenotypic variance (σ_P^2) calculation was based on additive genetic variance of sire (σ_S^2), maternal and common environmental variance (σ_C^2) and residual variance (σ_e^2) as $\sigma_P^2 = \sigma_S^2 + \sigma_C^2 + \sigma_e^2$. Then the heritability using the sire variance component was calculated as $h^2 = \frac{4\sigma_S^2}{\sigma_P^2}$. The maternal and common environmental/full-sib effect was calculated as $c^2 = \frac{\sigma_C^2}{\sigma_P^2}$.

All computations for the Model 2A and Model 2B were carried out using the ASREML software package (Gilmour et al., 2002). Variance and covariance components were estimated using restricted maximum likelihood. Convergence for log-likelihood of variance component estimation was considered satisfactory when

two successive rounds of interaction changed by less than 0.1%. All known parent to progeny information was included in the analyses through a relationship matrix.

Heritability and common environmental effects and correlation estimates were tested for significantly different from each other, or zero by using z-scores:

$$z = \frac{x_i - x_j}{\left(\sigma_i^2 + \sigma_j^2\right)^{0.5}}$$

Where, x_i and x_j are the estimates of heritability and common environmental effects, or genetic correlations for the two traits and σ_i and σ_j are their respective standard errors. Both x_j and σ_j were set to zero or one when test of an estimate was significantly different zero or one, respectively. The resulting z-scores were then tested against a large sample normal distribution.

6.2.4.3. Response to selection analysis

The least squares means estimated by the mixed model (Model 1) were used to calculate response to selection in each generation for growth-related traits. Response to selection for selective breeding line to control line was calculated as the difference of the least square means. In addition, the selection differential between selection and control lines was also calculated from the univariate estimated breeding values.

6.2.4.4. Estimates of realized heritability, selection differential and selection intensity

The realized heritability (h_r^2) is a value to quantify the degree to which change in a trait in a population can be achieved by selection. For each of the selected traits, response to selection was estimated as difference between the observed mean of the

selected progeny group and the observed mean of the control progeny group. The observed selection differential was estimated as the difference between the mean of the selected parents and the mean of the total number of individuals measured prior to selection. Realized heritability was calculated as the ratio of the response to selection and the observed selection differential (Falconer and Mackay, 1996). The response to selection and the observed selection differential was obtained from the least squares means of mixed model analysis.

Selection intensity (i) which is equal to the selection differential expressed in term of standard deviation by the following equation:

$$i = \frac{S}{\sigma_P}$$

Where,

i is selection intensity

S is selection differential

σ_P is the standard deviation of fish population before selection

6.3. Results

6.3.1. General summary data of selected and control fish

The estimated mean, standard deviation and coefficients of variation for body weight, standard length and height at times of measurement in the G_1 and G_2 generations are presented in Table 6.1. The first measurement was at the time of PIT tagging after separately family rearing in hapas. The second and third observations were communal rearing data of tagged fish. Height was only collected one time at final harvest because it was not the main trait in this breeding programme. There was a reduction in sample sizes of weight and length from the first to last times of measurement due to missing data caused by mortality and recording errors. Although the two batches of spawning in each generation were only seven days apart, there was high variation in age at PIT tagging and onwards (from 8.48% to 15.31%) in the G_2 generation. The age of fish at PIT tagging was 80 days for the first batch and 110 days for the second batch. This means that there was 30 days difference between batches to reach suitable size (5 to 10 g per fish) for PIT tagging although the spawning dates were only seven days apart. It is likely that the environment was a serious factor affecting fish growth and caused major environmental/full-sib effect on variance estimation of traits. The main environmental variance started in early spawning season (the end of winter season) when suddenly the weather changed with monsoon arrival and reduced water temperature, extending the time of egg incubation and perhaps affecting growth of fish in the second batch thereafter.

The mean and standard deviation for weight, length and height increased with ages of measurements. Means of weight and length at the first measurement in the G_1 were

lower than those in the G_2 even though the latter had a longer rearing time. However, growth of fish at the second measurement in the G_1 was higher at a similar age at the next measurement. The coefficient of variation (CV) was lowest for length and highest for weight. The CV at the second measurement was larger than that at the first and last measurements for weight and length in the G_1 and G_2 generations. The CV in the G_2 generation was greater than in the G_1 generation for all traits of interest and at different measurements.

Table 6.1. Sample size (N), mean, maximum (max), minimum (min), standard deviation (Std), coefficient of variation (CV %) of data for weight, length, height and age in the G_1 and G_2 generations.

Traits	Measurement	G_1						G_2					
		N	Mean	Std	CV	Min	Max	N	Mean	Std	CV	Min	Max
Weight (g)	1	2670	22.74	10.14	4.58	7.0	105.0	3010	32.24	21.18	65.71	6	190
	2	2485	82.05	40.57	49.45	14.0	411.0	2784	70.50	57.18	81.10	12	523
	3	2352	368.50	172.34	46.77	57.0	1578.0	2586	154.53	116.92	75.66	15	940
Length (cm)	1	2670	10.89	1.46	13.38	7.5	18.6	3010	11.87	2.19	18.45	7	22.7
	2	2485	17.31	2.53	15.36	10.0	30.8	2784	15.74	3.58	22.74	8.7	32.7
	3	2352	28.43	4.37	14.64	15.9	48.0	2586	20.36	4.37	21.46	10.2	43
Height (cm)	3	2352	7.68	1.27	16.57	4.2	12.0	2586	5.65	1.36	24.07	2.7	13.6
Age (day)	1	2670	136.94	2.99	2.19	134	140	3010	95.0	14.86	15.31	80	110
	2	2485	203.94	2.99	1.47	201	207	2784	183.0	14.86	11.91	168	198
	3	2352	413.94	2.99	0.71	411	417	2586	257.0	14.86	8.48	242	272

6.3.2. Prediction of fixed effects

General linear model (GLM) analysis on the growth-related traits of final harvest data revealed that the effects of sex and age were all significant ($P<0.05$). Line was only significant ($P<0.05$) for weight and length in the G_1 generation and length in the G_2 generation. In other words, there were differences in magnitude of effects between sex (male, female) and age (at PIT tagging) on weight, length and height of fish however differences between line (selection, control) was obtained for weight and length in the G_1 generation and length in the G_2 generation. The two-way interactions between these effects were non-significant and were removed from the model.

Regarding the R^2 value, the full model accounted for 4.3%, 3.9% and 5.1% of the observed variance for weight, length and height in the G_1 generation, respectively. The G_2 generation had higher R^2 values (17.8%, 27.2% and 24.8%) corresponding to weight, length and height although this high variation is likely due to environmental effects due to covariation with age. Age was significant for all traits and had the biggest effect in the two generations, suggesting that fish at stocking with different spawning dates were exposed to different environments. The estimates of fixed effects for line, sex and age by general linear model are presented in Table 6.2.

Table 6.2. The general linear model (GLM Procedure: SAS, 2002) estimates for the fixed effects of line, sex and age at third time measurement in the G_1 and G_2 generations.

		Effect					
		Line		Sex		Age	
Generation	Trait	F-value	P	F-value	P	F-value	P
G_1	Weight	3.6	0.013	12.2	<0.001	15.3	<0.001
	Length	2.9	0.037	7.4	0.009	8.9	<0.001
	Height	0.4	>0.05	3.5	0.041	7.8	<0.001
G_2	Weight	1.7	>0.05	3.2	0.014	23.5	<0.001
	Length	5.0	0.024	7.6	0.008	25.4	<0.001
	Height	0.6	>0.05	3.0	0.046	29.7	<0.001

6.3.3. Population characteristics

The least squares means (LSM) of weight, length and height for selection, control and reference lines in the G_1 and G_2 generations are shown in Figure 6.1, 6.2 and 6.3. The highest standard errors (S.E.) were observed in the reference population, followed by the control population. The LSM of traits in the selection population had the smallest S.E. In the G_1 generation, there were no significant differences between the selection and control lines for all traits of interest at three times of measurement except for body weight and length at final harvest. The mean weight and length at final harvest of the selection population were 30.35 g and 0.99 cm, respectively, greater than the control line ($P<0.05$).

Adjusted means of weight, length and height were not significantly different (P>0.05) between control and selected lines except length at the third observation in the G_2 generation. The reference population was higher than control and selection lines for most of the traits and measurement times. However, the interpretation of the results should be with caution because there were a very limited number of reference families and tested progeny compared with the selection and control lines.

Figure 6.1. Least squares means of weight at different measurements for each generation (G_1, G_2) and line (C: Control, S: Selection, R: Reference).

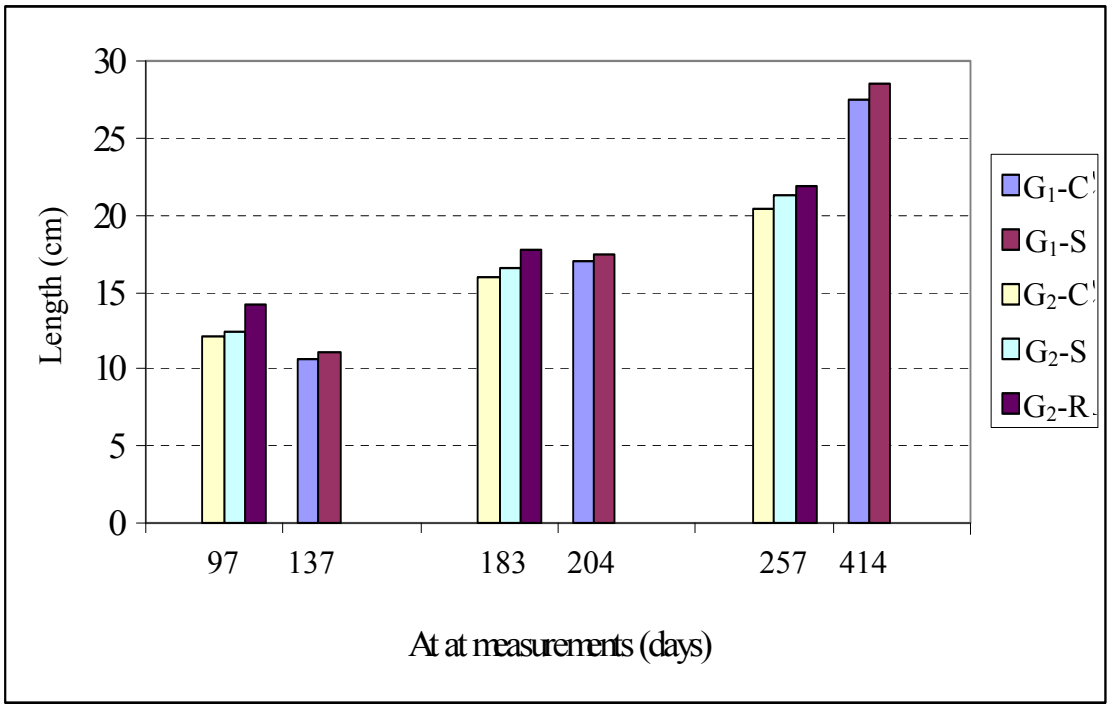

Figure 6.2. Least squares means of length at different measurements for each generation (G_1, G_2) and line (C: Control, S: Selection, R: Reference).

