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SOCIAL BEHAVIOUR AND GROWTH RATE VARIATION IN CULTIVATED TILAPIA (*OREOCHROMIS NILOTICUS*)

By

SUDHINDRA R. GADAGKAR

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

at

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Halifax, Nova Scotia
September, 1997

DALHOUSIE UNIVERSITY

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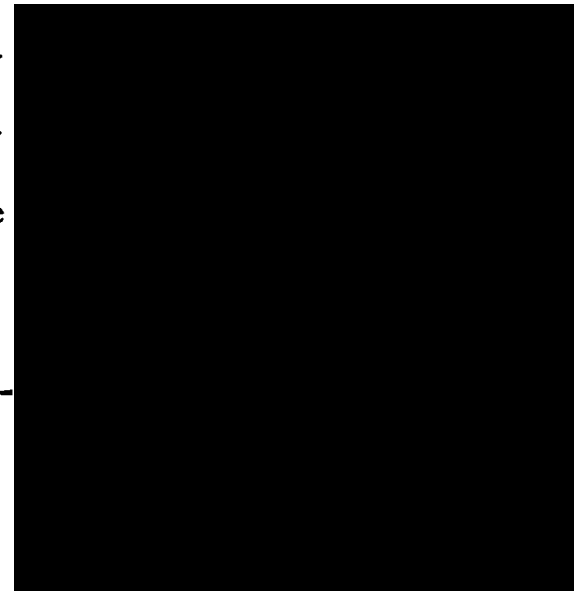

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DEDICATION

This Ph.D. thesis is dedicated to the memory of my father,

Shri. Ramarao K. Gadagkar.

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ABSTRACT

This study was undertaken to understand the behavioural causes of growth variation in cultivated fish and to study the genetics of agonistic behaviour *vis-a-vis* growth rate. The fish studied was a laboratory population of the Nile tilapia, *Oreochromis niloticus*, being maintained at the Marine Gene Probe Laboratory, Dalhousie University. Ten maternal half-sib families were produced by fertilizing the eggs of each female with the sperm from four different males. The fish within each half-sib family were all pooled together for the behaviour as well as growth experiments, in order to expose each fish to the full range of microenvironments within the half-sib family, and to eliminate replicate variance. Upon termination of the experiments, the male parent of each fish was determined using DNA fingerprinting using microsatellites.

The behavioural observations were made by randomly pairing fish from within each half-sib pool, soon after swim-up, and counting the number of aggressive and submissive behaviours displayed by each member of the pair. Two derived variables (net aggression, *viz.* aggression minus submission; and total agonistic activity, *viz.* aggression plus submission) were also constructed for each fish.

Growth experiments were conducted by rearing fish from the same 10 half-sib groups in each of two types of competitive environments, high interaction (HI), and low interaction (LI). Measurements included length, weight, and maturity status. Estimation of variance components and heritabilities was done by ANOVA as well as by DFREML (which can utilize pedigree information that was provided). Genetic correlations were calculated by correlating family mean values and by correlating breeding values.

The sire component estimates from ANOVA gave a low value of heritability for aggression, and moderate values, ranging from 0.240 to 0.391, for submission, net aggression and total agonistic activity. The DFREML estimates were very low for aggression and submission, but 0.131 and 0.258, respectively, for total agonistic activity and net aggression.

Sire component heritabilities from ANOVA as well as DFREML estimates gave high to very high values for all the growth traits from the HI environment (0.843 to >1.0, and 0.652 to 0.962, respectively, by the two methods) while the estimates from the LI environment ranged from 0.218 to 0.345 (ANOVA sire component) and 0.290 to 0.544 (DFREML). The extremely high values from the HI environment is attributed to the effect of the higher level of genotype-by-social microenvironment interaction in the HI environment.

Net aggression and winner/loser status were found to have significant positive correlations with subsequent growth, in both competitive environments, thus making these behaviours good predictors of growth. A path analysis revealed a strong dependence of growth on net aggression. It also revealed that aggressive behaviour was associated with poor growth - more so in the LI environment than HI. Submission, on the other hand, was associated with good growth more in the LI environment than in the HI environment. Finally, it is speculated that net aggression represents a behaviour that is under inadvertent selection during domestication.

LIST OF ABBREVIATIONS AND SYMBOLS

AGON	Total Agonistic Activity (AGR + SUB)
AGR	number of aggressive behaviours shown by fish in 5 m
ANOVA	Analysis Of Variance
ATP	Adenosine Tri-Phosphate
bp	base pairs
BRL	Bethesda Research Laboratories
COR-AGR	"Corrected" AGR score
COR-NET	"Corrected" NET score
DFREML	Derivative Free Restricted Maximum Likelihood
DIS	Extent of external damage caused by a disease
EDTA	Ethylenediaminetetraacetic acid disodium salt
FRP	Fibre Reinforced Plastic
ftp	file transferpProtocol
g	grams
h	hours
HI	High Interaction (environment)
K / RCF	Relative Condition Factor
l	litre
LI	Low Interaction (environment)
m	minutes
M	Molar (Normality)
MGPL	Marine Gene Probe Laboratory
ML	Maximum Likelihood
MME	Mixed Model Equations
MSS	Mean Sum of Squares
MST	Maturity Status
MTDFREML	Multiple Trait Restricted Maximum Likelihood

NET	Net Aggression (AGR minus SUB)
ng	nanograms
NIFI	National Inland Fisheries Institute, Thailand
PC	Personal Computer (IBM)
PCR	Polymerase Chain Reaction
PIT	Passive Induced Transponder
PNK	Polynucleotide Kinase
REML	Restricted Maximum Likelihood
s	seconds
SDS	Sodium Deudecyl Sulphate
SL / SLT	Standard Length (cm)
SSC	Salt Sodium Citrate
SUB	number of submissive behaviours shown by fish in in 5 m
TE	Tris EDTA
TL / TLT	Total Length (cm)
V	Volts
W/L	Winner / Loser status
WT / WGT	Weight (g)
YT	Yeast extract Tryptone

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I would like to place on record my deep sense of appreciation of my thesis supervisor, Dr. Roger W. Doyle, whose unfailing support and encouragement undoubtedly led to my completing this work. I am equally grateful to the International Development Research Centre (IDRC) of Canada, that has unstintingly provided me with financial support for most of the time it took me to complete my studies. Further financial support was provided by the Patrick Lett Fund, of the Department of Biology, Dalhousie University, for which I am very grateful. Part-time employment provided by Dr. Roger Doyle and Dr. Jim Wright is also gratefully acknowledged.

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Chapter 1

General Introduction

1.1 INTRODUCTION

Ever since the farming of food fish began, probably well before 500 BC (Pillay, 1990), the emphasis must have been to produce sufficient quantities at harvest. This is generally easily achieved when the harvest is meant to cater only to the homestead, as it still is in many parts of the third world. In eastern India, for instance, the culture pond is generally a multi-purpose backyard pond from which a fish or two are caught periodically, for home consumption (pers. obs.). It is easy to visualize a situation where, if the fish caught on any given day is sufficiently large for that day's meal supplement, it gets eaten; if not it goes back into the pond and another is fished out. Given this kind of subsistence farming, as long as one or two fish of fair size are found every time, the farmer does not have to be overly concerned about the size or growth rate of the other fish in the pond.

However, when the economic nature of the farm changes from subsistence to commerce, two important correlated changes occur, both of which concern the formerly neglected aspect of the growth rate or size of all the individuals in the culture pond. First, in order to make the effort of harvesting and marketing economically worthwhile, the farmer needs to harvest *all* the fish in the pond, on a predictable schedule. Next, since he plans to sell the fish, he needs to satisfy a third party, the buyer, towards which end the harvest (*i.e.*, all the fish) must look presentable and appealing. A harvest of fish, in order to be marketable must firstly, obviously consist of healthy individuals. Apart from good health, however, what adds good market value is uniformity of size of the harvested individuals. It makes good economic sense to be able to obtain a harvest of easily marketable, "table-size" fish, rather than a bunch of dissimilar sizes, a few perhaps very

large individuals (which sometimes could well be difficult to market, particularly when the market preference is for whole fish), and a vast majority of undersized fish (which would not fetch a good price anyway). In the polyculture of forage and piscivorous species a population is said to be *in balance* when the range in weights of the forage and piscivorous groups is narrow (Jhingran, 1991). This concept of "balance" in an aquaculture pond, while used by Jhingran (*loc. cit.*) in the context of culturing forage and carnivorous fish in the same water body, can be extended to any aquaculture situation since it involves efficient utilization (as far as the culturist is concerned) of the existing resources by the fish.

As long as the culture pond is not over-stocked, the fish are well-fed and are free of disease, the goal of obtaining healthy fish is usually achieved. The package-of-practices generally available for most cultivable species (*e.g.*, Jhingran, 1985) ensures this. However, what the package-of-practices does not presently assure is uniformity of growth. Lack of uniformity in growth rate among fish is because individuals in groups tend to grow differently rather than similarly (*e.g.*, Jobling and Baardvik, 1994).

1.2 GROWTH DEPENSATION

This differential growth rate among the individuals in a water body leads to a phenomenon termed *growth depensation* (Magnuson, 1962), a term used to denote the increase in the dispersion of sizes of the fish over time due to differences in growth rates. Individuals in a pond that have but minute size differences when young and small, exhibit a magnification of these differences as they grow, resulting in a whole range of sizes at

harvest. Though particularly well known in captive fish populations (Magnuson, 1962; Jobling and Wansvik, 1983; Koebele, 1985; Davis and Olla, 1987; Jobling and Baardvik, 1994), growth depensation is also known to occur in shrimp (Ra'anan and Cohen, 1984; Karplus *et al.*, 1989, 1991, 1992), amphibia (Rose, 1959), and even birds (Nelson, 1978).

In fish it is also seen that not only is there much variation in size among individuals, the size distributions are also typically skewed positively towards the larger fish (Nakamura and Kasahara, 1955; 1956; 1957; 1961; - all in Wohlfarth, 1977; Wohlfarth and Moav, 1972; Koebele, 1985; McCarthy *et al.*, 1992), that is, a few individuals grow to be large while the vast majority remain small. The disparity in size keeps getting progressively greater with the large fish becoming larger by growing at much faster rates when compared to the smaller individuals. This lack of uniformity and asymmetry in growth rate has considerable significance in commercial food-fish aquaculture where it is profitable to produce uniform sized fish (Jobling and Baardvik, 1994).

The phenomenon of growth depensation has provoked much scientific investigation and there is a growing body of literature devoted to understanding it (Brown, 1946, 1951, 1957; Magnuson, 1962; Allen, 1972; Koebele, 1985; Baardvik and Jobling, 1990; Huntingford *et al.*, 1990; Kamstra, 1993; Jobling and Baarvik, 1994, Volpato and Fernandes, 1994; Kadri *et al.*, 1996). Most studies have shown that much of the observed variation and skewness, while still a manifestation of the characteristics of the fish, can be altered quantitatively by manipulating the culture system. Such a change, however, is effected from without and is obviously transient in its effect - operational only as long as

the particular set of alterations in the environment is in effect. Further, since commercial aquaculture is of relatively recent origin, package-of-practices are constantly subject to change based on the results of ongoing scientific research. This can potentially necessitate efforts to be started afresh under the changed circumstances (every time there is such a change), in order to reduce variation and skewness in size distribution.

1.3 A SOLUTION FROM "WITHIN"

Traits that exhibit sufficient additive genetic variation are amenable for change by a process of domestication selection. Domestication selection involves artificial selection for traits more suited to the domestic environment provided by man. The process of domestication has brought about the enormous changes seen in domestic breeds of animals and plants when compared to their wild counterparts. It has been suggested that behavioural traits are among the first traits to be affected by the process of domestication (Mayr, 1963; Kohane and Parsons, 1988), usually effected by changes in the thresholds for those behaviours (*e.g.*, Price, 1978; Barnett *et al.*, 1979).

The question of what happens to agonistic behaviours, or thresholds thereof, during domestication of fish (particularly selection for fast growth), appears to be a contentious issue in the literature (*e.g.*, Moyle, 1969; Swain and Riddell, 1990; and Mesa, 1991, reporting an increase in aggression in their respective fish species; and Holm and Fernö, 1986; Doyle and Talbot, 1986; Robinson and Doyle, 1990; and Ruzzante and Doyle, 1991, 1993, presenting theoretical reasons and experimental evidence to the contrary). In any case there appears to be evidence of a correlated response in agonistic

behaviours to selection for fast growth. It then becomes important to assess the direction and magnitude of such genetic correlations for different fish species under different culture environments, in order to evaluate the potential for successful genetic selection for fast growth. This becomes especially important in view of the concern expressed that higher levels of aggression that might result as a correlated response to selection for fast growth may, as a consequence, lead to no net gain in biomass (Purdom, 1974; Weatherly, 1976; and Kinghorn, 1983).

If aspects of social behaviour can be found that are positively correlated with other desirable traits such as fast growth, resistance to disease, less stress during handling, *etc.*, then a directed and more enduring change can be effected by bringing about a genetic change through a process of domestication, ultimately leading to the development of superior varieties, *i.e.*, varieties with desired traits. Since genetic improvement of fish stocks is typically accomplished by selectively breeding the "best" parents, it therefore becomes important to study the observed variation among them and the reasons thereof.

1.4 OBJECTIVES OF THE PRESENT RESEARCH

The present study was undertaken to understand the connection between agonistic behaviours and growth rate in one of the most widely used species in aquaculture, the Nile tilapia (*Oreochromis niloticus*). The specific objectives included measuring agonistic behaviours in the fish in such a manner that while they represented responses to different social microenvironments, they could still be reflective of the overall behaviour of the fish, and to that extent, be considered "innate" to the fish. The second specific objective was

to understand the growth response of the same fish to different levels of competitive interactions. A final objective was to assess the genetic correlation between the behaviours studied and the growth responses in the different environments.

A major difficulty in studying closely linked traits such as behaviour and growth is that there is a feedback loop between these traits, wherein larger sizes usually confer behavioural advantages by means of dominance, and behavioural dominance in turn, usually reinforces growth rate differences through differential access to food, among other means. Thus phenotypic studies on either of these two traits typically suffer from the confounding effects of the other. The present study circumvented this feedback loop by splitting each brood of fish into three groups, and obtaining behavioural information from the first group, and growth data in two different competitive environments from the second and third groups, respectively. In other words, behaviour and growth were not assessed from the same fish, thus breaking the feedback loop. However, since the three groups were each full sibs of the other two, inferences drawn regarding the traits referred to the same sets of parents.

Further, in order to expose each fish to a large set of social microenvironments, each group of fish was obtained by crossing a female with each of four males, and pooling equal numbers from each of the four crosses. This procedure also eliminated replicate variance. At the termination of the experiments, parentage was established by DNA fingerprinting with microsatellites.

1.5 OUTLINE OF THESIS

Chapter 1: General Introduction

Chapter 2: Growth rate variation among cultured fish

Chapter 3: DNA fingerprinting using single locus microsatellites

Chapter 4: Statistical Methods

Chapter 5: Estimation of genetic parameters in cultivated tilapia (*Oreochromis niloticus*) using DNA fingerprinting. I. Agonistic behaviours

Chapter 6: Estimation of genetic parameters in cultivated tilapia (*Oreochromis niloticus*) using DNA fingerprinting. II. Growth in competitive and non-competitive environments

Chapter 7: Estimation of genetic parameters in cultivated tilapia (*Oreochromis niloticus*) using DNA fingerprinting. III. Genetic correlations between behavioural and growth traits

Chapter 8: General Conclusions

Chapter 2

Growth rate variation among cultivated fish

2.1 THE PHENOMENON OF GROWTH RATE VARIATION

Growth rate variation in poikilotherms has been under scrutiny for a long time, and there has been much speculation concerning the causative factors. Trying to understand the phenomenon in brown trout early this century, Dahl (1918, 1919), cited by Brown (1946), concluded that factors like food, degree of crowding, and egg size were important in causing size variation among fish. There were also several studies that seem to associate the different growth rates with abiotic factors such as level of hardness and pH of the water (*e.g.*, Southern, 1932, 1935; Frost, 1939, 1945; Swynnerton and Worthington, 1939). "Water-borne agents" causing growth depensation among crowded *Rana pipiens* tadpoles were described by Richards (1958), Rose (1960) and West (1960).

Aware that the growth of individual fish could be affected by a number of environmental (hydrological) factors, Brown (1946), studying the growth of brown trout, attempted to control them in order to see if there were also any genetic or social factors that might be responsible for the observed growth rate variation. Her experiments led her to conclude that the most important factor influencing the specific growth rates of individual fry was the size of a fish relative to that of the others in the tank. She suggested that a size hierarchy was established within groups, an individual's specific growth rate depending on its position in the order of decreasing weight. A similar result was reported by Allee *et al.*,(1948), based on their experiments with the green sunfish (*Lepomis cyanellus*). Interestingly, however, they also found that the mean growth of the fish was significantly higher when grown in groups than when either completely isolated, or

isolated but in visual contact with other fish. Exploring the social aspect of size hierarchy in his much-cited work, Magnuson (1962) noted that aggressive behaviour in competition for food and space was principally involved in causing growth variation among juvenile medaka.

Growth rate variation and in particular, skewed size distributions for fish within environments, was perhaps most vividly first described by the Japanese scientists Nakamura and Kasahara (1956, 1957, 1958 and 1961). Working with carp, they termed the biggest fish that fell to the right of the size frequency distribution *tobi koi*, or in its English translation, *shoot carp*. (These publications were later made available to the English speaking world by Wohlfarth, 1977.) This series of experiments by the Japanese scientists is especially noteworthy not only because it is one of the earliest known systematic studies of the phenomenon in fish but also because of its simplicity and the logical sequence in which questions were asked and answers sought.

The authors recorded some very interesting and suggestive results. First of all, they noted that the size distribution of newly hatched carp populations (symmetrical to begin with) became progressively skewed with time, the asymmetry setting in early (20 day old fry). This asymmetry was seen only in group-reared fish and not among those individually reared. (Allee *et al.*, (1948) also noted that their group-reared fish grew at different rates, but there is no mention of the disparity in growth rates of isolated fish, for comparison.) Further, they (Nakamura and Kasahara, *loc. cit.*) noticed that the appearance of shoot carp could be suppressed by adding a small number of slightly larger individuals, upon which the frequency distribution of the original sample would still be

symmetrical, but that of the entire group would be as skewed as the control. Finally, when they created a symmetrical distribution by removing the largest individuals, the remainder, when allowed to grow again, assumed an asymmetric distribution (just as Brown (1946) found with brown trout fry). The degree of this secondary skewing was found to be a negative function of the size and age of the fish.

That size grading of fish does not have a significant effect in changing the size-frequency distribution or in increasing total biomass has been shown in other studies too (*e.g.*, Jobling and Reinsnes, 1987; Wallace and Kolbeinshavn, 1988; Kamstra, 1993); it is interesting to note that Baardvik and Jobling (1990) actually found Arctic charr to suffer growth disadvantages from size grading.

Based on their findings, Nakamura and Kasahara (above references) concluded that the culture conditions in terms of the quality and quantity of food, determined the level of competition for the resource, and that the observed asymmetry and skewness in the size distribution is a result of this (unequal) competition.

These conclusions of competitive ability being associated with body size have been corroborated for various fish species: Arctic charr (Jobling and Wandsvik, 1983); chum salmon (Davis and Olla, 1987); pumpkinseed sunfish (Blanckenhorn, 1992); Atlantic salmon (Nortvedt and Holm, 1991, Thorpe *et al.*, 1992); tilapia (Koebele, 1985); steelhead trout (Abbott and Dill, 1989).

2.2 MACRO AND MICRO ENVIRONMENTS

The environmental variation that fish are subjected to can stem partly from changes in the macroenvironment. A *macro*environment (*e.g.*, Falconer, 1990; Yadava *et al.*, 1994) is defined by the macro-level conditions existing in the environment, such as the population density, physico-chemical characteristics of the water and soil, type of food and availability, and type of access to food resulting in contest/scramble competition. Each culture pond therefore, is a macroenvironment, where all the fish within it are subjected to the same macro-level conditions.

It is thus easy to visualize size or growth rate differences *among* ponds, that is, varying as a function of macroenvironment, even when it is the same set of genotypes that is grown in the different ponds.

What becomes really interesting is that considerable growth rate differences are usually seen even *within* ponds (that is, within the same macroenvironment) and *within the same brood* (Nakamura and Kasahara, 1955, 1956, 1957, 1961; Wohlfarth, 1977; Koebele, 1985). Since all the fish of any given brood are essentially of the same age and are all subjected to the same macroenvironment within the pond, it would seem that there must be minimal growth rate variation among such fish. However, there is an additional environmental component that has not yet been discussed.

This environmental component is the *micro*environment (*e.g.*, Yadava *et al.*, 1994) that each fish finds itself in. As mentioned above, the macro-level conditions within each pond constitute the macroenvironment of that pond, and this is the major cause of the environmental variation seen *among* ponds. The microenvironment, on the other hand, is

the environment that each fish finds itself in *vis-a-vis* the other fish in the same water body. Thus, the microenvironment for each fish includes all the fish that it comes in contact with, that is, all the fish in the water body except itself, and therefore the microenvironment is defined at the level of individual fish and is obviously unique for each fish. The differences in microenvironment are considerable: a small fish will find itself in the presence of large competitors, and *vice versa* for a large fish.

Since a fish pond is a water body full of fish sharing the same resources, the interactions between each fish and all the other fish define the microenvironment for that fish. These interactions can determine how well an individual is able to utilize a shared resource. Microenvironment can thus play a major role in causing growth rate differences among fish in an enclosed water body such as a fish pond, this variation reflecting individual differences in capacity to thrive in a shared environment.

It also becomes easy to see how very important genotype-environment interactions can become, if present, when the environment in question is the microenvironment; there are then as many environments as there are fish.

2.3 MICROENVIRONMENT AND COMPETITION

The microenvironment in a fish culture pond is perhaps most easily understood in terms of its manifestation - competition among the fish. Based on their extensive experiments with size variation in carp, Nakamura and Kasahara (1955, 1956, 1957, 1961; in Wohlfarth, 1977) concluded that it is the management of the culture system, especially with respect to the type and amount of food given, that determine the level of competition

for the resource and that the observed asymmetry in the size distribution is a result of this (unequal) competition. In other words, the growth response of fish to changes in the social microenvironment depends largely on the competitive conditions prevailing in the macroenvironment.

It is also believed that competition among fish has a snowball effect, where initial size differences, for whatever reason, can escalate into the commonly observed growth depensation, through a size-behaviour-growth feedback loop. This cyclical phenomenon, continually feeding upon itself, eventually results in the enormous size variation commonly observed. Purdom (1974), for example, reared plaice to maturity singly and in groups and found that the fish reared in groups quickly formed hierarchies and this resulted in size differences not seen among the fish reared singly. He found that the coefficient of variance (ratio of variance to the square of the mean) for length remained relatively constant over time implying that the range of sizes kept increasing as the fish grew bigger. Jobling and Wandsvik (1983) working with Arctic charr and using weight as a measure of size, found that the coefficients of variance for size actually increased with time (as predicted by Purdom, *loc. cit.*) and concluded that social interactions among the fish were responsible for the escalation in the value. Social behaviour (aggression) has been implicated in the suppression of growth in crustaceans too (Karplus *et al.*, 1986, 1992).

Some controversies in the literature

There is, indeed, little dispute in the literature that competition among fish is a major determinant of their relative growth performances within culture ponds. The points of debate, rather, centre around two main questions:

1) What is the causal relationship between dominance and size? Do dominant fish grow faster, or are bigger fish more dominant? In other words, are fish dominant because they are large, or do fish get to be large because they are dominant? Therefore, at harvest, are the biggest fish also the most aggressive and dominant, and is that why they are the biggest? Numerous studies have established the dominant status of larger fish, *per se*, usually more as an incidental observation. Jobling and Reinsnes (1987), for instance, found that the growth of small Arctic charr, in the absence of larger conspecifics, improved after size-sorting, indicating that the larger fish were dominant. A similar relationship between dominance and size in juvenile chum salmon has been reported by Davis and Olla (1987). Studying aggression and foraging behaviour in young-of-the-year brook charr (*Salvelinus fontinalis*), Grant (1990) noted that aggressive charr were 13% larger than non-aggressive conspecifics. Blanckenhorn (1990) found that dominant pumpkinseed sunfish acquired more food and gained significantly more body mass than subordinates. Nortvedt and Holm (1991) showed that better mean growth of Atlantic salmon in duoculture with Arctic charr rather than in monoculture (at the same density), was associated with reduced interactions among the salmon. Larger male red swamp

crayfish were significantly more dominant than similar sized ones when intruding upon mixed-sex settled communities of conspecifics (Figler *et al.*, 1995).

Most of the above studies were not undertaken with the specific goal of resolving the causal relationship between status and size. There are a few studies, however, that either have this as one of the objectives, or have the appropriate experimental setup in order to answer this question. There seems to be a consensus among the results of these studies that measurable size differences follow the establishment of status differences. The experiments of Koebele (1985) and Abbott and Dill (1989), with size-matched *Tilapia zilli* and steelhead trout, respectively, resulted in the dominant fish growing faster than its matched subordinate(s). These results must be treated with caution, however, since not only were the fish tested for behaviour as dyads (Abbott and Dill, 1989) or triads (Koebele, 1985), thus missing the influence of "group factors", if any (Nelissen, 1985), but they were also grown for the period of the experiments as dyads and triads, respectively. Physiological stress affects growth rates (Jobling and Wandswik, 1983; Pickering, 1990, 1993), and it is known that the level of stress that could arise as a consequence of interactions among conspecifics, in general, varies inversely with group size (McNicol and Noakes, 1984; Blanckenhorn, 1992; Brown *et al.*, 1992; Murphy 1993, but see Jorgensen *et al.*, (1993), whose experiments with Arctic charr seem to indicate a low level of agonistic interaction at the lowest density employed). There could well be a threshold level of stress, beyond which it could translate into reduced growth in the subordinates. Such high levels of aggression and stress may be unlikely even in the confined space of a culture pond, and thus extrapolation of the results reported by Koebele (1985) and Abbott

and Dill (1989), beyond the particular laboratory settings ("external validity", Altmann, 1974) is questionable.

The paper by Huntingford *et al.*, (1990) (see also Metcalfe *et al.*, 1989, and Metcalfe, 1993) gives more convincing proof of status being independent of relative size. There is no growth component in their experiment, only status *vs.* size. They tested individually marked Atlantic salmon parr in groups of 6 or 10, noting which individual of the group was dominant on the first day of the experiment, assigning it a rank of 1 and removing it from the tank. The following day, the rest of the group was tested similarly, and one fish (of rank 2) removed, and so on till all the fish were assigned ranks. Relative size and rank were then compared for each successive pair (i.e., fish of ranks 1 and 2, 2 and 3, and so on). They found that dominant status, in so far as their definition of dominance was concerned (an individual holding station near the only food inlet, acquiring the majority of food particles and making un-reciprocated attacks on its companions, was considered dominant), was independent of size, since the larger fish was dominant in only about half the cases. Size and status were linked, however, when the fish were tested after 5 months of feeding in a competitive environment, with the larger fish being dominant in 77% of the pairs. This link disappeared again when the fish were tested for a third time after a total of 12 months of rearing. These results suggest, as the authors mention, that in socially naive young fish, it is not size, but perhaps some inherent behaviour ("fierceness") that determines status and thus growth rate, in Atlantic salmon parr at any rate (but see Egglishaw (1967) for similar results while testing 0+ salmon and trout parr, and Bakker (1986) for sticklebacks). Such a causal relationship also provides a plausible explanation

for their finding the larger fish being mostly dominant when tested after 5 months of rearing, since the fish were no longer socially inexperienced. The disappearance of the link again at 1 year was, as explained by the authors, because it was only the fish that delayed smolting that were tested then, and such fish are known to be less aggressive than their siblings that choose to smolt aged 1+ (Metcalfe *et al.*, 1989, 1990; Metcalfe, 1991), and hence differential status may not result in differential growth.

A causal pathway between status and size also provides an explanation for the observation of Abbott *et al.*, (1985) in their study with steelhead trout, that subordinate fish failed to change rank even though their sizes were increased, relative to the dominants, by supplementary feeding.