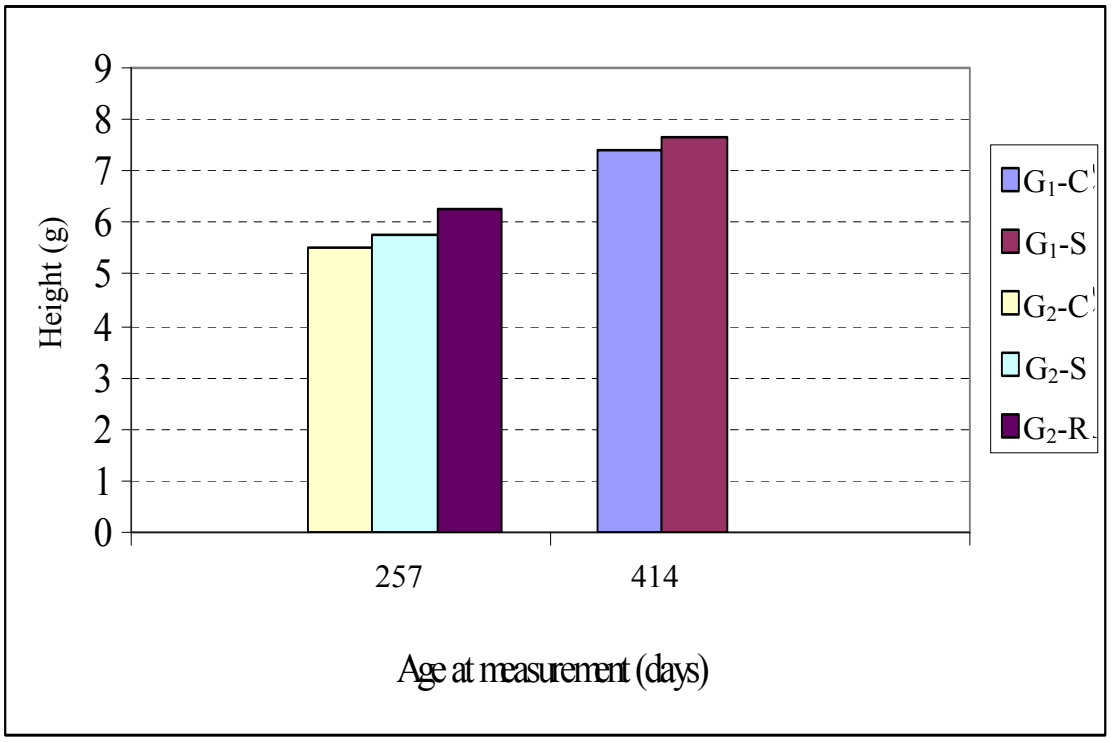

Figure 6.3. Least squares means of height at final harvest for each generation (G_1, G_2) and line (C: Control, S: Selection, R: Reference).

Growth of females and males showed no significant difference for weight, length and height at the first and second times of measurement in the G_1 and G_2 generations. However, females were significantly larger than males for all traits at the latest life stage assessed. Females were approximately 13.1%, 3.4% and 5.2% greater than males in weight, length and height in the G_1 generation. In the G_2 generation, weight, length and height of females were 13.3%, 5.5% and 4.2% greater than males. Standard errors of the estimates were similar in females and males for all traits of interest at a given age, however the standard errors in the G_1 were lower than in the G_2. They ranged from 0.07 to 10.59 in the G_1, and from 0.12 to 11.96 in the G_2. The adjusted means of traits for each sex in generations are presented in Table 6.3.

Table 6.3. Least-squares means (±S.E.) of traits by sex in the G_1 and G_2 generations obtained from the mixed model.

Traits	G_1		Proportion of differences (%)	G_2		Proportion of diferences (%)
	Female	Male		Female	Male	
Weight1 (g)	$23.26^{a} \pm 0.64$	$22.69^{a} \pm 0.68$	2.5	$35.36^{a} \pm 2.53$	$35.20^{a} \pm 2.52$	0.5
Length1 (cm)	$10.89^{a} \pm 0.09$	$10.82^{a} \pm 0.09$	0.6	$12.34^{a} \pm 0.25$	$12.22^{a} \pm 0.25$	1.0
Weight2 (g)	$83.05^{a} \pm 2.17$	$78.74^{a} \pm 2.37$	5.2	$77.99^{a} \pm 6.45$	$73.06^{a} \pm 6.39$	6.3
Length2 (cm)	$17.44^{a} \pm 0.15$	$17.09^{a} \pm 0.16$	2.0	$16.31^{a} \pm 0.36$	$16.11^{a} \pm 0.35$	1.2
Weight3 (g)	$377.72^{a} \pm 9.83$	$328.05^{b} \pm 10.59$	13.1	$173.04^{a} \pm 11.96$	$150.03^{b} \pm 11.43$	13.3
Length3 (cm)	$28.52^{a} \pm 0.25$	$27.54^{b} \pm 0.27$	3.4	$21.46^{a} \pm 0.41$	$20.29^{b} \pm 0.40$	5.5
Height3 (cm)	$7.74^{a} \pm 0.07$	$7.34^{b} \pm 0.08$	5.2	$5.77^{a} \pm 0.13$	$5.53^{b} \pm 0.12$	4.2

**Means with different superscript letters in the same line for each generation are statistically different.*

6.3.4. Genetic parameters

6.3.4.1. Heritability estimates

- The G_1 generation:

The variances, heritability and common environmental effect were estimated for weight, length and height in the G_1 generation by Model 2A (Table 6.4) and Model 2B (Table 6.5). The results of analysis for variances and heritabilities were quite consistent between the estimates from the two models. Very high variances were estimated for weight compared to length and height. There was an increase of variances with advancing ages for all traits of interest. Proportion of common environmental variance to total phenotypic variance ranged from 5% for weight at the first measurement to 12% for weight at the second measurement in Model 2A while it varied from 4% for height to 10% for weight at the last measurement in Model 2B. There were small change of residual error variance to total phenotypic variance that accounted for 57% at PIT tagging and 69% at the final measurement for weight in Model 2A and 57% to 68% respectively in Model 2B.

Estimated heritabilities were high at PIT tagging and lower but more constant at later stages. In Model 2A, heritability for weight was 0.38 at the first measurement then reduced to 0.23 at the final measurement. Similar levels of heritability for traits within each measurement age were observed in Model 2B. The standard errors of heritability were low and declined when age increased. The common environmental/full-sib effect (c^2) at the second and third measurements was larger than at the initial observation. The increase of c^2 may be considered as one factor to reduce heritability at later ages. Perhaps, food and environment competition caused higher environmental effect on growth of communal rearing tagged fish. The low and consistent standard errors found for c^2 reflect the low range of common environmental variation.

Table 6.4. Estimated additive genetic variance (σ_A^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h^2 ± S.E.) and common environmental effect (c^2 ± S.E.) of growth-related traits in the mixed models including individual and dam (Model 2A) as random effects in the G_1 generation.

Traits	σ_A^2	σ_C^2	σ_e^2	h^2	c^2
Weight1	41.4	5.3	63.7	0.38 ± 0.12	0.05 ± 0.05
Length1	0.81	0.16	1.37	0.35 ± 0.10	0.07 ± 0.05
Weight2	420.2	225.8	1235.5	0.22 ± 0.08	0.12 ± 0.04
Length2	1.45	0.70	4.60	0.21 ± 0.08	0.10 ± 0.04
Weight3	6887.1	2537.2	20745.1	0.23 ± 0.08	0.08 ± 0.04
Length3	3.88	1.86	13.74	0.20 ± 0.07	0.10 ± 0.04
Height3	0.45	0.09	1.17	0.26 ± 0.08	0.05 ± 0.04

Table 6.5. Estimated sire variance (σ_S^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h_S^2 ± S.E.) and common environmental effect (c^2 ± S.E.) of growth-related traits in the mixed models sire and dam (Model 2B) as random effects in the G_1 generation.

Traits	σ_S^2	σ_C^2	σ_e^2	h_S^2	c^2
Weight1	10.32	7.19	84.19	0.37 ± 0.12	0.06 ± 0.04
Length1	0.20	0.14	1.78	0.34 ± 0.10	0.06 ± 0.03
Weight2	105.52	170.68	1406.18	0.24 ± 0.08	0.10 ± 0.03
Length2	0.36	0.53	5.32	0.22 ± 0.08	0.08 ± 0.03
Weight3	1721.65	3040.55	24178.60	0.22 ± 0.08	0.10 ± 0.03
Length3	1.21	1.56	15.67	0.25 ± 0.07	0.08 ± 0.03
Height3	0.11	0.07	1.39	0.26 ± 0.08	0.04 ± 0.03

– The G_2 generation:

The variances, heritability and common environmental effect were estimated for weight, length and height in the G_2 generation by Model 2A (Table 6.6) and Model 2B (Table 6.7). Heritability and common environmental effect estimates by Model 2A were all slightly smaller than Model 2B except at the first time of measurement. The contribution of σ_C^2 for weight at PIT tagging was approximately 17% of the total phenotypic variance, and increased to 18% at the second measurement before reaching a maximum of 21% at the last time of measurement in Model 2B while it increased with advancing age and ranged from 15% to 22% for all traits at different measurements in Model 2A. The proportion of residual variance (σ_e^2) increased with age in both the models. The σ_e^2 of weight explained approximately 21% of total variation in the first measurement; however, it rose up to 50% in the last measurement estimated by Model 2A. In Model 2B, the contribution of σ_e^2 to total variance of weight was almost 22% at PIT tagging but doubled to 47% at the final measurement.

Weight had high heritability and length and height had moderate heritabilities at the final harvest. The heritability declined in magnitude as the age of measurement increased, from 0.64 to 0.31 at the first and third measurement, respectively, in Model 2A. Heritability estimates for weight also decreased from 0.61 at PIT tagging to 0.32 at the final measurement in Model 2B. Heritabilities were generally high and significantly different from zero ($P<0.05$). The standard errors of heritability estimates were generally proportional to the heritability value, thus if the heritability decreases, the standard error also declines. The common environmental (full-sib family, c^2) effect tended to increase with advancing age in the two models. The ratio of full-sib family and/or environmental effect to total phenotypic variance for all traits at the final measurement accounted by Model 2A were lower than those in Model 2B. The standard errors of c^2 were all low. There was also a trend of slightly decreasing standard error of c^2 when age increased, similar to observed standard errors for heritability.