The studies cited above have all shown, as cause or consequence, an association between dominant status and large size. There are instances, on the other hand, where competition between fish need not necessarily confer growth advantages to the dominant, or put the subdominant fish at a disadvantage, as Knights (1985) showed when he found that food consumption was the greatest in the middle 60% (by size) of most cultured populations. Further, examining the effect of mixing large and small elvers on mutual growth rates, Wickins (1987) found that after a period of mixing, more than a third of the large fish had been outgrown by the previously small fish. Baardvik and Jobling (1990), testing if size-sorting would disrupt dominance- and hence size-hierarchies in Arctic charr, instead found that size- sorted groups suffered a growth disadvantage due to high levels of interaction within them. It appears that there is a growth cost to dominance, especially if there are high energetic demands in order to maintain high rank. What appear to be such

growth costs have been reported in a number of fish species: in juvenile rainbow trout *Salmo gairdneri* (Newman, 1956; Yamagishi, 1962; Li and Brocksen, 1977; Metcalfe, 1986), in the goby *Odontobutis obscurus* (Yamagishi *et al.*, 1974), in the pygmy sunfish *Elassoma evergladei* (Rubenstein, 1981), in the tilapia *Oreochromis mossambicus* (Fishelson and Wise, 1983), and in medaka *Oryzias latipes* (Ruzzante and Doyle, 1991).

In general, however, it is a common enough observation that in an encounter with a conspecific, fish, as indeed most animals, are aggressive and dominant when bigger, and submissive when smaller. On the other hand, exceptions to this rule are not uncommon either; we can all recall occasions when, with a mixture of amusement and admiration, we have watched a smaller animal successfully chase away a bigger conspecific. It appears reasonable, then, to categorize animals into the following behavioural types: *pure aggressors* (an individual that always attacks, regardless of its relative size), *pure submitters* (one that always retreats regardless of relative size), and *contingent* (one that attacks when relatively large, and retreats when relatively small). In their game-theoretical model of the relationship between relative size and behaviour in fish, Doyle and Talbot (1986), however, have recognized a fourth behavioural phenotype, which they termed "uninvolved" (a "passive" fish that is a less aggressive variant of the contingent type). They theorized that in a resource-rich environment (as in most aquaculture situations), success in competition was irrelevant, and that a fish need neither be a pure aggressor nor a pure submitter, but ideally should be uninvolved in order to achieve fast growth. This model appears to have been empirically validated, as witness the results of selection experiments on medaka by Ruzzante and Doyle (1991, 1993), who reported a decrease in

agonistic interactions when selection for fast growth was performed in an environment of excess food, as also the negative phenotypic correlations between growth rate and food-related aggression in a hybrid tilapia reported by Robinson and Doyle (1990). It is possible that it was the success of this behavioural phenotype that resulted in the poorer growth of aggressive fish, in the references cited in the previous paragraph. Admittedly this is speculative since the experiments in those publications were not designed to answer this question. Nevertheless, the fact that in those experiments the largest fish were not the most aggressive, lends credence to the model of Doyle and Talbot (1986).

2) The second item of debate in the literature concerns the actual mechanism of competition in aquaculture ponds. There is ample evidence that in an aquaculture pond, some fish grow faster, at the expense of the others, since, on their removal from the water body, the hitherto slow-growing fish begin to exhibit faster growths, or on the addition of larger individuals, the growth rate of the original group is suppressed (e.g., Nakamura and Kasahara, 1957, 1961, in Wohlfarth, 1977). What is the mechanism of this phenomenon?

Competition can act in various ways. Koebele (1985), working on juvenile *Tilapia zilli* compared the three existing theories on the mechanism of competition among fish: i) the "physiological stress" theory put forth by Brown after her studies with brown trout (Brown, 1946, 1951, 1957) - that subordinate fish grow slower because they are stressed by the behaviour of the dominant fish; ii) the theory of "disproportionate food acquisition" of Magnuson (1962) - that relative growth rates of dominant and submissive fish were different only when food was limiting, at which times the socially dominant fish acquired more food at the expense of the subordinates; and iii) the "activity differences" theory of

Allen (1972) - that subordinate fish utilized food energy in evading the dominants rather than for growth. Koebele (*loc. cit.*) found that competition resulted in size differences among his fish, primarily through disproportional food acquisition due to the dominance-subordinance relationship.

This finding seems to have been corroborated in a number of studies. For example, McCarthy *et al.* (1992), using radiography to measure individual feeding in rainbow trout, found that dominant fish (those that obtained a greater share of the meal) had lower CVs for food intake than subordinate fish, and also that the range of CVs among all the fish decreased with increased rations to the group. As far as body weight was concerned, they only found a correlation between initial body weight and the share of the group meal in the low ration group, indicating that size had an effect on the feeding hierarchy when food was limiting. Other similar results have been reported by Thorpe *et al.*, (1990), (1992); Gotceitas and Godin (1992); Grant and Kramer (1992); Jobling and Baardvik (1994).

Based on these findings it would appear as if fish growth should be directly proportional to the amount of food ingested, and the observed variation of sizes within a pond simply attributable to differential success in feeding, reflecting corresponding differences in competitive ability. This is indeed the case when socially dominant fish restrict access to food to subordinates (the references listed in the previous paragraph). Significantly, these studies have also shown that improving access to food to all the fish by increasing the food rations reduces growth depensation.

Trying to ascertain the relationship between growth rate and agonistic behaviour when access to food was not restricted, Abbott and Dill (1989) tested 12 size-matched pairs of juvenile steelhead trout, and found that in 10 pairs the dominant fish grew faster than its paired subordinate. In the course of the experiment, both fish within pairs were separated during feeding and were fed equal rations. They found that the growth rates of the dominant fish were not exceptionally high, and therefore concluded that depression of subordinate growth rates was the main reason for the observed growth depensation.

Notwithstanding the actual mechanism of competition, it is clear that fish respond physiologically (i.e., growth) differently to the social microenvironment, based on the type of macro-level conditions prevailing in the pond in terms of competition for food. Further, competition among confined fish leading to a snowball effect could be due to small initial size differences resulting in a corresponding variation in competitive ability, or *vice versa*, where an initial "fierceness" (Huntingford *et al.*, 1990) confers an advantage to some fish, regardless of size, that then have better access to food, and consequently grow bigger.

Incidentally, initial size advantages could also be conferred simply by virtue of having hatched from larger eggs. For example, Quattro and Weeks (1991), studying the relationship between egg size and egg energetic content both within and among individual females of three *Poeciliopsis* strains, found high correlations between the two variables at all levels. Further, the relationship between egg size and the size of the offspring at hatching and the onset of exogenous feeding, has been seen in some stocks of walleye (*Stizostedion vitreum*) (Moodie *et al.*, 1989), and is well established in salmonids (Fowler, 1972; Thorpe *et al.*, 1984; Wood and Foote, 1990; Hutchings, 1991; Ferguson *et al.*,

1995). Skulason (1990), cited by Ferguson *et al.*, (1995), in fact noticed what appears to be a full cycle; smaller arctic charr produced smaller eggs, which in turn hatched into smaller progeny at the onset of exogenous feeding.

In any case, once a size differential has been achieved, either as a cause or consequence of behavioural status, status and size seem to become linked, leading to the observed phenomenon of growth depensation. The studies of Wohlfarth, (1972) and Wohlfarth and Moav, (1976), (1993) on the common carp, for example, have revealed a positive association between initial weight and weight gain.

In general, the animals within a macroenvironment are found to exhibit a variability in growth rate that is contingent upon the presence or absence of conspecifics. From most studies it appears that most individuals are capable of fast growth in the absence of larger individuals in the same water body. A superior aquaculture system would be one where this potential for fast growth is realized by all the individuals.

2.4 THE CAUSES OF VARIATION

In natural populations of plants or animals, only a fraction of those that are born live to create another generation. The rest die out or fail to reproduce, as a result of predation or in the competition for scarce resources. Indeed, this is an essential component of Darwin's theory of natural selection. On the other hand, resources are not limiting in the aquaculture situation, and every effort is usually made to keep mortality to a minimum. Even here, however, not all that are born get to reproduce. This is not merely because they die out but because they are not chosen (by man) as broodstock. Fish are

usually chosen as broodstock based on certain traits that they exhibit (domestication selection). In the case of food-fish aquaculture this trait usually is fast growth (but see Eknath and Doyle, 1985a; 1985b, for evidence of an inadvertent tendency to breed slower growing and later maturing individuals in India). The fitness of individuals in aquaculture is thus generally related to their growth rate.

If growth rate is the basis of broodstock selection by an aquaculturist, then the continued action of the selection process should lead to a reduction in the genetic variance of the trait. It is relevant to discuss Fisher's fundamental theorem (Fisher, 1958) here. This theorem states that the rate of increase of fitness (of an individual or a species) is equal to the genetic variance in fitness at that time. This implies that the genetic variance decreases over time. The trait under consideration here, growth rate, is intimately related to fitness. It is also genetically correlated with a certain suite of behaviours (e.g., Ruzzante and Doyle, 1991; 1993). It would then seem logical, from Fisher's fundamental theorem, to expect an increase in the mean and a loss of variance in growth rate as well as the correlated behaviours in the offspring of subsequent generations, especially in selection programs that have been operative for a long time. However, Fisher partitioned the total change in fitness over time into two terms: one caused by natural selection, and the other due to causes extrinsic to the population (the "environment"), and the fundamental theorem actually refers only to the change in fitness caused by natural selection (Frank and Slatkin, 1992). Fisher referred to the second term as change caused by the "deterioration of the environment", to indicate that this term is generally negative, since natural selection increases fitness but the total change in fitness is usually close to zero. But again, this

fact, that there is little change in fitness, does not by itself mean that there is no reduction in genetic variance - this can yet occur, since the theorem only refers to the change caused by selection.

We find however, as the studies cited here show, that growth rates continue to be variable and so do behaviours. This seemingly paradoxical situation can be explained by the fact that there are sources that generate and maintain variation.

Sources of variation

Apart from the additive and non-additive components of genetic variation, there is also the aspect of the sex of the individual. For example, fish such as tilapia are known to exhibit sexual dimorphism with respect to growth rate. As far as the non-genetic or environmental sources of variation are concerned, a major component may be the so-called "intangible" variation (developmental noise, Yamplosky and Scheiner, 1994), which simply refers to non-genetic variation whose cause is unknown (Falconer, 1989). This intangible variation can, in theory, be partly attributed to "environmental" causes, *i.e.*, sources that are external to the individual; and partly to sources pertaining to the individual, namely developmental variation, presumably caused by "errors" of development unique to each individual. The total intangible variation can be substantial - accounting for as much as 30 percent of the total phenotypic variance in human birth weight, for example (Penrose, 1954; Robson, 1955 - both cited by Falconer, 1989).

While genetic variance can be increased by the process of mutation, both genetic and environmental variance can be increased by disruptive selection (Falconer, 1989).

However, the amount of variation generated by mutation in a population is in general known to be as low as 10^{-3} V_E (Lynch, 1988), where V_E is the measure of variation in the trait caused by the "environment" (*i.e.*, non-genetic). Also, the type of selection practiced in aquaculture breeding programs is not disruptive, but uni-directional truncation selection. These factors therefore, do not play a major role in explaining the continued existence of variance in growth rate and behaviour. However, several recent studies have shown that additive genetic variation and heritability of morphological traits and fitness components can actually increase following periods of brief, but severe reductions in N_E, such as population bottlenecks or founder events (e.g., Nei *et al.*, 1975; Bryant *et al.*, 1986; Goodnight, 1988; Lopez-Fanjul and Villaverde, 1989; Willis and Orr, 1993). Laboratory and aquaculture populations can often be considered such bottlenecks at the time of their initiation, and thus account for the continued existence of variation.

Further, when genotypes are subjected to environmental changes, mean values may change due to GE-interaction, thus exhibiting *phenotypic plasticity* (the capacity of a genotype to alter its phenotype in response to changes in the environment - Bradshaw, 1965). Phenotypic plasticity can be a solution to the problem of individual adaptation to environmental heterogeneity (Bradshaw, 1965; Schlichting, 1986; Semlitsch, 1993; Day *et al.*, 1994; Via *et al.*, 1995; Behera and Nanjundiah, 1995) and allows diverse genotypes to have similar phenotypic responses. This reduces precise matching of genotypes to environments, thus permitting the maintenance of genetic diversity in the face of uniform selection pressures (Sultan and Bazzaz, 1993). Instances of phenotypic plasticity in fish abound in the literature (e.g., "stunting" in tilapia - Lowe-McConnell (1982), Noakes and

Balon (1982); the "shooting" phenomenon in carp - Wohlfarth (1977) and the references therein; and the bimodal pre-smolt growth distribution of salmon - Thorpe *et al.*, (1982)). Many geographically variable traits in many vertebrates have been attributed to phenotypic plasticity rather than microevolutionary processes (e.g., life history traits in frogs - Berven, 1982, fish - Stearns, 1983, and squirrels - Dobson and Murie, 1987; and morphological characteristics in birds - James, 1983, and snakes - Madsen and Shine, 1993).

A phenotypically plastic trait can provide for increased tolerance of environmental variations (Via *et al.*, 1995). In an aquaculture environment, effort is generally made to provide uniform macro-level conditions to rearing ponds; the differences among them being usually either age- or density-related. However, within environments it is the differential growth rates and behaviours among fish that provide heterogeneity in the environment, and as fish tend to respond to this heterogeneity, it is easy to see that the social microenvironment is perhaps the most variable environment of all to any fish, both temporally and spatially. Perhaps that is why, as an adaptation to this heterogeneity, a fitness related trait such as growth rate has remained so plastic within macroenvironments, and is being maintained as an evolutionary consequence of competition (Robinson and Wilson, 1994). This continued existence of variation in growth rates also indicates that plasticity in body size is being selected, perhaps indirectly as a correlated trait. If growth rate is under direct selection, and there exists a covariance between growth rate and plasticity in growth rate, then it points to the presence of genetic differences in the reaction norm (range of the trait across the changing environment) of the trait, and thus, a chance for the reaction norm to evolve.