Table 6.6. Estimated additive genetic variance (σ_A^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h^2 ± S.E.) and common environmental effect (c^2 ± S.E.) of growth-related traits in the mixed models including individual and dam (Model 2A) as random effects in the G_2 generation.

	Model 2A				
Traits	σ_A^2	σ_C^2	σ_e^2	h^2	c^2
Weight1	90.4	28.8	22.8	0.64 ± 0.18	0.15 ± 0.06
Length1	1.08	0.63	0.81	0.43 ± 0.12	0.16 ± 0.08
Weight2	724.8	318.6	461.1	0.48 ± 0.12	0.17 ± 0.09
Length2	1.96	2.02	2.47	0.30 ± 0.07	0.20 ± 0.07
Weight3	5753.3	5303.6	7213.4	0.31 ± 0.08	0.19 ± 0.06
Length3	4.54	5.89	9.07	0.23 ± 0.05	0.21 ± 0.04
Height3	0.55	0.78	1.01	0.23 ± 0.06	0.22 ± 0.06

Table 6.7. Estimated sire variance (σ_S^2), common environmental variance (σ_C^2), residual variance (σ_e^2), heritability (h_S^2 ± S.E.) and common environmental effect (c^2 ± S.E.) of growth-related traits in the mixed models including sire and dam (Model 2B) as random effects in the G_2 generation.

Traits	σ_S^2	σ_C^2	σ_e^2	h_S^2	c^2
Weight1	21.36	28.0	70.16	0.61 ± 0.15	0.17 ± 0.08
Length1	0.31	0.77	1.60	0.41 ± 0.11	0.15 ± 0.08
Weight2	202.91	366.66	839.13	0.50 ± 0.12	0.18 ± 0.06
Length2	0.50	2.14	3.25	0.31 ± 0.07	0.17 ± 0.06
Weight3	1525.21	5688.4	10338.46	0.32 ± 0.08	0.21 ± 0.06
Length3	1.15	6.2	11.73	0.23 ± 0.05	0.20 ± 0.05
Height3	0.15	0.7	1.33	0.26 ± 0.05	0.22 ± 0.04

6.3.4.2. Genetic and phenotypic correlations between traits

Phenotypic and genetic correlations between growth-related traits at the same and different ages were all positive and from moderate to high in the two generations (Tables 6.8 and 6.9). In general, genetic correlations were higher than those of phenotypic correlations. Furthermore, correlations in the G_1 generation were greater than in the G_2.

- In the G_1 generation:

Phenotypic correlations of growth-related traits within and between measurements were moderate to high, ranging from 0.51 between length 1 and weight 3 (or height 3) to 0.93 between length 1 and weight 1. Phenotypic correlations tended to decrease with increasing interval of ages. The results showed that correlation of length 1 reduced from 0.93 with weight 1 to 0.78 with weight 2 and to 0.51 with weight 3. Furthermore, weight and length showed higher phenotypic correlation compared to those of weight and length with height. The estimated standard errors were all low and varied from 0.004 to 0.026 for all studied correlations.

Very high genetic correlations were observed between and within growth-related traits of interest. Correlations of traits at the same age and successive age interval were stronger than those between further apart ages. The lowest genetic correlation was found between length 1 and height 3 (r_g = 0.63), whereas the highest correlation was 0.93, observed between weight 1 and length 1, and weight 3 and height 3. Standard errors of the genetic correlations were from 0.018 to 0.055 and did not differ much between traits and between measurement periods.

- In the G_2 generation:

Phenotypic correlations ranged from 0.34 between length 1 and height 3 to 0.90 between weight 1 and length 1. The correlations of the same trait and between traits at the first and second ages were higher than at the final measurement. Standard errors of phenotypic correlations were low, from 0.005 to 0.017, for relative traits of interest.

Higher genetic correlations were obtained between traits of close ages than larger interval measurements. The correlations between weight and length (from 0.62 to 0.92) were greater than between these traits and height (from 0.50 to 0.92). The standard errors were relatively low and consistently for studied correlations at different times of measurement.

The highest genetic correlations were found between length 2 and weight 1 (0.92), length 2 and length 1 (0.93), and length 2 and weight 2 (0.90) in the G_1 generation. The same correlations observed between these traits were 0.86, 0.90 and 0.92 respectively in the G_2 generation. However, genetic correlations of traits were reduced at the last measurement.

Table 6.8. Phenotypic (above) and genetic (below the diagonal) correlations (±S.E.) between traits in the G_1 generation.

	Weight1	Length1	Weight2	Length2	Weight3	Length3	Height3
Weight1		0.93 ± 0.012	0.85± 0.008	0.88 ± 0.018	0.70 ± 0.015	0.73 ± 0.015	0.61 ± 0.024
Length1	0.94 ± 0.022		0.78 ± 0.013	0.81 ± 0.016	0.51 ± 0.027	0.58 ± 0.024	0.51 ± 0.026
Weight2	0.93 ± 0.026	0.87 ± 0.028		0.88 ± 0.004	0.75 ± 0.009	0.72 ± 0.012	0.72 ± 0.022
Length2	0.94 ± 0.026	0.93 ± 0.021	0.90 ± 0.029		0.73 ± 0.011	0.81 ± 0.008	0.72 ± 0.013
Weight3	0.73 ± 0.038	0.64 ± 0.046	0.84 ± 0.036	0.82 ± 0.037		0.85 ± 0.010	0.82 ± 0.009
Length3	0.85 ± 0.042	0.72 ± 0.055	0.81 ± 0.033	0.88 ± 0.030	0.91 ± 0.025		0.81 ± 0.011
Height3	0.67 ± 0.047	0.63 ± 0.050	0.84 ± 0.034	0.82 ± 0.036	0.94 ± 0.018	0.90 ± 0.027	

Table 6.9. Phenotypic (above) and genetic (below the diagonal) correlations (±S.E.) between traits in the G_2 generation.

	Weight1	Length1	Weight2	Length2	Weight3	Length3	Height3
Weight1		0.90 ± 0.008	0.70 ± 0.012	0.84 ± 0.015	0.64 ± 0.014	0.80 ± 0.016	0.48 ± 0.017
Length1	0.92 ± 0.021		0.80 ± 0.010	0.74 ± 0.007	0.66 ± 0.017	0.74 ± 0.014	0.34 ± 0.016
Weight2	0.82 ± 0.023	0.90 ± 0.021		0.84 ± 0.012	0.34 ± 0.009	0.62 ± 0.010	0.62 ± 0.010
Length2	0.86 ± 0.020	0.90 ± 0.012	0.92 ± 0.018		0.60 ± 0.009	0.44 ± 0.006	0.62 ± 0.009
Weight3	0.76 ± 0.036	0.78 ± 0.043	0.52 ± 0.026	0.64 ± 0.032		0.64 ± 0.005	0.76 ± 0.005
Length3	0.88 ± 0.022	0.84 ± 0.052	0.74 ± 0.026	0.62 ± 0.024	0.80 ± 0.016		0.74 ± 0.006
Height3	0.56 ± 0.033	0.50 ± 0.041	0.78 ± 0.030	0.76 ± 0.013	0.92 ± 0.014	0.86 ± 0.022	

6.3.5. Response to selection

Table 6.10 shows response to selection calculated as a percentage of the difference between selection and control lines in the G_1 and G_2 generations. In the G_1 generation, the responses to selection were positive but low for all traits especially for length (2.9% to 3.5%) and height (3.3%). The G_2 generation analysis resulted in higher response to selection for weight at the first and second time of measurements but decreased slightly at the final observation. Overall, responses in all traits to selection were lower at the initial and second measurements than at the final harvest except for weight at the final measurement in the G_2 generation. The difference between selection and control lines was high for weight but was low for length and height. In addition, the responses to selection of the selective breeding line were consistent across ages of measurement in the both generations.

Table 6.10. Responses to selection (%) per generation estimated by Mixed Model (Model 1) for the difference between the selection and control lines in the G_1 and G_2 generations.

Traits	G_1	G_2
Weight1	2.6	8.3
Length1	2.9	2.9
Weight2	6.3	9.3
Length2	3.2	3.8
Weight3	8.2	7.9
Length3	3.5	4.1
Height3	3.3	4.5

6.3.6. Realized heritability

The realized heritability was estimated based on selection intensity, selection response and selection differential at the final harvest (Table 6.11). The selection intensity in G_1 and G_2 generations were 6.3% and 7.0% respectively. The selection response and selection differential were all high in weight. A low realized heritability for weight (0.10) was obtained in the G_1 generation, however it increased to 0.24 in the G_2 generation. The realized heritability for length and height ranged from 0.21 to 0.23 and was quite consistent between the two generations. The realized heritability estimates were all lower than heritabilities estimated by the animal model (Model 2A). The moderate realized heritability for growth-related traits shows that the offspring of the selected parents differ from the original population as a result of selection.

Table 6.11. Estimated selection intensity (i), selection differential (S), response to selection (R) and realized heritability (h_r^2) of weight, length and height at final harvest in the G_1 and G_2 generations.

Traits	G_1				G_2			
	i (SD)	S	R	h_r^2	i (SD)	S	R	h_r^2
Weight3 (g)	1.784	313.39	30.35	0.10	0.465	54.97	13.25	0.24
Length3 (cm)	1.000	4.40	0.99	0.22	0.898	3.86	0.87	0.23
Height3 (cm)	0.861	1.10	0.25	0.23	0.914	1.24	0.26	0.21

6.3.7. Estimated breeding values

The univariate analysis of breeding values for growth-related traits by lines (selection and control) is presented in Table 6.12. Estimated breeding values were smallest at stocking and highest at the last measurement in both the G_1 and G_2 generations. The estimated breeding values (EBV) of growth-related traits were all positive and higher in the selection line compared to the control. The EBVs and its standard errors obtained from univariate analysis in the G_1 were slightly higher than that in the G_2. However, the proportions of difference between two lines were lower in the G_1 generation compared to the G_2 generation.

Table 6.12. Univariate estimated breeding values (±S.E.) of traits by lines in the G_1 and G_2 generations.