In the study of the genetics and evolution of reaction norms, one of the points of debate seems to centre around the specific genetic basis for phenotypic plasticity (Schlichting and Pigliucci, 1995), that is, whether or not there are genes governing the phenomenon. Via (1993), for instance, argues that phenotypic plasticity itself is not a trait and there are no genes for plasticity since selection, being within environments, simply favours a different phenotype in each environment, and thus plasticity is a by-product of and not the target of selection. However, if the environment under consideration is the social microenvironment, then selection does take place among environments, and therefore, plasticity is perhaps not simply an incidentally occurring phenomenon. For example, variation in body size and age among stocks of chum salmon in British Columbia, presumably allowing them to exploit the range of spawning habitats present there (Beacham and Murray, 1987), environmentally cued metamorphosis in amphibians (Semlitsch, 1987; Newman, 1988), and seasonal polyphenisms in insects (Moran, 1992), among others, are well-documented cases of adaptive phenotypic plasticity. Phenotypic plasticity of a trait can therefore, itself be considered a trait.

Controversy also exists regarding whether reaction norms evolve directly as a result of selection or indirectly, through the evolution of separate mean phenotypes in each separate environment (Scheiner, 1991, 1993; Schlichting and Pigliucci, 1993; Via 1993a, 1993b; Via *et al.*, 1995). In the context of an aquaculture setting where selection, based on size and attainment of sexual maturity, is generally truncation selection, the evolution of reaction norms is indirect, as a correlated response to the main trait(s) under selection.

2.5 COMPETITION (OR THE LACK THEREOF ?) AS A LIFE HISTORY STRATEGY

Fast growth may lead to early maturity (Borrowsky, 1973; Sohn, 1977; Farr, 1980; Campton and Gall, 1988; Karplus *et al.*, 1991; Clarke and Blackburn, 1994). Thorpe *et al.*, (1983) showed that fast growth and early maturation in Atlantic salmon were genetically linked. A later study by Rowe and Thorpe (1990) too found that the biggest Atlantic salmon males were the first to mature, but also that subsequently their growth rates were lower than those of non-maturing males, thus resolving a controversy about whether maturing males were the largest or the smallest parr (e.g., Saunders and Sreedharan, 1977; Gjerde, 1984). While reviewing the life history tactics of 28 species of darters, Paine (1990) found that the primary pattern of life history traits was associated with body size; large fish grow faster, mature at a larger size, are more fecund, and have longer reproductive and life spans. This perhaps explains the presence of competition-like effects even when no restrictions are imposed on the resources provided, such as food, as reported by Allee *et al.*, (1948); Molander-Swedmark (1957); Nagoshi (1967); Purdom (1974); Koebele (1985); Abbott and Dill, 1989; and Ryer and Olla (1995). In other words many individuals appear forced to reduce their growth rate below their potential maximum value in the presence of conspecifics, even when food is relatively abundant.

In general it appears as if age and size at maturity are under genetic control (Thorpe *et al.*, 1983; Kallman, 1983; Gjerde, 1984; Trexler and Travis, 1990), but it has also been shown that environmental factors are important too. There is evidence in salmonids, for instance, that in the presence of abundant food, fish tend to mature when

younger and smaller, and conversely, in times of scarce food, maturity was delayed until the fish were older and bigger (Thorpe *et al.*, 1990, and the references therein; Clarke and Blackburn, 1994), and perhaps better equipped for competition - the two opposing, so-called r and K selection strategies (MacArthur and Wilson, 1967; Pianka, 1970; 1994). Other environmental factors found to influence age and size at maturity are stocking density (Sehgal and Toor, 1995) and photoperiod (Sower *et al.*, 1984).

Mention must also be made of the r and K strategies and the "stunting" phenomenon encountered in the intensive culture of tilapias. Lowe-McConnell (1975, 1982), summarizing a number of studies on the growth and maturation of tilapias throughout their range, reports on the plasticity that these species exhibit in growth and reproduction: individuals of a species when in large and deep lakes, can delay their maturation until they are older and larger, whereas in smaller water bodies such as floodplain pools and fish ponds, they breed when younger and smaller - the "dwarf" or "stunted" fish. Fryer and Iles (1972) and Noakes and Balon (1982) opine that the term "stunting" is a misnomer; that the problem is not of inhibited growth but of accelerated ontogeny (sexual maturation). This shift towards a more altricial life style, with a shorter interval of somatic growth and an earlier onset of sexual maturity, according to Noakes and Balon (1982), is an evolutionary response of the fish to an environment that is different from that of their fluvial ancestors. In any case, it is clear that the smaller body size is an environmentally induced phenotype.

In this context of the influence of environment on life history traits such as age and size at maturity, it is interesting to note the work of Campton and Gall (1988) on

mosquitofish, where they found that, when reared individually males matured significantly earlier and at a much smaller size when compared to those reared in groups. The authors attributed the observed differences to the presence or absence of behavioural interactions affecting the neuroendocrine control of the maturation process. A similar finding has been that of Bushman and Burns (1994), who detected a social control of maturation in the males of the swordtail characin.

Thus, plasticity in growth rate seems generally associated with an environmental correlation between the two life history traits, age at maturity and size at maturity. When the macroenvironment is largely unchanging and conducive to fast growth, as in a culture pond, the decision to adopt different life history strategies is dependent more on the perception of the social microenvironment by the fish. In a resource-limiting environment a fish can adopt a strategy of depriving conspecifics of food and thus benefit by growing faster. This is the strategy seen in nature and is usually achieved by aggression (Fausch, 1984, Metcalfe, 1986). If by being dominant, a fish can stress other fish into refusing food, it will of course gain at the expense of the others. This has been seen to occur. Brown (1946) observed that growth depensation occurred in juvenile brown trout despite unlimited food, leading her to hypothesize that physiological stress differences between dominants and subordinates were responsible. Koebele (1985) in his study testing three mechanistic hypotheses of growth depensation in tilapia (*Tilapia zilli*), found that in one of his treatments, fish that were in visual contact only with other fish consumed less food. Subordinate salmon and trout (Symons, 1971) and lobsters (Nelson *et al.*, 1980) have

been found to reduce their feeding and show fear even when not being threatened and when there was excess food.

In a resource-rich environment such as a culture pond, this strategy could be potentially maladaptive, as it is not necessary to aggressively contest for food; sufficient food is usually made available to all by broadcasting. The high energetic demands of the dominant fish coupled with the distraction from feeding that maintenance of high social status entails, could result in potential loss of growth opportunities for the dominants, as seen in the results reported by Yamagishi (1962), Yamagishi *et al.*, (1974), Li and Brocksen (1977), Rubenstein (1981), and Ruzzante and Doyle (1991). Under such circumstances, it is perhaps logical to expect that those that happen to have a low-risk, energy-saving strategy ("uninvolved" ?, Doyle and Talbot, 1986) for this environment of plenty, are the ones that gain in terms of somatic and reproductive growth. Examining the trade-offs between growth and mortality rates mediated by foraging activity, Werner and Anholt (1993) too have developed a model predicting adaptive responses for activity levels and foraging speed when individual growth rate and mortality are functions of these variables. They predict that if growth rate decreases with activity level, then speed or activity levels should decrease with increase in resources.

Adaptive behavioural strategies are important as they can chart the course in which the stock could evolve under domestication. Domestication is an evolutionary process in which emphasis has shifted from natural to artificial selection (Hale, 1969), and behavioural change is likely to be a major feature in its early phases (Kohane and Parsons, 1988). Doyle and Talbot (1986) have shown in their model that as long as 1) food is

abundant and 2) the behaviour of a fish towards its conspecifics depends upon its relative size (as is usually the case, Noakes, 1978; Turner and Huntingford, 1986; but see Huntingford *et al.*, 1990), the fish that grow best are those that are neither excessively aggressive nor submissive, but those that are "uninvolved", that is, fish that essentially ignore each other. Based on their model, Doyle and Talbot (1986) predict that, under the conditions mentioned above, artificial selection for fast growth should lead to a decrease in aggression among fish, and therefore in net gain in biomass, contrary to what seems to be generally feared (*e.g.*, Purdom, 1974; Weatherly, 1976; Kinghorn, 1983). This model is intuitively appealing, and some empirical evidence exists to validate its predictions (Holm and Ferno, 1986, cited by Swain and Riddell, 1990; Robinson and Doyle, 1990, Ruzzante and Doyle, 1991, 1993). However, there also appears to be some empirical evidence of aggressive behaviour increasing as a consequence of domestication (*e.g.*, Moyle, 1969; Swain and Riddell, 1990; and Mesa, 1991, all of whom found higher levels of agonistic behaviour among domestic than among their wild counterparts, in brown trout, coho salmon and cutthroat trout, respectively).

Empirical evidence both validating as well as disproving the predictions of Doyle and Talbot (1986) have been dealt with in detail in his review by Ruzzante (1994). Ruzzante (*loc cit.*) refutes the interpretation of the results in the three papers (above) that have reported greater aggression among domestic stocks, on grounds of 1) insufficient evidence (only one out of two replicates showed a difference between the stocks - Moyle, 1969), 2) inappropriate methodology in eliciting behaviours (use of mirror-image stimulation - Swain and Riddell, 1990), or 3) inappropriate comparison between wild and

domestic fish (widely different concomitant variables - Mesa, 1991). While these criticisms may be valid, it is obvious that the consequences of domestication are far from clear in fish. Clearly more work needs to be done in a variety of competitive environments and under different selection goals before we have a better understanding.

It appears, however, that in terrestrial animals, the process of domestication generally provides an environment for increasing thresholds for agonistic behaviour, as witness the reports of a reduction in aggressive behaviour in laboratory rearing of wild Norway rats (Price, 1978; Barnett, *et al.*, 1979), and a change to "calm behaviour" in foxes (Belyaev, 1979; and Belyaev and Borodin, 1982, both references cited by Kohane and Parsons, 1988). In his essay on the behavioural aspects of animal domestication, Price (1984) goes further, to state: "Along with a reduction in the intensity and frequency of aggressive behaviours in captive animal populations, there appears to be a corresponding decrease in the intensity and frequency of submissive behaviours".

Returning to the specific issue of the evolutionary consequences of selection for fast growth in the domestication of fish, there are, in fact, a number of studies that show that there is no necessary relationship between dominance and fast growth. High energetic costs of aggression in *Cyprinodons* have been recorded by Feldmeth (1983). Studying the aggressive behaviour and feeding of Atlantic croaker, black drum and striped mullet in mono- and polyculture, Gibbard *et al.* (1979) found that the largest individuals were not necessarily also the most aggressive ones. Magnuson (1962) in his study with medaka noted that as long as food was limited, large fish were dominant and aggressive, but at high densities and when food was unlimited, the dominants lost their aggression as

well as growth advantages. He therefore concluded that aggression as a strategy did not confer growth advantages when there was no need to compete for food. Knights (1985) found that food consumption was the greatest in the middle 60% (by size) of most cultured populations. Assessing the dominance relationships between pairs of Atlantic salmon parr during their first year of life, Huntingford *et al.*, (1990) found that the larger of the two fish was dominant in only 54% of the pairs tested. Indeed, under most culture conditions there seems to be a significant growth cost to social dominance, and this has been shown to occur in a variety of species: rainbow trout (e.g., Newman, 1956), tilapia (Fishelson and Wise, 1983), *Odontobutis obscurus* (Yamagishi *et al.*, 1974), *Salmo gairdneri* (Newman, 1956; Li and Brocksen, 1977; Metcalfe, 1986), *Oreochromis mossambicus* (Fishelson and Wise, 1983; Nelissen, 1985; Turner, 1986) and *Odontobutis obscurus* (Yamagishi *et. al.*, 1974).

Thus when the resource (food) is not limiting, there seems to be no real advantage to being aggressive, especially if it is energy consuming, and if competitive interactions escalate into wars of attrition.

2.6 ADAPTIVE STRATEGIES AND THEIR INHERITANCE

It is obvious that competitive ability among individuals sharing a resource such as a culture pond, is unequally distributed. In the short term, success might depend upon the ability to defend a coveted territory or to obtain sufficient food in the face of competition, by whatever means, "involved" or "uninvolved", but the ultimate currency in which the success of an individual is gauged is in terms of its life-time fitness. This includes the

individual's viability and survival as an egg and juvenile, its survival and growth from the juvenile through the sexually mature adult phase, the number and quality of offspring it produces (as a female) in its lifetime, or the number of eggs it successfully fertilizes (as a male), and any parental care bestowed upon the offspring.

Strategies that are adopted in the attainment of short- or long-term goals are adaptive if they ultimately contribute towards increasing the fitness of the individual. For example, if the short-term goal is obtaining sufficient food, then the behaviours that aid in attaining this goal are adaptive if the consequent fast growth that this may lead to also culminates in early maturity and successful reproduction. As seen in the foregoing sections, fast growth in fish is associated with different behaviours depending upon the type of the competition regime there exists for obtaining food. I now again cite some examples available in the literature, of fast growth being associated with each of three different behaviours: aggressive or dominant (e.g., Koebele (1985); Jobling and Reinsnes (1987); Abbott and Dill (1989); Grant (1990); Figler *et al.*, (1995)), submissive (e.g., Newman (1956); Yamagishi *et al.*, (1974); Li and Brocksen (1977); Fishelson and Wise (1983); Nelissen (1985); Metcalfe (1986); Turner (1986); Huntingford *et al.*, (1990)), and "uninvolved" (e.g., Ruzzante and Doyle, 1991, 1993; Robinson and Doyle, 1991).

If a behaviour (be it dominance, submission, or indeed, indifference) in an individual that is invoked in response to the behaviour or even the mere presence of a potential competitor, has any bearing on its success in attaining short or long term goals, it can be termed the individual's competitive ability. Is there a genetic basis to this competitive ability? Can it be inherited?