Traits	G_1		G_2	
	Control	Selection	Control	Selection
Weight1 (g)	1.54 ± 0.24	1.65 ± 0.23	1.62 ± 0.22	1.70 ± 0.23
Length1 (cm)	0.11 ± 0.02	0.115 ± 0.03	0.14 ± 0.02	0.145 ± 0.04
Weight2 (g)	2.98 ± 0.67	3.20 ± 0.80	2.46 ± 0.44	2.71 ± 0.58
Length2 (cm)	0.22 ± 0.02	0.23 ± 0.03	0.20 ± 0.02	0.21 ± 0.04
Weight3 (g)	9.90 ± 1.62	10.80 ± 2.30	7.40 ± 1.01	8.10 ± 1.16
Length3 (cm)	0.94 ± 0.08	0.98 ± 0.05	0.92 ± 0.02	0.93 ± 0.04
Height3 (cm)	0.15 ± 0.006	0.16 ± 0.008	0.096 ± 0.01	0.10 ± 0.03

6.4. Discussion

6.4.1. Phenotypic variation

In the present study, the coefficient of variation (CV) at the second measurement was higher than that at the first and third measurements for weight and length in the two selection generations. In addition, the CV in the G_1 generation was lower than that in the G_2 generation for all traits of interest. The change of CV is in agreement with other studies although these researchers grew families separately until harvest. For example, Wang et al. (2006) reported that CV at 8 months of age was slightly higher than that at 20 months of age for five growth-related traits in common carp. In rainbow trout, the CV for body weight decreased from 49% to 22% at 168 days and 364 days of age respectively (Su et al., 2002).

The graphs (Figures 6.1, 6.2 and 6.3) clearly show differences of growth performance between the G_1 (control and selection lines) and the G_2 (control and selection lines) indicating that environment and grow-out conditions influenced the performance of fish. Growth performance of the fish in each generation was also highly variable due to selection, environment and yearly condition as well as management method. They were also the main factors that caused high coefficients of variation for growth-related traits. Although interval of the spawning date between families in each generation were very close (seven days apart) and number of fish in each family were equally stocked in hapas during the period of nursing, variation of initial (PIT tagging) growth traits were still very high. Change of weather in the spawning season is considered as one of the main effects, which suddenly reduced water temperature and caused longer incubation time for families hatched in one batch then appeared to affect their growth

in later life stages. Furthermore, variation in growth performance within family may be due to the competition for food during separate rearing in hapas, since high density rearing (2.000 individuals in $1m^2$ fine net hapas) was applied for three weeks. The slower development meant later tagging and start to communal rearing for second batch in the G_2 generation as shown in Table 6.1. Common carp is a bottom feeder and growth performance is very sensitive to stocking density in earthen pond. Since larger fish (family) in the first batch were tagged and stocked first in the pond they had better feeding and grew faster. Therefore, the observed variation was high at the start of communal rearing (PIT tagging) and increased even more when fish were larger.

The yearly conditions including environment and management practices may effect the growth of fish between generations. Growth performance of fish in Table 6.1 showed that size at tagging in the G_1 was lower even at higher age compared to in the G_2 but fish size in the G_1 was larger than that in the G_2 in the next measurements at the same age. Furthermore, this separate early rearing experiment was designed to compare with communal early rearing method (see Chapter 5) so different times of measurement and management were applied corresponding to the communal early rearing method (the differences will be discussed in Chapter 7).

6.4.2. Common environmental/full-sib effects

The differences among means of full-sib groups could be due to dominant gene action, maternal environmental effects and/or common environmental effects, as well as differences in the average breeding values of parents. The implementation of a genetic improvement programme using family-based selection for fish species with early separate rearing requires intensive investment in facilities and labor cost because

families are hatched and reared separately for a period of time until reaching a suitable size for physical tagging (Fishback et al., 2002). Separate early family rearing also caused variable stocking densities due to different survival rates between families that may confound the estimation of additive genetic parameters as well as breeding values for the selection of potential broodstock. In the current study, the same number of larvae in each family was taken (by volume) to stock in uniform $1m^2$ (1m × 1m × 1m) fine net hapas. After three weeks of nursing, the numbers of fry in each family were equalized and they were transferred to $5m^2$ (2.5m × 2m × 1m) plastic net hapas. Separate family rearing continued until tagging at 5-10 g. Variable survival rate was observed during the first nursing periods that ranged from 0.8% to 59.6% and from 0.6% to 30.9% in the G_1 and G_2 generations respectively. In addition, about 6.2% to 7.2% reduction of sample number in each measurement of communally grown tagged fish due to mortality and recording information error caused further reduction in family size. This, therefore, may increase the environmental/full-sib effects on the evaluation of growth traits.

The phenotype of progeny is determined not only by its own genotype and the random environmental conditions it experiences during development, but also by the environment provided by its parents. The environment provided from the mother usually contributes considerably more to offspring phenotype than that from the father, and this is generally referred to as a maternal effect. In this study, the variance due to maternal effect was not separated from common environmental effect. However, full-sib families were reared separately in hapas for at least 80 days in the G_2 generation and 134 days in the G_1 generation so this may have reduced any maternal effect on later communal growing of tagged fish. For most fish species, maternal effects are largely caused by egg size and quality which primarily influence

growth at early stages of development (Gjedrem, 1983; Gjerde, 1986) and decrease with advancing age (Henryon et al., 2002). Although the long separate family rearing may have had reduced any maternal effect, it might be expected to inflate common environmental effects in the current study.

Growth traits of fish species differ between genders. In this study, females were approximately 13.2% in weight, 4.4% in length and 4.7% in height larger than males at the last measurement. Kocour et al. (2007) found a significant effect of sex in all growth traits of common carp which is consistent with our results. In some species, males are larger than females, e.g. in tilapia (Ponzoni et al., 2005; Rutten et al., 2005), rainbow trout at one year old stage (300 g) (Bonnet et al., 1999), and catfish (Goudie et al., 1994) while females are larger than males in European eel (Roncarati et al., 1997), perch (Fontaine et al., 1997), Atlantic halibut (Imsland and Jonassen, 2004) and silver barb (Pongthana et al., 1999). In all of these species, sex should be included as a fixed effect in quantitative genetic analysis.

There were no significant interactions between effects for any growth-related traits found in this study. This demonstrated relatively small effect of dominance and epistasis on growth of the fish in the current study.

6.4.3. Heritability estimates

The estimates of heritability reported in the literature for separate family rearing of common carp are highly variable, ranging from less than 0.01 to 0.58 for weight and from 0.04 to 0.55 for length (Vandeputte, 2001). For other fish species, heritability ranged from 0.24 for females to 0.61 for males of mean body weight in tilapia (Velasco et al., 1995), from 0.41 to 0.60 for weight at harvest in Atlantic salmon

(Fjalestad et al., 1996), and from 0.35 for body weight and 0.53 for body length in rainbow trout (Henryon et al., 2002). In the present study, the lowest obtained heritabilities were 0.23 ± 0.08, 0.20 ± 0.07 and 0.23 ± 0.06 for weight, length and height at the final data collection, respectively. These estimates are in the same range as previous studies in common carp. Kocour et al. (2007) obtained high heritabilities of 0.70 for body weight and 0.69 for standard length in communal early rearing common carp at the third growing season (mean 1,549 g) using the molecular pedigree technique, while a heritability of 0.3 for both weight and length was found in juvenile stage (eight weeks) applying the same analysis method (Vandeputte et al., 2004). Similar estimates of heritability for growth rate were found by Nenashev (1966) (0.34-0.44) and Nagy et al. (1980) (0.48), even though their experimental designs could have produced upwardly biased estimates. Other studies based on separate family rearing showed lower heritability estimates, e.g. 0.11 for body weight of androgenetic common carp (Tanck et al., 2001), although the restricted feeding regime applied might have prevented potential genetic differences in individual growth rates from being fully expressed. In addition, the shock treatment in androgenesis might induce an increased amount of additional environmental variation in morphological traits due to embryonic damage. Wang et al. (2006) reported that heritability was from 0.14 to 0.3 for growth-related traits at final harvest of carp, however this was estimated based on sire component and it was suggested that other genetic effects, such as non-additive effects and dam effects, would be present in the experiment. The number of parents, families and family size in the present study were substantially higher compared to all previous studies. The medium heritability found in our study indicates substantial additive genetic variation for selection of growth-related traits. In general they are reliable because their associated standard errors were

relatively small. However, the estimated heritability was likely confounded with environmental effects since information of single generation(s) were used in the analyses.

Common environmental (full-sib family) effects (c^2) were all lower at tagging and slightly higher at the last measurement, ranging from 0.05 to 0.22. In general, estimation of c^2 in the early development stage of fish usually includes large common full-sib effect and reduces at communal rearing stage (Vandeputte et al., 2002). Therefore, full-sib family effects, as observed in the present study, most likely reflect only common environmental effects and non-additive (dominance) effects. The slight increase of c^2 within each generation and large different of c^2 between two generations were considered as being due to selection, environment and yearly condition as well as management method that have been mentioned and discussed in section 6.4.1. Furthermore, the possible explaination for the considerable full-sib family effects is that communally rearing the fish with high density and different tagged size in the same pond could alter fish behaviour resulting in differential competition common to full-sib families. The trend of full-sib family effect increased with advancing age may reflect an accumulation of the effect of competition between families and an increase of non-additive genetic effects as the fish grew larger. A similar observation was also made (increasing of c^2 due to declining maternal effect, thinning or culling family and environmental competition) in three different lines of rainbow trout (Su et al., 1996). Our observation of c^2 with REML analysis are similar to results in other species such as estimates in tilapia growth traits ranging from 0.09 (Gall and Bakar, 2002; Maluwa et al., 2006) to 0.21 (Rutten et al., 2005; Nguyen et al., 2007). In addition, a relatively low c^2 (0.06) was found for body weight in

rainbow trout (Kause et al., 2003). However, their results were only observed in one measurement and there was no monitoring for the change of c^2 with age.

The heritabilities were very high at the initial measurement (PIT tagging), however they declined with advancing age while common environmental variance increased. The larger common environment variance in later ages may contribute to higher total phenotypic variance so the proportion of additive genetic variance was reduced. In addition, our analyzed results showed a significant effect of sex on growth performance so the effect of sexual maturation on heritability estimates may be considered. The change of heritability due to sexual maturity was reported in rainbow trout, where it decreased before maturation and increased after spawning (Crandell and Gall, 1993). A reduction of heritability with advancing age was also obtained in common carp (Wang et al., 2006) and Atlantic salmon (Gjerde et al., 1994), however no reasons were suggested for this.

6.4.4. Genetic and phenotypic correlations

The estimated phenotypic and genetic correlations between body weight and length in this study were moderate to high and comparable to previous studies in common carp (Vandeputte et al., 2004; Wang et al., 2006; Kocour et al., 2007). Although the correlations between height and weight, and height and length were lower than between weight and length, these were still much higher than those reported by Wang et al. (2006). These estimates of genetic and phenotypic correlations for growth traits were all positive, and decreased in magnitude as the age of measurement increased. This contradicts findings of increasing correlations with age in other species due mainly to pleiotropy (Falconer and Mackay, 1996) so it is probable that the same genes control traits at different life stage, e.g. rainbow trout (Elvingson and

Johansson, 1993; Su et al., 2002), Atlantic salmon (Gjerde et al., 1994) and chinook salmon (Winkelman et al., 1991). In addition, Crandell and Gall (1993) reported that the estimates of genetic correlations between post-spawning weight of rainbow trout females and body weight at earlier ages were all positive, and increased in magnitude as the age of measurement approached to the age of sexual maturity. High genetic correlations indicate that the same genetic factors control both traits. The observed correlations reflect that selection for high body weight would result in greater correlated increase in length than in height in common carp. In addition, selection decisions may be taken based on the second measurement or even at PIT tagging.

6.4.5. Selection response

The current study found a substantial selection response for growth-related traits by mixed model estimates, in agreement with the results from communal rearing presented in Chapter 5. No genetic gain during five generations of mass selection for growth trait of carp was found by Moav and Wohlfarth (1976). An average improvement of 6% to 7% per generation has been obtained for most species but some other studies indicated average genetic gains of over 10% per generation by applying separate family rearing and selection in coho salmon (Hershberger et al., 1990) and tilapia (Gall and Bakar, 2002; Ponzoni et al., 2005). Vandeputte (2001) estimated an improvement of 20% in each generation by simple mass selection of the best 3%, assuming that the growth trait of carp had heritability of 0.3 and the phenotypic coefficient of variation was 30%.

The performance of the selected lines in a selective breeding programme tended to improve with advancing generations but a genuine control line did not change much in most studies, for instance, in coho salmon (Hershberger et al., 1990). In tilapia,

Gall and Bakar (2002) reported 20% phenotypic improvement of selected fish in each generation compared to the base population. In the present study, the response in each generation of selection, as the difference between the selection and control lines, ranged from 3.3% to 8.2% for growth-related traits at the final harvest. This suggested that selected lines were improved over generations.

In the present study, response to selection may still be biased since there are also other factors which may have had influence on body traits so the control stock should be maintained. In practice, the actual phenotypic changes corresponding to the expected response to selection are only achieved when the common environmental/full-sib family effects are identified (Gall et al., 1993). The common environmental/full-sib family effects at separate early rearing stage may be shown by subsequently increasing coefficient of variation for growth performance, likely due to dominant, at later measurements in the current study.

6.5. Conclusions

The heritability estimates for growth-related traits at different ages were moderate to high. The common environmental effects accounted for a larger proportion of total variation than under communal early rearing. The relatively high heritabilities for body weight, length and height at the final measurement indicate that genetic improvement of these traits could be successfully achieved using separate early rearing. Positive and high genetic and phenotypic correlations between traits suggest that growth performance of the fish could be improved by selection on any of the traits. The responses to selection that were achieved in this study indicate that the performance of common carp could be significantly improved by conventional selective breeding.

Chapter 7. General Discussion, Summary of Research Findings and Future Perspective

7.1. Introduction

The main purposes of breeding programmes for fish are to increase the profitability and sustainability of aquaculture. Mostly, these have been successfully achieved by using pedigree information to maximize effective population sizes and to use information from relatives to increase the accuracy of predicting breeding values for all traits included in the breeding objective. This method assumes that phenotypes are explained by a large number of genes with small effects and random environmental deviations. In selective breeding programmes using pedigree information, molecular markers have been used primarily for parentage assignment when tagging individual fish is difficult and to reduce common environmental effects from rearing families in separate hapas or tanks. However, the appropriate method depends on availability, estimates of genetic parameters and cost-effectiveness of the techniques.

There are various approaches to improve the rate of selection gain. Most involve maximizing the correlation between the desired improvement (selection objective: merit or profit) and the way fish are measured or ranked for selection (selection criterion: phenotype, or index of information from many relatives and traits). Each selection programme should analyze cost/benefit of the options, calculating total costs and cost per unit of genetic gain from different types of selection. For instance, if there is a single trait of primary economic importance with moderate heritability, mass selection can be as efficient as using information from relatives (Toro and Lopez-Fanjul, 1998). The benefit evaluation of a selection programme should consider

temporal, biological and technical constraints as well as genetic constraints. For remote and developing countries, the availability of genetic management and technical skills should also be taken into account, especially for complicated genetic programmes requiring extensive and accurate record and keeping (Tave, 1995).

Investment in a breeding programme can provide a high rate of economic return since genetic gain is cumulative, permanent and sustainable. Nearly all the genetic gain contributes to the national economy, especially in countries where a pyramid breeding structure is well established to disseminate improved genotypes from the nucleus either directly or indirectly to commercial production. Although genetic gain is never lost if the population is well maintained, its value needs to be discounted to express all returns and cost in terms of net present value (Hill, 1971). Ponzoni et al. (2007) evaluated investment in a genetic improvement programme in tilapia and reported that the economic benefit ranged from 4 to 32 million US$, and the corresponding benefit to cost ratio was 8.5 to 60. The substantial returns clearly indicated that it is beneficial to invest in breeding programmes.

So, assessment of selection methodology in a selective breeding programme is a complex process that requires quantitative genetic prediction and economic analysis, mainly focusing on three aspects: (1) the returns are realized because this determines the value of a unit of improvement and the genetic parameters to be applied; (2) the technology is applied because this determines the rate of gain and the flow of genes to the sector in which the return is gained and the direct costs of implementing the technology; (3) the source of returns includes the estimation of genetic value and the accuracy of the estimated genetic value. These will be integrated in this section.

7.2. General discussion on efficiency of separate early rearing (SER) and communal early rearing (CER) in the selective breeding programme

7.2.1. The methods of rearing for selective breeding programme

The efficiency of family-based selection relies on the fact that the environmental deviations of the individuals tend to cancel each other out in the mean value of the family. This selection method has more advantages than other types of selection when environmental deviations constitute a large part of the phenotypic variance. It is therefore necessary to reduce the common environmental component to a minimum by standardization of the environment for all families as far as possible (Gjedrem, 2005). Thus, individuals from all families should be tagged as early as possible before communal rearing together in the same tank, pond or cage. There are a great number of different physical tagging methods for fish and shellfish, including metal and plastic tags with numercial and/or other information that are attached to fins, jaw, tail or gill cover by wire or string. The most promising type of tag is electronic tags (PIT tag), which may be inserted into the body cavity or muscle. In reality, there are no completely satisfactory physical tags available for fish and shellfish because it can not meet five requirements for satisfying marking methods as described by Refstie and Aulstad (1975): (1) the method should be applicable for small animals; (2) the tag should not influence the growth rate of the animal; (3) the method should not be expensive; (4) the method of tagging should require little labour; and (5) the mark should be readable past the time for recording. Therefore, family-based selection based on physical tagging has to accept a minimum period of separate rearing before tagging, which is likely to introduce some common environmental effects.

In the communal early rearing method, when communally reared fish are big enough for tagging using PIT tags, they are traced to ascertain their parents and relatives by using highly variable molecular markers. Parents and their progeny were genotyped so the parentage of each communally reared offspring can be identified. The success of parentage assignment using molecular markers allows communal rearing of all families from the very early larval stage. This simplifies key steps in the selective breeding programme since the early communal rearing of all family can alleviate confounding effects caused by environmental factors on phenotypic and genetic parameters.

7.2.2. Parentage analysis

The molecular markers were used to establish the pedigree of fish communally reared from the early larvae stage. Seven highly polymorphic microsatellite loci were used for traceability analysis of two generations of common carp in the selective breeding programme. The mean of effective number of alleles at the seven loci used for parentage analysis in the G_1 and G_2 generations were 9.26 and 9.03 respectively, which assigned parentage (allowing for up to two mismatches) of 96.8% of the G_1 generation and 96.2% of the G_2 generation. In an other study, 95% successful assignment of 550 offspring to a single parental pair from a complete factorial cross of 24 sires and 10 dams was reported using 8 microsatellite loci with a mean of effective number of alleles of 7.75 in selective breeding programme of common carp (Vandeputte et al., 2004). Typing errors (mainly due to technical causes), heterozygotes for adjacent alleles and null alleles in the present study appeared to be much lower than found in Atlantic cod (Herlin et al., 2007) and Atlantic salmon

(O'Reilly et al., 1998) because more markers were available for common carp and these used were selected from the best markers of previous studies.

The estimation of effective population size (N_e) based on molecular assignment of offspring to families is useful information to conduct effective breeding programme. The variable full-sib family size, number of family and unequaly sex ratios had major impacts on the effective population size (N_e) that reduced the N_e to less than the census population size (N). Actually, the produced breeders were over than estimated and inbreeding rates were less than 1% in each generation.

7.2.3. Phenotypic variation

There were large differences in size of fish between CER and SER (Figures 7.1 and 7.2) although stocking density of those methods were equalized at all cultured stages from nursing to grow-out. The other management techniques were very similar between CER and SER. In addition, progeny in each generation were derived from the same families (same parents). However, the CER fish were three times heavier than the SER in the two selection generations at any of the three measurements. Otherwise, the length of CER fish was almost doubled that of the SER fish in the first two measurements and one half higher at the final measurement. These differences may be due to the habitat and feeding habits of the common carp as a bottom feeder, hapas are not favourable for nursing in the conditions implemented in SER. It also shows that separate full-sib family rearing was seriously effected by the environment. Therefore, one of the most obvious advantages in CER was to save one year interval for each selection generation, since the fish can be mature and ready for spawning after one year old, which could not be obtained under SER.

The reference population was only significantly higher than the control and selection lines for traits at first measurement in SER. There was a significant different between the reference and control lines at first and second measurements in CER. Thus the full-sib family rearing might cause differences at early stages in SER. Perhaps, the observed higher mortality of reference families caused lower density in the period of separate family rearing so they may grow better before tagging in SER. However, there were a very limited number of reference families and progeny compared with the selection and control lines.