A recent debate centered on the inheritance of social dominance brought out opposing viewpoints, beginning with the publication of two articles that demonstrated an inter-generational predictability in social dominance. Dewsbury (1990) reported that dominance relationships among male deer mice, *Peromyscus maniculatus*, mirrored the dominance relationships of their fathers, while Moore (1990), studying genetic variation in social behaviour in the cockroach, *Nauphoeta cinerea*, noted that typically, dominant males produced dominant offspring and subordinate males produced subordinate offspring. These claims of demonstration of the inheritance of dominance were refuted by Capitanio (1991, 1993). Using the analogy of emergent properties and integrative levels in General Systems theory (Feibleman, 1954; Boulding, 1968; Salthe, 1988), he pointed out that social dominance and genetic transmission are at different levels of analysis, and that therefore, the concept of inheritance of dominance does not even arise. The findings of Dewsbury (1990) and Moore (1990) were also challenged by Barrette (1993) on the grounds that social dominance is not the property of an individual but an attribute of an interacting dyad, and hence cannot be inherited. In their replies to these challenges the original authors reiterated their interpretation of their findings, by means of the following arguments: even though inheritance is at the level of genes, behaviours are inherited since genes influence behaviours (Dewsbury, 1991; Moore, 1991), the concept of the extended phenotype (Dawkins, 1982) can be used to better understand social dominance (Dewsbury, 1993), and by clarifying the difference between behavioural interactions and the 'aptitude to dominate' and pointing out that the trait 'aptitude to dominate' is the property of an individual and can be inherited (Moore, 1993).

It appears that ever since Schjelderupp-Ebbe (1922) introduced the concept of the peck-order, which later came to be called dominance, the term, while seemingly straightforward in meaning, has actually been a rather controversial and elusive concept in terms of definition, in the literature. An extensive review of the various definitions of the term dominance in the literature has recently been published by Drews (1993), who himself has proposed yet another, a "structural" definition, which he describes as a synthesis of the "essence" of dominance. Drews (1993) maintains that the term dominance, as understood say, by aggressiveness, as opposed to dominance as an attribute of an interaction, are two distinct concepts, and that while the former is (at least conceptually) heritable, the latter is not, as it refers to a relationship between two individuals and is therefore a relative measure.

Dominance and submissiveness are end results, the final products of processes triggered in response to stimuli provided by others. While meaningful only in the presence of other individuals, dominance is still a manifestation of the "ability to dominate" of an individual, which in turn is probably determined by a host of interacting factors that include age, sex, physiology, size and behaviour. In juvenile Atlantic salmon, it has been shown that the ability to dominate can be independent of size, being determined instead, by behavioural qualities such as "fierceness" (Huntingford, 1990; Metcalfe, 1993). These qualities obviously belong to individuals and are very likely genetically determined and heritable, at least in part. This simply means that given the same visual/chemical stimuli, the offspring would behave in much the same manner as the parent towards the source of

the stimuli. As a consequence it is also likely to be ultimately dominant or submissive, as the case may be.

Many behaviours have been shown to be genetically determined. For example, Hedrick and Reichert (1989) found that differences in foraging behaviour between two funnel web spider populations were not environmentally determined. Berthold *et al.*,(1990) and Helbig (1991), studying migratory behaviour in blackcaps, produced hybrids between the German and Cape Verde populations and noted that the behaviour of the hybrid birds was like neither parent but rather, in between. Behavioural differences have even been shown to be associated with single-gene differences (e.g., de Belle and Sokolowski, 1987). Indeed, some mutant alleles have been named to reflect their behavioural consequences (Alcock, 1993): for example, the following mutant alleles in fruit flies: "stuck" (males with this gene fail to dismount after the normal 20 minutes of copulation), "coitus interruptus" (males with this allele disengage after just 10, not 20, minutes of copulating), and "bang-sensitive" (a sudden jolt causes flies with this allele to become paralyzed). Adaptive, hereditary differences have been found in the feeding behaviour of coastal and inland populations of garter snakes (Arnold, 1980).

Aggressiveness, which has been used as a basis of determining dominance (Wilson, 1975) has been shown to have a genetic component in various animals: chickens, *Gallus domesticus* (Guhl *et al.*, 1960); domestic dogs, *Canis familiaris* (Scott and Fuller, 1965); mice (DeFries and McClearn, 1970, 1972); silvereyes, *Zosterops lateralis* (Kikkawa *et al.*, 1986); sticklebacks. *Gasterosteus aculeatus* (Bakker, 1986); and *Drosophilla* (Hoffman,

1988, 1989; Hoffmann and Cacoyianni, 1989). Craig *et al.*, (1965) and Hoffmann (1988, 1989) have shown that dominance ability itself can be inherited.

Certainly, dominance and subordinance are not fixed roles or strategies, as dominants behave as subordinates and *vice-versa*, depending on the circumstances (McGuire *et al.*, 1984). However, there could be a size and/or behaviour threshold in response to which the animal makes the switch in role. In other words, this threshold could represent a discontinuity in an otherwise continuous character (a physiological mechanism that brings out the behavioural response of the fish to stimuli provided by other fish), the "liability" (Falconer, 1989). This switch-eliciting threshold, in much the same manner as a trait such as susceptibility to disease, or cold tolerance, could very well vary among individuals, and could indeed be heritable. An animal with a high threshold for a switch between dominance to submissiveness, can be termed dominant. If the response to threshold is heritable, its offspring would then be called dominant too.

The animals with which an individual cohabits, serve to determine its microenvironment, invariably rather heterogeneous. Each individual receives signals from its cohabitants that vary both in space and in time. As seen earlier, once size differences (status-related or unrelated) are established in a population, this serves to increase the variation in size (and perhaps behaviour), and thus the heterogeneity in the social microenvironment among the fish, over time. The behaviour and growth response of each individual to this changing environment, in turn, determine the extent of heterogeneity in the environment. This feedback can lead to reduced heterogeneity in the population only when the responses of individuals do not contribute to it, that is, neither being growth-

inhibited themselves, nor inhibiting the growth of other fish. The best candidates for this purpose, at least theoretically, appear to be the "uninvolved" fish of Doyle and Talbot (1986). Selection for such behaviour may lead to reduced variation in growth rates and behaviours.

"Passiveness" as a behavioural strategy has been described in some mammals such as rodents (Benus *et al.*, 1991) and pigs (Hessing *et al.*, 1993; but see Jensen *et al.*, 1995). It is therefore possible that passiveness or "uninvolvedness" is used as strategy by fish too. If "uninvolved" fish also do actually exist then it will be interesting to 1) look at size and age at maturity of these fish and assess if indeed the behaviour is related to a life history trait, and if so, relate it to fitness, *i.e.*, estimate the phenotypic selection (Endler, 1986; Alatalo, 1990), by statistical methods recently developed (Lande and Arnold, 1983; Arnold and Wade, 1984a; 1984b), and 2) to assess the level of plasticity in growth rate in these fish (reaction norm). Ideally the growth rate should be rather aplastic, thus conferring a uniformly high growth rate among all such fish, irrespective of the social micro environment that they are subjected to. It is then that the strategy of "uninvolvedness" would pay dividends in terms of reproductive fitness inasmuch as growth rate is associated with sexual maturity and breeding.

Chapter 3

DNA fingerprinting using single locus microsatellites

3.1 INTRODUCTION

The term microsatellite refers to that part of the DNA wherein a di-, tri- or tetra-nucleotide motif is repeated in tandem. The most commonly occurring microsatellites are the dinucleotide repeats $(AC)_n$, $(AG)_n$ and $(AT)_n$ (Rafalsky and Tingey, 1993). These microsatellites are thought to be distributed throughout the eukaryotic genome, generally spaced at 7-10 kilobase intervals (Tautz, 1989; Wright, 1993).

Variability among the alleles of a microsatellite is due to variation in the number of repeats. This variation is manifested as size differences when the amplified product is electrophoresed in a suitable medium. Alleles are codominant, so that a heterozygote can be easily distinguished from a homozygote. Microsatellites also follow a simple Mendelian inheritance (Harris *et al.*, 1991; Queller *et al.*, 1993). These factors make them an ideal molecular tool in the study of pedigrees.

Any given microsatellite represents a minuscule portion of the total genome, and to be visualized, it is necessary to selectively amplify the particular piece of the genome containing the microsatellite, before it can be detected and analyzed. For this process, the microsatellite itself and a portion of the two flanking regions of the genome are first isolated (by cloning) and sequenced. Complementary sequences to the flanking regions are then generated to give the two "primers". One of the two primers is radio labeled. The DNA isolated from the fish is then placed in a cocktail consisting of both primers including the labeled primer, an enzyme, free deoxynucleotides and all the other necessary reagents.

The mixture is then placed in a thermocycler that can be programmed to change the temperature of the sample for specified lengths of time. The temperature is cycled between three points - a high, melting temperature (90-94°C) such that the two strands of the DNA separate to give single strands; a low primer-specific annealing temperature, where two single strands (*e.g.*, the labeled primer and one strand of the input DNA) can anneal; and 72°C, a temperature where the enzyme is active, and the free nucleotides in the cocktail are added to the shorter of the two annealed strands complementary to the template provided by the longer strand. One run through these three temperature points constitutes one cycle and results in near-doubling of the existing number of copies of that part of the input DNA containing the microsatellite, as defined by the sequence of the two primers. Typically the reaction is run through up to 40 cycles in the thermocycler, resulting in an enormous increase in the number of copies of the microsatellite.

Next, the product is electrophoresed through a gel with marker sequence of known molecular weight. If radioactive, the gel is fixed, dried and exposed to an X-ray film on which the alleles at the locus appear as bands, the migration distance depending on the size (length) of the alleles. The size of the alleles is then scored relative to the known marker sequence.

3.2 DEVELOPMENT OF DNA MICROSATELLITE PRIMERS

Nine tilapia microsatellite primer pairs which had already been developed at MGPL (Ambali, 1996), eight from *Oreochromis shiranus*, and one from *O. niloticus*, were available for this study. Of the eight *O. shiranus* primer pairs, six (Os-7, Os-7R, Os-25,

Os-64, Os-74 and Os-75) could be used with *O. niloticus* DNA. These six plus the single *O. niloticus* primer pair (On-15) were used initially in this study. As the population being studied had low levels of polymorphism (two alleles at two loci, three at one locus, five at three loci and six alleles at one locus), it was necessary to develop more probes to address the question (of pedigree identification) at hand. The procedures used for the development and use of the *O. niloticus* primers are detailed below.

3.2.1 *Extraction of DNA*

A sample of about 0.5 ml of blood was drawn from the caudal vein of a fish using a syringe rinsed with 0.5 M EDTA (to prevent rapid clotting), and preserved in 0.5 ml absolute ethanol. DNA from this sample was extracted using the phenol extraction procedure (Sambrook *et al.*, 1987), as described below.

The blood/ethanol suspension measuring two hundred and fifty microlitres, was washed two times with 1 ml high TE buffer (100 mM Tris-Cl, 40 mM EDTA) by spinning it for ~10 s in a microcentrifuge (Eppendorf) and decanting the supernatant. The pellet was re-suspended in 500 µl. extraction buffer (0.1 M Tris-Cl pH 8.0, 0.1 M EDTA, 0.25 M NaCl, 1% SDS) and 100 µg/ml proteinase K (ICN Biomedicals), and incubated for 18 h at 55°C. To the cell lysate, 500 µl. buffer-saturated phenol was added and mixed for ~10 m, after which it was spun in a microcentrifuge for 5 m. The aqueous phase was then gently decanted into a clean tube and re-extracted with buffer-saturated phenol. The aqueous phase was transferred to a clean tube, and extracted with 500 µl. chloroform.

The next aqueous phase was decanted to a clean tube. Two volumes of ethanol was added, thoroughly mixed, and the mixture incubated at -20°C for at least 2 h. The precipitated DNA was recovered by spinning the mixture in a microcentrifuge for ~5 m and discarding the supernatant. The DNA pellet was washed with 1 ml 70 % ethanol. The DNA pellet was then dried in a speedvac for ~5 m and dissolved in 50 µl. TE (10 mM Tris-Cl, 1 mM EDTA).

3.2.2 *Preparation of a size-selected library*

Fifty micrograms of the isolated genomic DNA was digested with 100 U each of P*al*I, R*sa*I, H*inc*II and A*lu*I (Pharmacia) according to the manufacturer's recommendations. The digested DNA was electrophoresed in a 1% low melting point agarose gel and the fragments in the appropriate size range were recovered from the gel using a phenol freeze-fracture procedure as described below. The portion of the gel containing the 300-700 bp fragments was finely chopped, placed in a Corex tube and frozen at -80°C. Three volumes of buffer-saturated phenol were added to the solidified gel, the tube sealed with parafilm and vortexed until the chopped gel fragments were completely dissolved. This mixture was spun in a Beckman J2-21 centrifuge at 10,000 g for 20 m. The aqueous phase was decanted to a second Corex tube and the phenol extraction repeated. The aqueous phase was again transferred to a clean tube and extracted with an equal volume of chloroform. The final aqueous phase was precipitated by adding glycogen (as a carrier) at 20 µg/ml, NaCl to a final concentration of 0.2 M, and two volumes of ethanol. The mixture was frozen at -80°C to precipitate the DNA, which

was recovered by centrifugation in a Beckman J2-21 centrifuge at 30,000 g for 30 m. The supernatant was discarded and the DNA pellet resuspended in 0.5 ml TE, vortexed and transferred to a 1.5 ml microfuge tube. The DNA was precipitated again, as above, and resuspended in 20 µl TE. A 5 µl aliquot of this was run on 1% agarose mini-gel to estimate the quantity recovered for cloning.

3.2.3 *Cloning*

The DNA isolated from the gel was ligated into a commercially prepared vector, as described below. Ligation was done using the DNA ligation mix consisting of 40 ng of the vector pUC 18 S*ma*I/BAP (Pharmacia), 500 ng of the digested insert DNA, 1 X ligation buffer (10 mM Tris-HCl pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$), and 2 U of T4 DNA ligase (Pharmacia) in a total volume of 25 µl. The reaction was incubated at 16°C for 20 h. Five µl of the ligated DNA was used to transform 100 µl of MAX efficiency DH5α competent cells (BRL) according to manufacturer's recommendations. The final mixture was shaken at 225 rpm at 37°C for 1 h and spread in volumes of 50 µl on 2X YT medium (1.6% Bacto tryptone, 1 % yeast extract, 0.1 M NaCl) with 100 µg/ml ampicillin.