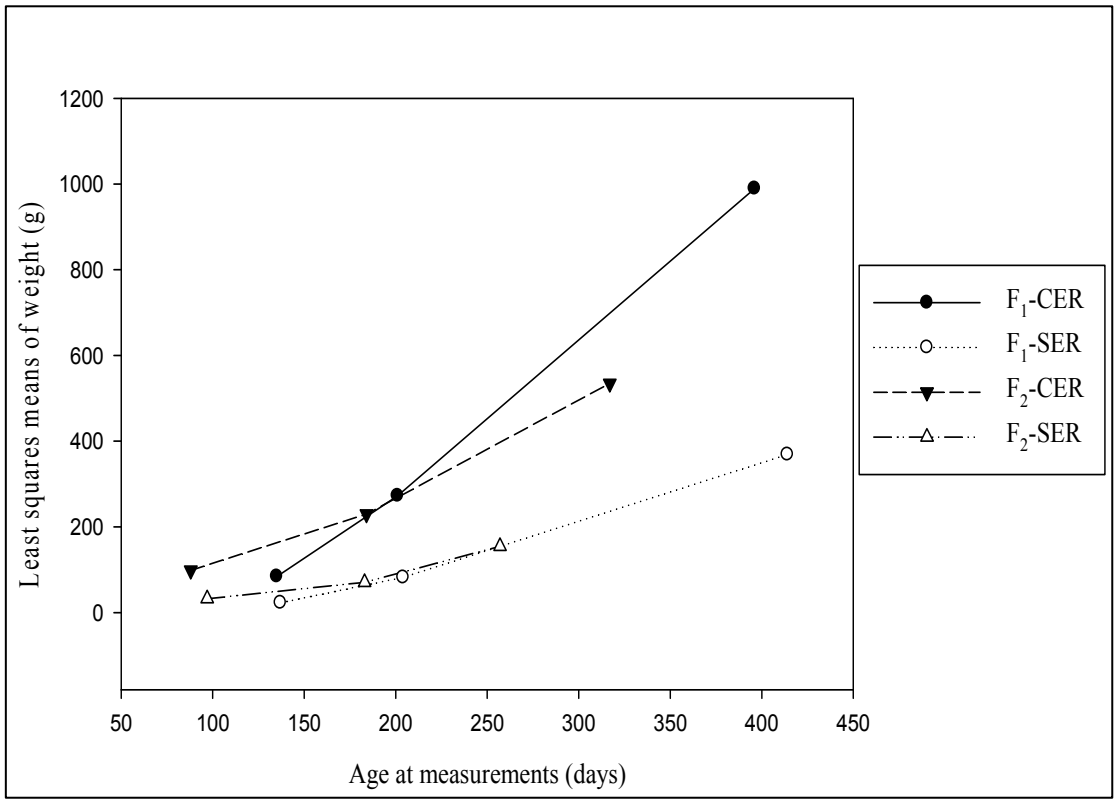

Figure 7.1. Least squares means of weight at different measurements of selection population in each generation (G_1 and G_2) and rearing method (Communal early rearing: CER, Separate early rearing: SER).

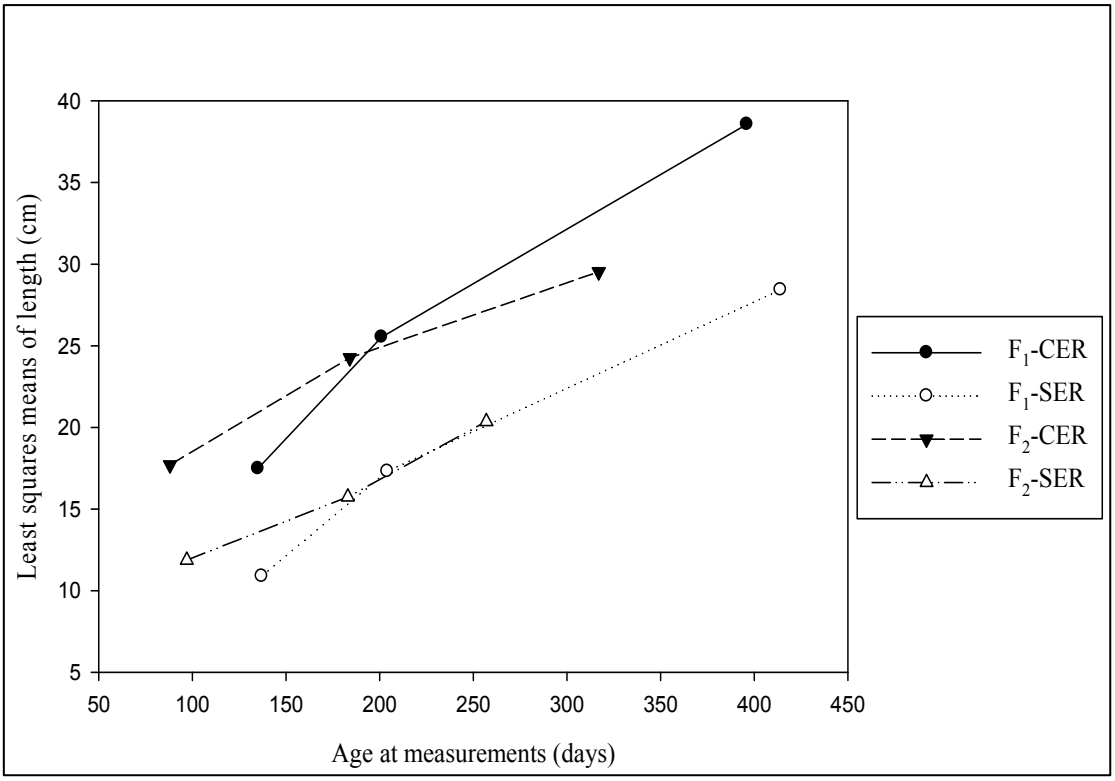

Figure 7.2. Least squares means of length at different measurements in each generation (G_1 and G_2) and rearing method (Communal early rearing: CER, Separate early rearing: SER).

7.2.4. Genetic parameters

- Common environmental effects (c^2)

One of the main constraints facing breeding programmes for fish is that at hatching fish are too small to be tagged individually. Application of the animal model approach ideally requires tagging a constant number of individuals from each family with passive integrated transponders (PIT tags) when they become sufficiently large after a period of individual family rearing. However, this system of early management creates high common environmental effects for full-sib families, e.g. 0.21 in tilapia (Rutten et al., 2005; Nguyen et al., 2007). The common environmental effects ranged from 0.05 to 0.22 for common carp in our study on separate early rearing. However, it

is worth noting that estimated additive genetic variance might be confounded with common environment effects due to separate family rearing. To overcome this problem, mixtures of equal numbers of hatched progeny from different families can be reared communally to reduce the environmental effects to almost zero. Common environmental effects were considered to be zero in other studies which applied communal early rearing in common carp (Vandeputte et al., 2004, 2008; Kocour et al., 2007).

- Genetic heritability (h^2)

Both CER and SER rearing methods gave relatively moderate heritabilities, in agreement with previous studies in common carp (Vandeputte et al., 2004, 2008; Wang et al., 2006; Kocour et al., 2007) even though number of parents, families and family size were higher in the present study compared to these others. The heritabilities in CER were generally not higher than in SER for the studied traits (Figures 7.3 and 7.4). This may be due to the analysis of SER where the genetic parameters estimated could be confounded with common environment effects. The heritabilities of growth-related traits are reasonable high at the first measurement and decrease to moderate at the last measurement. The decline in heritability may be an uncorrectable effect related with sexual maturity (e.g. Crandell and Gall, 1993).

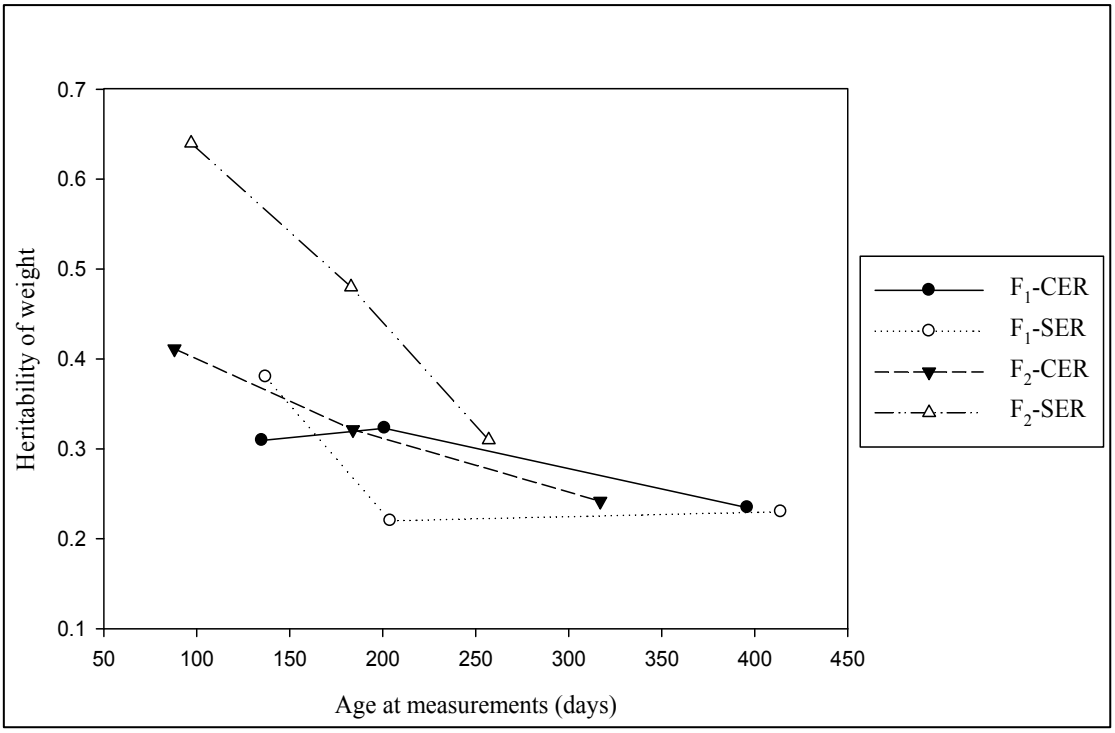

Figure 7.3. Heritability estimates of weight at different measurements in each generation (G_1 and G_2) and rearing method (Communal early rearing: CER, Separate early rearing: SER).

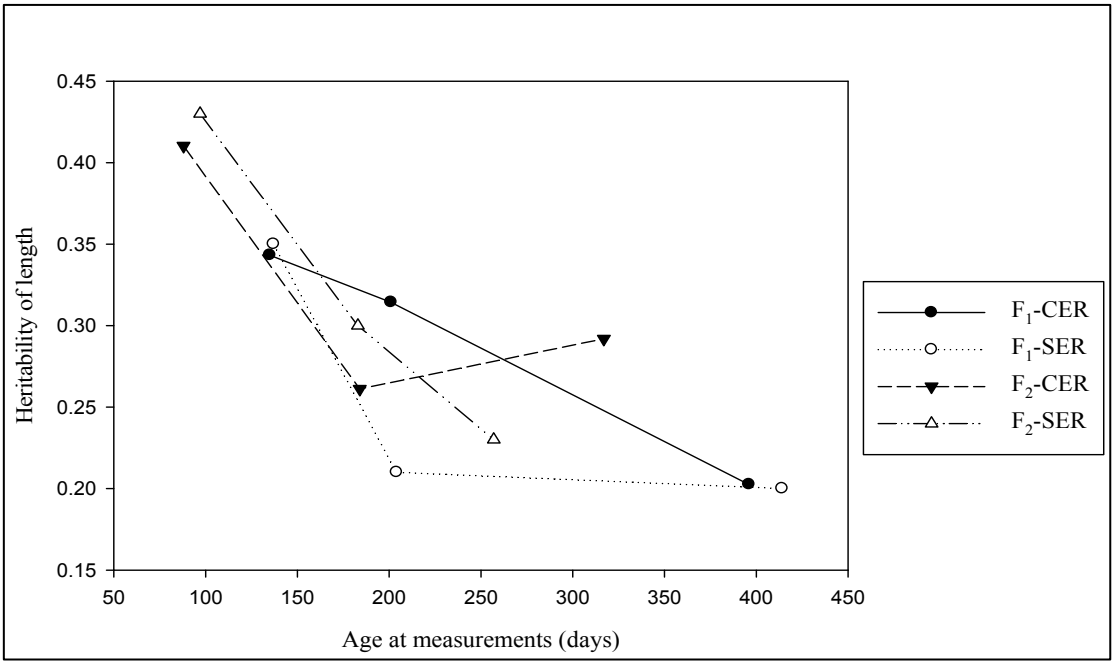

Figure 7.4. Heritability estimates of length at different measurements in each generation (G_1 and G_2) and rearing method (Communal early rearing: CER, Separate early rearing: SER).

7.2.5. Responses to selection

In the current study, seven microsatellite markers were used to identify a high proportion of progeny. Furthermore, estimated heritabilities were all fairly high for growth-related traits in CER and SER selection methods. The second selection generation had higher selection response than that in the first selection generation. The selection responses under CER were almost double those under SER for weight and length (Figure 7.5 and 7.6). While response to selection tended to decrease from the first measurement to final harvest in CER it increased in magnitude with age increase in SER. The selection response may be related to size or stage of development and final harvest size of fish in CER, which was triple that of SER. However, the response to selection in SER could also be reduced due to introducing some 'noise' to family means that caused lower accuracy in the selection estimates.

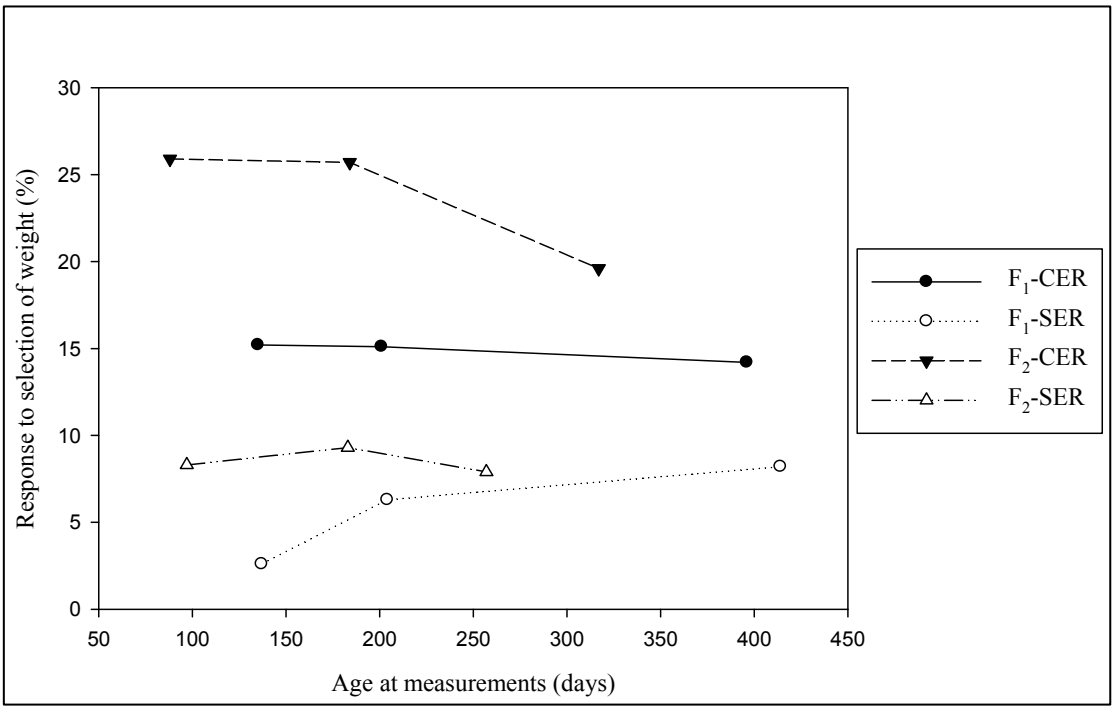

Figure 7.5. Response to selection of weight at different measurements in each generation (G_1 and G_2) and rearing method (Communal early rearing: CER, Separate early rearing: SER).

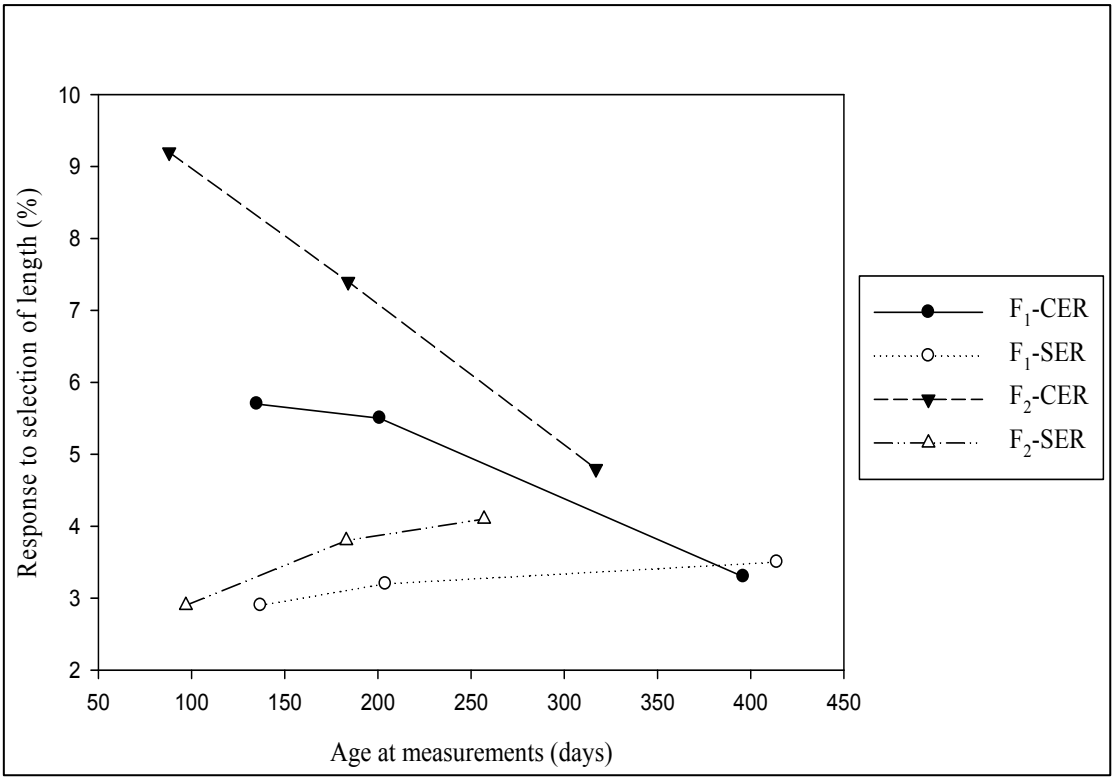

Figure 7.6. Response to selection of length at different measurements in each generation (G_1 and G_2) and rearing method (Communal early rearing: CER, Separate early rearing: SER).

No published research could be found concerning comparison of response to selection between CER and SER in fish and shellfish. The only research to refer to is in livestock, by Dodd et al. (2005), who ran a simulation to evaluate selection response of livestock and found that the use of DNA marker based parentage assignment for genetic evaluation was associated with lower selection response than by using the traditional pedigree recording. This decrease in response was caused by number of factors, included trait heritability and the number of DNA markers used (efficiency of parentage assignment).

7.2.6. Benefit of the breeding programme (further details in the Appendix)

The economic benefit (EB) and benefit to cost ratio (BCR) under different biological (heritability values, response to selection, accounting for feed intake), economic (initial investment, annual cost, discount rate, price of fish) and operational (year of first return, reproductive efficiency) parameters were calculated based on parameters estimated from the selective breeding programme of common carp at the Research Institute for Aquaculture No.1, Vietnam. The parameters were written in models and SAS software was used for all the calculations. A paper resulting from this is included as an Appendix (Ponzoni et al., 2008). This section discusses the study in relation to the rest of the thesis.

7.2.6.1. Costs and benefits evaluation of CER and SER

The full cost of any method is very difficult to measure, and also may not translate well among institutions. In the current study, all activities and expenditure were carried out in Vietnam except genotyping so all costs were calculated based on local prices and transferred to US dollars (Table 7.1). The following estimations relied on the CARP-II ADB funded project carried out at the Research Institute for Aquaculture No.1 during the period from year 2005 to 2008. The overall cost for one generation of selection in SER was almost 14.4% higher than in CER. In SER, feed and labour were the main expenditure that consumed 29.2% and 27.8% of total cost respectively. The labour and feed used were calculated from two years of management including hapas, nursing and communal rearing of tagged fish until selection at harvest size. It is also noticeable that the calculation is based on the same number of experimental fish in

SER and CER, thus the PIT tag cost using in CER and SER are the same. The current study estimated that the cost of consumables for genotyping (primers, plastic tubes and plates, reagents) including failed assays for the work presented in this study was US$ 5.6 per sample for the two multiplexes. Since two multiplex PCRs for a total of seven microsatellite markers were efficiently assignment and DNA extraction was a fixed one-time cost, each sample required two genotyping runs. Therefore, the total cost of genotyping (technician and material costs) 1362 progeny to 136 parents in one generation was US$ 8,389, that contributed 55.6% of the total cost in CER. Our expenditure for genotyping is lower than some other studies since we did multiplex PCRs of three and four loci and perhaps had lower labour costs. Withler et al. (2007) reported that US$ 12 per sample for microsatellite DNA assignment of 2911 progeny to parents enabled communal rearing in Atlantic salmon selective breeding programme. Vandeputte et al. (2008) reported that cost lies in fish number, not number of families and estimated genotyping costs ranged from 7.5 euros to 15 euros per sample. Hayes et al. (2007) studied the optimization of marker assisted selection for abalone breeding programme and concluded that the cost of genotyping was US$10 per animal. Using rapid DNA extraction methods and multiplex PCR techniques brought the costs of generating genotypes to approximately £ 5 per sample in gilthead seabream (Brown, 2003).