3.2.4 *Colony screening*

The colonies were lifted onto Hybond-N (Amersham) filters and the filters were processed according to the manufacturer's specifications. The colonies were screened for the presence of a specific dinucleotide (AC_n) repeat. A synthetic oligonucleotide, (GT_{15}),

synthesized on an Applied Biosystems 390 DNA synthesizer, was used as a probe. Eighty nanograms of the oligonucleotide was end-labeled with 0.5 µl 10 µCi/µl (γ ^{32}P) ATP (ICN), 5 U of T4 PNK (Pharmacia), 0.5 µl T4 kinase buffer (Pharmacia) and 2.5 µl sterile ddH_2O, incubated at 37°C for 30 m, and heated to 70°C for 10 m to denature the enzyme.

The filters were incubated in pre-hybridization solution [6.25 ml 20X SSPE pH 7.6 (174 g NaCl, 27.6 g $NaH_2PO4.H_20$, 7.4 g EDTA to a total volume of 500 ml), 2.5 ml 50X Denhardt's (5 g Ficoll-Type 400, 5 g polyvinyl pyroline, 5 g BSA (pentax fraction) to a total volume of 500 ml), 10% (w/v) SDS to a total volume of 100 ml containing 100 µg/ml t RNA] at 65°C for 1 h before addition of heat-denatured (70°C for 5 m) radio labeled probe. The hybridization reaction was carried out in a Hybaid hybridization oven at 60°C overnight. The excess probe was then washed off the filters in three washes: first with 2X SSC, 0.1% (w/v) SDS, for 15 m at room temperature, a second wash with 1X SSC, 0.1% SDS for 15 m at room temperature, and finally with 1X SSC, 0.1% SDS at 60°C for 15 m. The filters were then wrapped in cling-wrap and subjected to autoradiography (Kodak XAR5 X-ray film) at -80°C with one intensifying screen, overnight.

The colonies were then aligned with the images on the film and 48 individual putative positive colonies (that show up as clear and distinct dark dots on the film) were found and picked using sterile tooth picks, and grown overnight in YT broth containing 100 mg/ml ampicillin. Templates for sequencing were prepared from the putative positive clones using the Speedprep procedure of Goode and Fenstein (1992).

3.2.5 *Sequencing of clones*

DNA from the putative positive colonies were sequenced using the ^{T7}Sequencing™ kit (Pharmacia LKB Biotechnology) following the manufacturer's instructions, and electrophoresed through a 8% denaturing polyacrylamide gel containing 7.8 M urea, using 1X Tris-borate-EDTA buffer (10X TBE: 0.9 M Tris Base, 0.9 M boric acid, 20 mM EDTA pH 8.3) at 1600 V for 2.5 h. After the run, the gels were fixed in a solution of 10% acetic acid and 10% methanol, for 30 m and then dried onto a Watman 3MM paper under vacuum for 2 h. The dried gels were autoradiographed overnight at room temperature.

3.2.6 *Designing of primers*

From the sequences obtained clones containing microsatellites and sufficient flanking sequence were identified and primers designed using the following criteria: close proximity to the repeat array, equivalent GC content for both forward and reverse primers, close to 50% GC content overall, at least one C or G at the 3' end, and a length of ~20bp. An initial annealing temperature for the primers was estimated based on the following calculation:

$$T_{ann} = 4 \times (G + C) + 2 \times (A + T) - 6$$

From the initial PCR results the annealing temperature was either reduced (if the signal was weak) or increased (if the product was "dirty") until an optimum was found.

Based on the method described here two primers were developed using *Oreochromis niloticus* DNA (Table 3.1).

Table 3.1. Microsatellite primers designed using tilapia (*Oreochromis niloticus*) DNA from the sequences flanking the chosen dinucleotide repeats, and their respective optimum annealing temperatures.

Primer On-3	**Annealing temp.**
Forward sequence: 5′ - CCAGCCCACTGGCACC - 3′	
Reverse sequence: 5′ - ACTCTGAGCGTGAGGACC - 3′	51°C
Primer On-13	
Forward sequence: 5′ - GTCTGTGATATTACCATCAC - 3′	
Reverse sequence: 5′ - ACATGAAGAAACACATCTGC - 3′	51°C

3.2.7 *PCR amplification*

For amplification, a cocktail consisting of 1X PCR buffer (10mM Tris pH 8.3, 50 mM KCl, 1 mM MgCl2, 0.01% gelatin), 400 μM each dNTP, 200 μM each primer, 0.25 U T*aq* polymerase and 25 ng of extracted genomic DNA, were added to 1 μM of one (reverse) γ^{32}P-labeled primer. This mixture was overlaid with a drop of mineral oil. Amplification was carried out in a Biosycler™ oven (Bios Corporation) or an MJ PTC-100 thermocycler (MJ Research, Inc.). The samples were first cycled 7 times through the following series: denaturation at 94°C for 1 m, annealing at primer-specific temperature for 30 s, and extension at 72°C for 1 s (30 s for the MJ). These were followed by 35-40 cycles through the series: denaturation at 90°C for 30 s, annealing at primer-specific temperature for 30 s, and extension at 72°C for 1 s (30 s for the MJ).

The amplified products were then electrophoresed through an 8% denaturing polyacrylamide gel, fixed, dried, autoradiographed, and sized relative to an M13 sequencing ladder.

Chapter 4

Statistical methods

4.1 INTRODUCTION

Traits such as size (length, weight), where the differences among individuals are on a continuous (as opposed to discrete) scale, are known as quantitative or metric traits. The presence of such differences among individuals means that they have to be measured on the trait, rather than categorized by it as in the case of a qualitative trait. Both qualitative and quantitative traits have a genetic as well as non-genetic basis. The genetic basis of quantitative differences among individuals, however, is slightly different from that of qualitative differences. For example, while genes govern the inheritance of quantitative traits too, their effects on the phenotype are frequently small when compared to non-genetic effects and other non-heritable genetic effects. Therefore, quantitative differences among individuals are only partially ascribable to heritable differences. Also, unlike qualitative traits, quantitative traits are generally influenced by the genes at many loci (polygeny). Hence differences among individuals are continuous and are not segregated into Mendelian ratios. Nevertheless, the study of the genetics of quantitative traits has, as a basic premise, the assumption that the inheritance of quantitative differences is dependent on genes that have the same properties and are subject to the same laws of transmission as those that govern qualitative traits (Falconer, 1989, p.1).

4.1.1 *The concept of Value*

As mentioned earlier, individuals are measured on quantitative traits. In order to understand the components that make up each of these measurements and to be able to compare the measurements of different individuals, we need the concept of *value*.

Genes at a QTL (quantitative trait locus) in a population, as at any locus, generally occur in alternative forms, known as alleles. The particular arrangement of alleles at a given locus is known as the genotype of the individual at that locus. At each locus, there could be two (in diploid organisms such as fish) representations of the same allele (such a genotype being called a homozygote), or the alleles could be different (heterozygote). If we consider two alleles (A_1 and A_2) at a locus, there can then be two possible homozygous genotypes (A_1,A_1 and A_2,A_2), and one heterozygous genotype (A_1,A_2).

The alleles at a locus not only have a direct influence on the trait in question, they also interact with each other to produce a joint effect. In the case of homozygotes, the genotype is such that the two alleles interact so as to influence the trait in the same direction, whereas in the case of heterozygotes, the interaction can be more complex: the combined effect of the two alleles could be anywhere on a continuum that overlaps the range of effects of the two homozygous genotypes. This effect depends upon the degree of dominance of one allele over the other.

Genotypic value

The effect or influence of a genotype at a locus on a trait can be thought of as the genotype conferring a certain value on the trait. This value, theoretically, is the effect of the genotype alone, without the influence of the environment. Alternatively, if we assumed that the performance of an individual could be measured in the whole range of conceivable environments that are normal for the species, then the average of all the measurements would be the true value of this individual that is determined by its genotype.

This value is known as the *genotypic value* of the trait for an individual possessing that genotype. As Falconer (1989, p. 112) notes, genotypic value is really a theoretical concept, easy enough to visualize but practically impossible to determine, except in certain special circumstances such as if we are dealing with a single locus and the various genotypes are somehow easily distinguishable, or if each genotype is represented by clones or highly inbred lines.

In the absence of an interaction between the two alleles at a locus, the genotypic value is also then the *breeding value* of the individual, which is twice the mean deviation of all its offspring from the population mean, when it is mated at random to a number of individuals from the population. (The breeding value of an individual gives an idea of the kind of offspring expected from an animal, and hence is of practical importance.) If however, there is an interaction between the two alleles within the locus, it then means that the combined effect is not simply the additive effect (the sum of the effects of the two alleles taken singly). This is because one of the two alleles is dominant, and this effect causes a deviation from the additive effect, known as the *dominance deviation.* Therefore, when we consider the alleles at a single locus, the genotypic value is the sum of the breeding value and the dominance deviation. Symbolizing the genotypic value by G, the breeding value by A and the dominance deviation by D, we have,

$$G = A + D \qquad \ldots(4.1.1)$$

Since a quantitative trait can be affected by the genes at many loci, the "genotypic value" of an individual for a given quantitative trait is therefore actually the aggregate or

the combined effect that would typically be elicited by the particular array of relevant genotypes in that individual, in an environment normal for that population.

Just as genes can interact within loci to produce dominance deviations, they can also interact among loci, rendering the aggregate effect not simply additive. This interaction among the alleles across loci is known as *epistasis*. There can of course, be interaction between two, three or more loci. Rather than attempt to distinguish among these different levels of interaction, we simply consider the aggregate of all these interaction effects as epistasis, symbolized by I.

Thus, for all loci together,

$$G = A + D + I \qquad ...(4.1.2)$$

where A refers to the sum of the breeding values at each locus, D is the sum of all the dominance deviations, and I is the sum of all the interactions among loci (epistasis).

There can be much variation in this value (G) among individuals. The joint effect of all the loci in an individual, depending upon the particular assemblage of alleles at the different loci, can be anywhere on a wide range. Further, as a population can consist of a number of alleles for each locus, there can be a variety of possible genotypes at each locus, and thus, a huge variety of joint effects. The extent of genetic diversity among individuals in a population that is not highly inbred, is therefore usually fairly substantial, such that practically no two individuals are genetically identical, even as far as the loci for one particular metric trait are concerned. This, then, is the genetic basis of quantitative variation among individuals; the differences in genotype, manifested as the differences in genotypic values.

Environmental deviation

As mentioned earlier, another significant factor that affects the expression of a quantitative trait is the influence of the environment, that is, all influences that are not genetic in origin. This includes maternal effects, effects of common environment, age, nutrition, hydrological factors, *etc.* If the genotypic value can be thought of as a value conferred upon the individual by its genotype (that is, from "within"), then the environment can be thought of as a factor that adds to or subtracts from the genotypic value (that is, an influence from "without"); this influence is called the *environmental deviation.*

As the environment comprises all influences that are non-genetic in nature, its effect is fairly complex. Some effects of the environment are fixed or permanent in nature (*e.g.*, physical defects or injuries), while some others are more dynamic and transitory, and thus temporary in nature (*e.g.*, daily food intake). Broadly speaking therefore, the environmental effects can be categorized as permanent (E_p) and temporary (E_t).

$$\text{Thus, } E = E_p + E_t \qquad \ldots(4.1.3)$$

Phenotypic value

For any given trait on an individual, the measure of the phenotype (known as the *phenotypic value*, P), which is the actual observation or measurement made, is the final manifestation of the combined effect of the genotype and the environment, and all the components thereof.

Therefore, we have,

$$P = G + E, \text{ (assuming G and E are independent)} \quad ...(4.1.4)$$

Or, expanding both G and E, we have,

$$P = (A + D + I) + (E_p + E_t) \quad ...(4.1.5)$$

GE Interaction

Frequently, G and E are not quite independent. If the expression of the genotype remains the same, whatever the environment, then G and E are said to be independent. In such a case, therefore, it is valid to speak about a "good" genotype or "poor" genotype since their effect is consistent in relation to other genotypes, in any variation of the environment. If, on the other hand, the genotypic value changes with variation in the environment, then G and E are not independent, but rather, they interact to produce yet another effect. This additional effect is known as the *genotype x environment interaction*, or *GE-interaction* for short. Such an interaction can be inferred, for instance, if the ranking (relative performance) of two strains or species changes with a change in the environment. The presence of GE-interaction has been demonstrated in many fish species: *e.g.*, common carp (Wohlfarth *et al.*, 1986), catfish (Dunham *et al.*, 1990), paradise fish (Gerlai and Csanyi, 1990), tilapia (Uraiwan, 1990; Romano-Eguia and Doyle, 1992), rainbow trout (Gjedrem, 1992), Arctic charr (Elvingson, 1992), and Atlantic silverside (Lagomarsino and Conover, 1993).

GE-interactions could arise due to differences in *environmental sensitivity* (the response of a genotype to changes in the environment). Some genotypes may respond

greatly to a certain change in the environment while some others may show a limited response. For example, Mather and Jinks (1982), comparing ten inbred lines of the tobacco plant (*Nicotiana rustica*) in eight different environments, found that two genotypes that had nearly equal means when averaged over all environments, however, displayed different environmental sensitivities, since one was taller in good environments and the other was taller in poor environments. Such information can be vital to a farmer, who can then choose the genotype suitable for his environment, thus avoiding costly changes in the environment.

The farmer, or indeed even the experimenter, has little control over the presence/absence of a GE-interaction, in a given situation. (It may be possible, however, to change the extent of GE-interaction by selection; see Doyle *et al.*, 1991.) GE-interaction becomes important when individuals of a population are reared in different environments (Falconer, 1989), as for instance in India, where commercial fish farms rear fish obtained as seed from Government seed production centres (Srivastava *et al.*, 1993). These seed production centres generally maintain a limited broodstock and employ the technique of hypophysation to produce fish seed for distribution. In such a case, we have a situation where the same set of genotypes is being subjected to different environments. In the absence of GE-interaction, then, there will be only one "best" genotype, and that genotype would be superior in all environments. However, in the presence of GE-interaction, the concept of a "best genotype" makes sense only in the context of the environment it is grown in.

Since a new effect is generated when G and E are not independent, this effect, the GE-interaction effect, symbolized by I_{GE}, needs to be added to the right hand side of Equation (4.1.5).