Table 7.1. Estimated costs of SER and CER methods for one selection generation in the selective breeding programme.

Items	Cost of selection methods (US$)	
	SER	CER
Operational expenditure		
Labour (technical and field workers)	8,000	2,000
Technician for genotyping	-	4,667
Energy (fuel and electricity)	3,200	560.0
Feed	7,640	2,765
Others (transportation, hapas, seining net…)	5,000	1,500
PIT tags and detector	3,600	3,600
Chemicals for genotyping (DNA extraction is a fixed one-time cost, PCR, fragment analysis)	-	8,389
Subtotal	27,440	23,481

Dodds et al. (2005) evaluated genetic selection using parentage information from genetic markers and reported that when growth rate was the trait simulated, the advantage of marker assisted section over non-marker assisted selection increased as more progeny per family were genotyped. This was true regardless of age at selection. Two generations of selection on early growth rate resulted in greater genetic gains than one generation of selection on growth rate when the number of progeny per family was more than five such in our case. However, the validity depends on the heritabilities of growth rate and early growth rate, and the genetic correlation between traits.

7.2.6.2. Economic parameters for the selective breeding programme

Among the economic parameters studied (initial investment, annual running cost, discount rate, fish and feed prices), the price of fish and feed costs had large effects on economic benefit (EB) and benefit to cost ratio (BCR). The current study shows that in order to capture full economic benefit from genetic improvement programmes, planners and policy makers should develop synergistic strategies to market aquaculture products. As production increases, the price of fish may go down. Thus in order to remain competitive, fish farmers and producers need to increase efficiency of production through adopting genetically improved stocks along with improved nutrition and management practices. Feed often accounts for 60 to 70% of the total production costs. EB and BCR from the breeding programme were highly sensitive to feed costs. So in order to sustain aquaculture and to increase profit of fish farmers, research in the area of nutrition should focus on the development of balanced low cost diets through efficient utilization of local feedstuff resources.

7.2.6.3. Operational factors

In common carp, induced breeding has become a common spawning practice in hatcheries to produce fry to supply farmers. This system was considered as the standard procedure. In general, the techniques are relatively simple and the cost of setting up an incubator system is low. At present, the adoption rate is approximately 10% of the total national population of common carp, but the proportion of improved fish used by the industry is expected to increase in coming years since the culture area for common carp is expanding. In addition, local producers are interested in the improved carp of RIA 1 because of their superiority over available strains under a wide range of on farm testing environments, with respect to growth rate, survival and yield per unit area. The current study shows that EB and BCR increased linearly with the adoption rate, indicating that in order to fully capture the economic benefit from

genetic improvement programmes, the dissemination of the improved fish to commercial production should be carried out in a systematic manner to ensure that high quality of seed reaches farmers and producers. Ponzoni (2006) and Nguyen and Ponzoni (2006) discuss strategies for effective dissemination of improved fish strains. Despite using the lower limit of improved fish contributing only 10% to the current total national production, EB and BCR ranged from 11 to 226 million and 22 to 420 million US$, respectively. Both EB and BCR would increase by a factor of 10 if the production sector cultured 100% of improved fish from the breeding programme in the country (606 million fish marketed annually).

7.2.6.4. Chance of success

The selection programme in common carp in RIA 1 has been carried out under a standard pond environment. Most likely, there will be a little loss in genetic gain in other prevailing environments, at least for growth performance. The estimates of genetic correlations between expressions of body traits in a range of environments reported in the literature are close to unity (ranging from 0.70 to 0.99) across a number of species such as rainbow trout (Sylven et al., 1991), tilapia (Ponzoni et al., 2005), rainbow trout (Fishback et al., 2002; Kause et al., 2003), white shrimp (Gitterle et al., 2005) and pacific oysters (Swan et al., 2007). In order to minimize G×E effects in breeding schemes, a number of strategies can be applied. First, G×E effects can be reduced through the choice of a selection environment that is as close as possible, or identical to, practical production. Second, the measurement of traits should be standardized to avoid G×E as a consequence of differences in trait definition. Third, breeding schemes could record performance of relatives in the production environment, and a combined genetic evaluation of the data recorded in both environments may alleviate G×E effects, thus reducing the loss in genetic gain (Mulder and Bijma, 2005).

7.3. Summary of research findings and concluding remarks

The current study results suggested that the genetic improvement programme of common carp at RIA 1, Vietnam can be conducted efficiently by both methods of communal early rearing (CER) and separate early rearing (SER). The microsatellite marker-based family assignment can be used to improve the accuracy of estimation of heritability and relative performance of different families during selection. The early communal rearing, even at hatching stage, does not need intensive labour for management or huge facilities for separate family rearing such as tanks and hapas. It also reduces common environmental effects including environmental and maternal effects in the selection programme. More specifically, there are some concluding remarks:

- The present investigation demonstrated that the seven microsatellite loci used showed high polymorphism and satisfactory parentage assignment in the studied population of common carp. These molecular markers were used to establish the pedigree of fish communally reared from the early larvae stage.

- The synthetic common carp base population showed relatively moderate heritability estimates for harvest weight that are in the range in other reports for common carp. The high estimates of heritability are likely to be due to additive genetic effects and also suggest that rapid gains could be achieved through selective breeding for growth rate in common carp.

- This study demonstrated the application and effectiveness of molecular parentage assignment as a tool in genetic selection in common carp. The estimates of heritability for body traits were moderate to high. It is suggested

that direct selection on body weight is the better selection criterion compare to length and height in order to improve growth performance of common carp.

- The present investigation revealed that the common environmental effects accounted for a larger proportion of total variation in separate early rearing selection method. The high heritabilities for body weight and height at the final measurement indicate that genetic improvement of these traits could be successfully achieved. The responses to selection were achieved in this study indicating that performance of common carp is significantly improved by conventional selective breeding.

7.4. Future perspectives

Economic benefit (EB) and benefit to cost ratio (BCR) decreased with the magnitude of the G×E (i.e. with the decrease in the genetic correlations between homologous traits in the selection and production environments). Furthermore, both EB and BCR from the genetic improvement programme depend on other factors, which can be categorized in three groups: i) biological (heritability and feed intake), ii) economic (initial investment, annual recurrent cost, discount rate, price of fish and feed cost) and iii) operational (year when first return is realized, adoption rates of the improved fish by the production sector). The level of heritability affected EB and BCR, with greater heritability being associated with greater EB and BCR. Accounting for feed intake in breeding objectives avoided an overestimation of EB and BCR. Generally, the economic efficiency of the breeding programme was almost insensitive to initial investment and annual cost. Increasing the discount rate by three times reduced EB and BCR by a factor of only 1.4 and 2.0, respectively. The price of fish and feed costs had a substantial effect on EB and BCR. However, the greatest contribution to

variations in EB and BCR came from increases in adoption rates of the improved fish by the industry. The risk programme failure due to technical reasons was extremely low. The present study recommends that even under the most conservative assumptions, and in the presence of G×E interaction, genetic improvement programmes are highly beneficial from an economic viewpoint. Thus it is suggested that the present selective breeding programme should be continued.

The economic benefits from a genetic improvement programme in carps are substantial, indicating that it is worth while investing in such activities from a national perspective. Furthermore, expanding to other farmed aquaculture species of economic importance would be justified. The efficiency of the programme, however, depends on several factors. Of particular importance are reproduction rate of female breeders and adoption rate by the production sector, which determine the number of fish of the improved strain that reach the production systems and are later available for sale. For carp species, improvement in reproduction rate can be easily implemented by taking advantage of induced breeding together with artificial incubation in both the nucleus and hatcheries. Dissemination of the improved fish is a key component in fully capturing all economic benefits from genetic improvement. The high sensitivity of the economic benefits to biological parameters (heritability and feed intake) and to genotype by environment interaction due to re-ranking effects also suggest that the design of breeding programmes should aim to minimize systematic effects, choosing appropriate testing environments.

At research and commercial facilities, common carp are grown in hapas, indoor tanks and inland ponds. Selective breeding programme in separate early rearing (SER) requires that families are stocked in hapas or tanks in early stages when small fish can

not be marked by physical tags. However, most commercial production of common carp is from ponds where fish are grown soon after hatch and is close to the communal early rearing (CER) method (using molecular genetic marker for parentage assignment).

Application of CER method using genetic marker for selective breeding of common carp can optimize accuracy of selection, increase response and shorten generation of selection. Optimization deals with the high costs of genotyping to obtain pedigree information, which have been solved by application of multiplex polymerase chain reaction (PCR) for three and four markers.

The Department of Genetics and Selection at RIA 1 is funded by the National Biotechnologies Programme which supports modern facilities and funds for molecular genetics studies. Therefore, this study proposed using molecular genetic techniques for further improving the on-going breeding programme in common carp in Vietnam. Application of microsatellite markers for communal early rearing approach can reduce the impact of common environmental effects and give evidence of adequate additive genetic variation as well as its benefit to enable selective breeding programme in common carp with a reduced generation time.

Growth rate is currently targeted as the major quantitative trait in the selective breeding programme of common carp. The primary objectives aim at faster growth, larger size at harvest and shorter culture period because growth rate is the trait of highest economic value to farm production. In the future, the common carp culture may be developed more intensively and be required to produce new products by the market so other health and carcass traits such as disease resistant, food conversion

efficiency and fillet yield may become relatively more economically important. Such traits can then be considered to be included in the breeding programme. The health and carcass traits are difficult to select because phenotypic data are recorded from relatives but not from candidates for selection. Thus information from sibs or pedigree is very important to predict the breeding value.

In family-base selection, CER was demonstrated to be more efficient for the present selective breeding programme, where molecular markers were used to maintain pedigree information. Furthermore, the physical tagging (SER) also proved that it could be beneficial both in economic and biological parameters to achieve acceptable rates of genetic gain and minimize rates of inbreeding. Therefore, SER could be implemented in any generation if required e.g. for evaluation of other traits (produced from CER), and the future breeding programme could switch back from CER to SER at any time if it is required for efficient selection of other traits such as larger/more constant number of fish are required per family for testing.

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Appendix

Publication:

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