That is, $P = (A + D + I) + (E_p + E_t + I_{GE})$ ...(4.1.6)

Or, assuming an interaction of both permanent and temporary environmental effects with the genotype, we have,

$P = (A + D + I) + [(E_p + E_t) + (I_{GE(p)} + I_{GE(t)})]$ ...(4.1.7)

4.1.2 ***Variances:***

Variation in fish size, whether within or among populations or environments (*e.g.*, culture ponds), is a ubiquitous phenomenon (e.g., Purdom, 1974; Koebele, 1985; Jobling and Baardvik, 1994; Ryer and Olla, 1995). It is measured variously as the *variance*, that is, the mean square deviation of the phenotypic value of the trait of all the individuals under consideration (or its square root, the *standard deviation*), the *coefficient of variation* (the ratio of the standard deviation to the arithmetic mean, expressed as a percentage), or the *coefficient of variance* (ratio of variance to the square of the mean). The value that is measured is the observed value, which is the phenotypic value. Thus we have the phenotypic variance (V_P) or the variance of the phenotypic values.

The various terms that comprise the phenotypic value P of an individual are shown on the right hand of Equation (4.1.7). Assuming that these terms are all independent, then the variation seen among individuals must be due to the variation in each of these terms.

That is,

$$V_P = (V_A + V_D + V_I) + [(V_{Ep} + V_{Et}) + \{V(I_{GEp}) + V(I_{GEt})\}] \quad ...(4.1.8)$$

Correlation

It will be noticed that in Equations (4.1.6), (4.1.7) and (4.1.8), the GE-interaction terms have been bracketed along with those pertaining to the environment rather than the genotype. This is appropriate because, as Falconer (1989) mentions, although the term arises due to a variable response of the genotype, the source of the variation is environmental. Therefore, Equation (4.1.7) is simply an expansion of Equation (4.1.4), even when G and E are not independent.

That is, $P = G + E$

Similarly, Equation (4.1.8) can be reduced to:

$$V_P = V_G + V_E \quad ...(4.1.9)$$

However, it can sometimes happen that the genotypic value and the environmental deviation are correlated. For example, certain genotypes may obtain a superior environment simply by virtue of their possessing those genotypes. Such a situation could potentially arise in a fish farm if the farmer, for any reason, allots a preferential treatment to certain strains of the species. Even without any deliberate discrimination by the farmer, however, a covariance between genotype and environment is likely to arise in a culture pond since some fish, by virtue of their possessing superior genotypes, successfully compete against other fish and thus gain preferential access to food, etc. Thus, even in

experimental setups, where care is usually taken in the experimental design to randomize the environments among the genotypes/strains being tested, such a covariance is likely to occur.

This correlation between G and E adds on twice the covariance (Cov_{GE}) between the two, to V_P.

Thus, $V_P = V_G + V_E + 2(Cov_{GE})$ ...(4.1.10)

This equation can be expanded based on Equation (4.1.8), in order to specify the various components. As mentioned before, it is only V_P, the observed variation among the individuals, that is available, *i.e.*, directly measurable.

4.1.3 *Heritability*

A genetic selection programme involves the selective breeding of certain individuals in order to improve the value of the trait in question, in subsequent generations. To accomplish this one must first be able to determine which individuals are to be chosen for breeding, *i.e.*, the individuals with the best breeding values. Unfortunately, it is only the phenotypic value of a trait that is directly measurable, and the breeding values of individuals can only be deduced. Before this is accomplished, however, it is possible to assess the degree of association between phenotypic and breeding values, in a population. This degree of correspondence tells us how closely the phenotypic values reflect the breeding values. This is important because it is the breeding values that determine the influence of the phenotypic values on the next generation (Falconer, 1989).

The degree of association between phenotypic values and breeding values of a trait in a population, thus tells us how heritable that trait is, in that population, and its measure is known as the *heritability in the narrow sense*, or simply, the *heritability* of the trait. The term heritability is also used in another sense, the "broad" sense, where it refers to the extent of genetic determination of a trait. Thus heritability in the broad sense is simply the proportion of phenotypic variance that is genetic in origin, and therefore includes additive, dominance and epistatic variation. Heritability in the narrow sense, on the other hand, refers only to that portion of the genetic variance that is additive, and is expressed as the ratio of the additive genetic variance of the trait to its phenotypic variance. It is symbolized by h^2.

$$\text{Thus,} \quad h^2 = \frac{V_A}{V_P} \qquad \ldots(4.1.11)$$

A large value of heritability can be taken to imply that there is scope for genetic selection for the trait in question, while low values render a trait unsuitable for improvement.

4.2 ESTIMATION OF VALUES AND VARIANCES

Genetic improvement by selection requires an understanding of the genetic properties of the population (Gall and Huang, 1988). Apart from summary values for the population such as heritability and genetic correlations for traits of interest, it is also greatly desirable to be able to predict the breeding value of each of the individuals in the

broodstock. (Incidentally, since the term prediction has been customarily used for random factors such as breeding values while "estimation" is generally reserved for fixed factors, this convention will be followed here too.) In aquaculture, unlike livestock with the concept of stud bulls, farms with breeding facilities maintain both sexes as broodstock. Therefore the potential for genetic improvement of a farmed fish population is potentially double that of livestock, if breeding values of both sires and dams are known. The prediction of breeding values thus plays an important role in any genetic improvement program.

Earlier procedures for the prediction of breeding values, such as those of Smith (1936) and Hazel (1943), were of limited practical use due to their excessive assumptions about the data (Kennedy, 1981). Henderson (1963, 1973) later developed BLUP (Best Linear Unbiased Prediction) - a less restrictive prediction procedure which has since become the standard method for prediction of breeding values, particularly in animal breeding. Even this method, however, requires that the genetic and phenotypic variances of the trait under consideration be known without error. Unfortunately, in practice, this stipulation can rarely be adhered to, particularly for new populations or new traits. The alternative is to obtain estimates of the variance components and then predict breeding values from the same data (Kennedy, 1981), with the result, of course, that the analysis is not truly BLUP. In the strictest sense, in any case, most analyses are never truly BLUP because most models are never true models but only operational models that approximate the true model with guessed values for the variance-covariance parameters (Schaeffer, 1993).

The general mixed linear model can be represented as follows:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Zu} + \mathbf{e} \qquad ...(4.2.1)$$

where,

$\mathbf{y}$ = an $n \times 1$ vector of observations,

β = a $p \times 1$ vector of unknown fixed effects associated with $\mathbf{y}$ by matrix $\mathbf{X}$,

$\mathbf{u}$ = a $q \times 1$ vector of random effects associated with $\mathbf{y}$ by matrix $\mathbf{Z}$, and

$\mathbf{e}$ = an $n \times 1$ vector of residuals.

$\mathbf{X}$ is a known, $n \times p$ incidence matrix, and $\mathbf{Z}$ is a known, $n \times q$ incidence matrix, that assign the various effects to $\mathbf{y}$.

Both $\mathbf{u}$ and $\mathbf{e}$ are assumed to have null means and

$$\mathrm{Var}\begin{pmatrix}\mathbf{u}\\ \mathbf{e}\end{pmatrix} = \begin{pmatrix}\mathbf{G} & \mathbf{0}\\ \mathbf{0} & \mathbf{R}\end{pmatrix} \qquad ...(4.2.2)$$

where we denote the $(n \times n)$ variance-covariance matrix for the vector $\mathbf{e}$ of residual errors by $\mathbf{R}$, and the $(q \times q)$ variance-covariance matrix for the vector $\mathbf{u}$ of random effects by $\mathbf{G}$.

Next, since $\mathbf{X}\beta$ is fixed, assuming that $\mathbf{u}$ and $\mathbf{e}$ are uncorrelated gives the $(n \times n)$ variance-covariance matrix for the vector of observations, $\mathbf{V} = \mathrm{V}(\mathbf{y}) = \mathrm{Var}(\mathbf{Zu}) + \mathrm{Var}(\mathbf{e}) = \mathbf{Z}\mathrm{Var}(\mathbf{u})\mathbf{Z}' + \mathrm{Var}(\mathbf{e})$. Since $\mathrm{Var}(\mathbf{u})$ is denoted by $\mathbf{G}$, and $\mathrm{Var}(\mathbf{e})$ by $\mathbf{R}$,

$$\mathbf{V} = \mathbf{ZGZ}' + \mathbf{R} \qquad ...(4.2.3)$$

The first term accounts for the contribution from the random effects while the second term gives the variance due to the residual effects.

As mentioned above, the mixed model takes into consideration both fixed effects as well as random effects. For the mixed model given by Equation 4.2.1, it can be shown that the BLUE (best linear unbiased estimator) of β is

$$\hat{\beta} = \left(\mathbf{X}'\mathbf{V}^{-1}\mathbf{X}\right)^{-1}\mathbf{X}'\mathbf{V}^{-1}\mathbf{y} \qquad \ldots(4.2.4)$$

with $\mathbf{V}$ given by Equation 4.2.3. Similarly, Henderson (1963) showed that the BLUP (best linear unbiased predictor) of $\mathbf{u}$ is

$$\hat{\mathbf{u}} = \mathbf{G}\mathbf{Z}'\mathbf{V}^{-1}\left(\mathbf{y} - \mathbf{X}\hat{\beta}\right) \qquad \ldots(4.2.5)$$

Both these equations involve the computation of the inverse of the variance-covariance matrix $\mathbf{V}$. This may not be particularly difficult for small sets of data. However, since the dimension of the matrix in question is dictated by the number of observations, analysis of large data sets becomes very severely constrained by the difficulty in inverting large matrices. This need for inversion was bypassed in a major contribution by Henderson (1950, 1953, 1963, 1973, 1984). He developed a neat and compact method of jointly obtaining both $\hat{\beta} = \text{BLUE}(\beta)$ and $\hat{\mathbf{u}} = \text{BLUP}(\mathbf{u})$ by solving his *mixed-model equations* (MME):

$$\begin{pmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z} + \mathbf{G}^{-1} \end{pmatrix} \begin{pmatrix} \hat{\beta} \\ \hat{\mathbf{u}} \end{pmatrix} = \begin{pmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y} \end{pmatrix} \qquad \ldots(4.2.6)$$

Equation 4.2.6 would generally require the inversion of a far smaller matrix than **V**. Only the matrix on the left in the above equation needs to be inverted. Recalling that **X** and **Z** are of dimension $n \times p$ and $n \times q$ respectively, $\mathbf{X}'\mathbf{R}^{-1}\mathbf{X}$ can be seen to be a $p \times p$ matrix, $\mathbf{X}'\mathbf{R}^{-1}\mathbf{Z}$ is $p \times q$, $\mathbf{Z}'\mathbf{R}^{-1}\mathbf{X}$ is $q \times p$, and $\mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z} + \mathbf{G}^{-1}$ is $q \times q$. Therefore, the dimensions of the matrix that needs to be inverted in Equation 4.2.6 is $(p + q) \times (p + q)$. These dimensions should, in general, be considerably less than those of **V** (which is an $n \times n$ matrix).

Secondly, as pointed out by Lynch and Walsh (1997), if **R** and **G** are diagonal then $\mathbf{R}^{-1}$ and $\mathbf{G}^{-1}$ are trivial to obtain. Therefore, while at first glance Equation 4.2.6 looks considerably more formidable than Equations 4.2.4 and 4.2.5, which it replaces, it should actually be computationally far simpler, especially in cases with large data sets.

4.2.1 *Estimation of variance components*

It should be noted that it is an implicit assumption in the equations above that the variance components are known. This is rarely true in practice, and therefore, the resulting predictors are not BLUP. However as the estimates approach the true variances, the resulting predictors approach BLUP (Kennedy, 1981). Obviously then, an appropriate method of estimation of the variances must be chosen.

Kennedy (1981) has reviewed various methods of variance component estimation, which include the ANOVA type methods 1, 2 and 3 of Henderson (1953), maximum likelihood (ML) method of Hartley and Rao (1967), restricted maximum likelihood estimation (REML) procedure of Patterson and Thompson (1971), minimum norm

quadratic unbiased estimation method (MINQUE) of Rao (1970, 1971), and variations on MINQUE. Some of these methods are briefly reviewed below:

4.2.1.1 *Analysis of Variance (ANOVA) estimators*

One of the most common methods of obtaining estimates of variance components is by analyzing the resemblance between relatives. For example, the fact that half sibs share one-fourth of their alleles could be exploited to deduce that the genetic covariance between them should yield an estimate of $\frac{1}{4}V_A$. Similarly, the covariance between full sibs should give an estimate of $(\frac{1}{2}V_A + \frac{1}{4}V_D)$.

An appropriate mating design such as a nested design, where each of several members of one sex are mated with several members of the other sex, chosen at random, would allow calculation of the covariances by partitioning the total variation into variation between half sibs, between full sibs and among the offspring. This is typically done by the standard analysis of variance (ANOVA) technique, after which the observed mean squares are equated to the respective expected mean squares. ANOVA itself has several advantages. It is a widely used standard technique that is conceptually easy to understand, and computationally simple. There are also a number of commercially available computer programmes that can be employed to handle large data. A theoretical justification of ANOVA is that it yields unbiased estimators for the variance components even for data that are not normally distributed.

However, while ANOVA can be a very handy tool for the analysis of data from well controlled experiments, it has some major practical limitations. Field situations and

experiments with live organisms frequently end up with very unequal sample sizes. Unfortunately, while ANOVA can handle small inequalities, severe imbalance in the data renders it unusable. Although modifications have been suggested to account for unbalanced data (Henderson, 1953, Searle *et al.*, 1992), their sampling properties are not well known (Lynch and Walsh, 1997). Secondly, after the expected mean squares are equated to the observed mean squares and the calculations done, it is fairly common to be confronted with negative estimates of variance components. Finally, many experiments are conducted with well pedigreed stocks and there is no known way in which information from relatives can be combined with ANOVA to yield better estimates of variance components.

4.2.1.2 *Maximum Likelihood (ML) estimators*

Variance component estimation by maximum likelihood methods was first suggested by Hartley and Rao (1967). Their method was subsequently simplified by Henderson (1973) in an algorithm based on the mixed model, that produces joint ML estimates of variance components, fixed effects and conditional means of the random variables.

The maximum likelihood approach seeks to obtain the value of a parameter that is most likely to have produced the given observations or data. While we generally think of a probability density function as describing the probability of obtaining a specific value (of the data or observations, **y**) given the parameter, say Θ, the probability density function can alternatively be considered as describing the likelihood that a given value of Θ

underlies the data, given **y** (Lynch and Walsh, 1997). In this alternative interpretation, the density function, usually denoted as $l(\Theta|\mathbf{y})$, is called the *likelihood function* for Θ given the observed data **y**, treating l as a function of Θ with the data vector **y** fixed. Of course, Θ is also a vector if there is more than one parameter. The *maximum* likelihood estimate (MLE) of the unknown parameter(s) is the value of Θ corresponding to the maximum of $l(\Theta|\mathbf{y})$. In other words, the MLE is the value of the parameter that is most likely to have produced the data.

The maximum likelihood of a function is generally easier to find when we take the natural log of the function and work with the resulting *log-likelihood*, normally denoted by L to distinguish it from l, the likelihood. The maximum of the log-likelihood function is found in the usual way by taking derivatives and equating it to zero.

Maximum likelihood (ML) estimators of variance components have many advantages - they are efficient and consistent, place no special demands upon the distribution (except normality, but see Harville, 1977 and Smith, 1980) or balance of data, can utilize information from relatives, have the smallest error asymptotically, and are translation invariant (that is, not affected by changes in the fixed effects). They also have the advantage of being non-negative, but, consequently, are also biased. The procedure fails to account for the loss in degrees of freedom resulting from the estimation of fixed effects, and as a consequence, leads to biased estimates of variance components, particularly that of the residual variance σ_e^2, which is downwardly biased. A large bias in σ_e^2 can in turn, lead to a substantial downward bias in the estimation of the variance of

any random factor (such as that of the breeding values, the additive genetic variance σ_a^2), particularly if the random factor has a small number of levels (Kennedy, 1981).

4.2.1.3 *Restricted Maximum Likelihood (REML) estimators and DFREML*

A modification of ML that eliminates the above bias by taking into account the degrees of freedom needed for estimating the fixed effects, can perhaps be traced back to paper by Cunningham and Henderson (1968). However, it is generally associated with the publication by Patterson and Thompson (1971), who introduced it as a "modified maximum likelihood". This procedure maximizes only the portion of the likelihood that does not depend on the fixed effects. That is, it makes use of a linear transformation of the vector **y**, such that the fixed effects are removed from the model. Being thus a *restricted* version of ML, it has since been generally called restricted maximum likelihood estimation or REML. The elimination of bias by REML is analogous to the removal of bias in the estimation of a variance component by dividing by the degrees of freedom instead of by the sample size (Lynch and Walsh, 1997).

Both REML and ML are iterative procedures and have similar desirable properties (such as non-negativity of estimates), and under a completely random model, both procedures result in the same estimates. However, although REML is computationally more demanding, it is usually the preferred method due to the elimination of bias as mentioned above.

Henderson (1986) described the use of REML in animal and reduced animal models, and his method involves the inversion of the coefficient matrix of the mixed model

equations (Equation 4.2.6). While this is a simpler task than attempting to invert the variance-covariance matrix **V**, it can still become a formidable task for large data sets. However, methods have been developed that avoid matrix inversion. Of these, a procedure (DFREML) proposed by Smith and Graser (1986) and Graser *et al.* (1987) instead, uses a one-dimensional search involving the variant part of the log likelihood to find the maximum of the function.

Consider the mixed linear model (Equation 4.2.1) again, where $V(\mathbf{u}) = \mathbf{G}$ and $V(\mathbf{e}) = \mathbf{R}$. In animal breeding applications, β is a vector of any fixed effects associated with records in **y** by **X**, but **u** is a vector of breeding values, with $Var(\mathbf{u}) = \mathbf{G} = \mathbf{A}\sigma_a^2$, where **A** is the numerator relationship matrix (NRM) and σ_a^2 is the additive genetic variance (variance of breeding values). Finally, $V(\mathbf{e}) = \mathbf{R} = \mathbf{I}\sigma_e^2$, with σ_e^2 being the residual variance.

The ML/REML approach involves working with a log likelihood function for the estimation/prediction of the parameters. Harville (1977) and Searle (1979) proposed the following form of the likelihood function which helps in a derivative-free approach to REML.

$$L = -0.5\left[\text{constant} + \log|\mathbf{R}| + \log|\mathbf{G}| + \log|\mathbf{C}| + \mathbf{y}'\mathbf{P}\mathbf{y}\right] \qquad ...(4.2.7)$$

where,

the constant = $(n - p)\log(2\pi)$, with n being the number of records and p the rank of the part of the coefficient matrix due to fixed effects,

$\mathbf{P} = \mathbf{V}^{-1} - \mathbf{V}^{-1}\mathbf{X}\left(\mathbf{X}'\mathbf{V}^{-1}\mathbf{X}\right)^{-1}\mathbf{X}'\mathbf{V}^{-1}$ (the generalized residual sum of squares),

with $\mathbf{V} = \mathbf{Z}\mathbf{G}\mathbf{Z}' + \mathbf{R}$ (the variance of **y**), and

$$\mathbf{C} = \text{the submatrix} \begin{pmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z} + \mathbf{G}^{-1} \end{pmatrix} \qquad ...(4.2.8)$$

of the coefficient of the general MME:

$$\begin{pmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z} + \mathbf{G}^{-1} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{y}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{y}'\mathbf{R}^{-1}\mathbf{Z} & \mathbf{y}'\mathbf{R}^{-1}\mathbf{y} \end{pmatrix},$$

where **X**, **Z**, **R** and **G** are as defined in Equations 4.2.1 and 4.2.2.

Derivative methods to obtain the unknowns **G** and **R** involve derivatives of L with respect to unique variances and covariances in these matrices, and require non-linear iteration (Boldman *et al.*, 1995).

The derivative-free method, DFREML, proposed by Smith and Graser (1986) and Graser *et al.* (1987), however, is simpler in that it basically tries different **G** and **R** (i.e., σ_e^2 of $\mathbf{R} = \mathbf{I}\sigma_e^2$, and σ_a^2 of $\mathbf{G} = \mathbf{A}\sigma_a^2$) until the combination that maximizes the log likelihood, L (Equation 4.2.7), is found for the data , **y**.

In Equation 4.2.7, $\log|\mathbf{R}|$ and $\log|\mathbf{G}|$ are relatively straightforward to calculate (since, with $\mathbf{R} = \mathbf{I}_n\sigma_e^2$, $\log|\mathbf{R}| = n\log(\sigma_e^2)$, and with $\mathbf{G} = \mathbf{A}\sigma_a^2$, $\log|\mathbf{G}| = \log|\mathbf{A}| + q\log(\sigma_a^2)$, where q is the order of **A**). The strategy proposed by Smith and Graser (1986) to calculate the difficult terms $\log|\mathbf{C}|$ and $\mathbf{y}'\mathbf{P}\mathbf{y}$ is to obtain them simultaneously during the absorption of the animal model equation by Gaussian Elimination (GE).

Incorporating the ideas of Smith and Graser (1986), Meyer (1988a &b, 1989, 1991) developed a series of DFREML computer programs. The programs include calculation of $\mathbf{A}^{-1}$ using the rules of Quaas (1976), and the search strategy for updating **R** and **G** for the MME is the simplex method of Nelder and Mead (1965). These programs were extremely efficient - they increased by five to ten fold the number of equations that could be managed with REML estimation, and reduced computation time by at least as great a factor (Boldman *et al.*, 1995).

Boldman *et al.*, (1995) have written a set of programs collectively called MTDFREML, modifying the DFREML programs of Meyer, that are even more efficient. Briefly, their method is developed as explained here. Boldman and Van Vleck (1991), based on the suggestion of Misztal (1990), incorporated sparse matrix routines instead of GE, in the calculation of the log likelihood, L. Their strategy was based on Choleski factorization using sparse matrix routines in the sparse linear equations package SPARSPAK (George *et al.*, 1980; George and Ng, 1984; Chu *et al.*, 1984). The Choleski factorization procedure involves finding a lower triangular matrix, **L**, the Choleski factor, such that, for a symmetric positive definite matrix **C**, $\mathbf{LL}' = \mathbf{C}$.

For the MME of the form $\mathbf{Cs} = \mathbf{r}$, the two difficult terms $\log|\mathbf{C}|$ and $\mathbf{y}'\mathbf{Py}$ of the log likelihood function L, can be calculated using Choleski factorization (instead of GE) in the following manner. First, find **L** such that $\mathbf{LL}' = \mathbf{C}$, so that $\mathbf{LL}'\mathbf{s} = \mathbf{r}$. Now let $\mathbf{d} = \mathbf{L}'\mathbf{s}$, so that $\mathbf{Ld} = \mathbf{r}$. The vectors **d** and subsequently **s** are then easy to calculate. From **s**, $\mathbf{y}'\mathbf{Py}$ can be calculated as $\mathbf{y}'\mathbf{R}^{-1}\mathbf{y} - \mathbf{s}'\mathbf{r}$. Now since $\mathbf{C} = \mathbf{LL}'$, $\log|\mathbf{C}| = \log|\mathbf{L}| + \log|\mathbf{L}'|$.

Next, the log determinant of a lower triangular matrix is simply $\sum\log(l_{ii})$ where the l_{ii} are the diagonals of **L**. Also, since **L** and **L′** are square matrices, their determinants are the same and therefore, $\log|\mathbf{L}| = \log|\mathbf{L}'|$. Thus, $\log|\mathbf{C}| = 2\sum[\log(l_{ii})]$. The other steps in the Boldman and Van Vleck (1991) algorithm are the same as with the original DFREML programs and make use of the Simplex routine in updating **R** and **G** to maximize L.

Boldman *et al.*, (1995) report decreases of computing time of 200 to 900 times with the SPARSPAK-Choleski strategy, over that of GE. They mention that the main reason for the efficiency of SPARSPAK is because the package needs that any particular data structure be reordered only once; it remembers the reordering when the coefficients of **C** and the RHS's, updated for new guesses of **R** and **G**, are entered each round.

The SPARSPAK factorization requires that the coefficient matrix be of full rank. This implies that constraints would need to be imposed on the coefficient matrix. Determination of the proper constraints, however, is difficult and therefore, modifications have been made in the Choleski factorization of SPARSPAK so that constraints are imposed automatically, and depend only upon the order of the rows and columns in the matrix. Since the order is determined only once, the constraints remain the same unless additional dependencies are added or removed.

The set of programmes MTDFREML were used in this study to obtain estimates of the additive genetic variance and heritability of the trait(s) in question.

Chapter 5

Estimation of genetic parameters in cultivated tilapia (*Oreochromis niloticus*) using DNA fingerprinting. I. Agonistic behaviours.

ABSTRACT

The growth rate of fish, especially in confined waters such as culture ponds, is known to be influenced greatly by behavioural interactions with conspecifics in the water body. The social microenvironment of a fish, which is essentially the set of behaviours elicited by the fish on account of its behavioural/size status, is thus an important determinant of its growth rate. The variation in social microenvironments typically introduces a cyclical feedback loop, wherein differences in social microenvironments leads to differences in growth rates, which in turn leads to further variation in social microenvironments, ultimately resulting in considerable size variation among the fish at harvest. Since there is bound to be some level of growth rate and behavioural variation in any group of fish, rather than attempting to achieve uniformity in growth rates among the fish through management, a more efficient way would be to attempt to reduce the excessive sensitivity to the social microenvironment by a process of genetic selection.

The present chapter deals with evaluating the feasibility of such an exercise by estimating the heritability of the observed agonistic behaviours (Aggression and Submission) as well as two derived variables (Net Aggression, *i.e.*, no. of aggressive acts minus no. of submissive acts, and Total Agonistic Activity, *i.e.*, no. of aggressive acts plus no. of submissive acts). The behavioural observations were done by testing 48-96 randomly chosen pairs of fish from within each of ten maternal half-sib pools of fish. The fish tested were presumed to be behaviourally naive, as the observations were done soon after swim-up. The male parent of the fish from each half-sib group was determined using DNA fingerprinting with microsatellites.

The estimation of variance components was done using the conventional ANOVA as well as the recently developed DFREML technique. The sire component estimates from ANOVA gave a low value of heritability for aggression, and moderate values, ranging from 0.240 to 0.391, for submission, net aggression and total agonistic activity. The DFREML estimates were very low for aggression and submission, but 0.131 and 0.258, respectively, for total agonistic activity and net aggression. It is suggested that there is potential for genetic selection, for at least some of these behaviours, in this stock of fish.

5.1 INTRODUCTION

The growth rate of a given stock of fish, especially in confined waters, is known to be not merely a function of the amount of food provided, but is highly variable even among the fish reared in the same water body and therefore, presumably with equal access to the food provided. Even fish that belong to the same brood and that are stocked when similar in size, begin to quickly show size differences. This variation in size magnifies over time leading to a phenomenon known as growth depensation (Magnuson, 1962). Growth depensation has been observed in many species of commercial importance in food fish culture, such as carp (Wohlfarth, 1977: the papers therein); Arctic charr, *Salvelinus alpinus* (Jobling and Wandsvik, 1983; Jobling *et al.*, 1993); pumpkinseed sunfish, *Lepomis gibbosus* (Blanckenhorn, 1992); chum salmon, *Oncorhynchus keta* (Davis and Olla, 1987); Atlantic salmon, *Salmo salar* (Nortvedt and Holm, 1991, Thorpe *et al.*, 1992); coho salmon, *Oncorhynchus kitsutch* (Fagerlund *et al.*, 1981); rainbow trout, *Oncorhynchus mykiss* (Li and Brocksen, 1977; Metcalfe, 1986); steelhead trout (Abbott and Dill, 1989), and tilapia, *Tilapia zilli* (Koebele, 1985).

There appears to be a social component in the behavioural repertoire of the fish that can selectively inhibit some fish from feeding even when food is available, in the presence of larger, more dominant fish (*e.g.*, Koebele, 1985). Such an inhibition leads to poor growth performance in those fish, and this in turn, translates into reduced profits for the farmer. This reduction in potential biomass can take on alarming proportions when one considers that it is not a few fish that appear to be growth-inhibited, but that the

majority is usually affected, with only a few fish, apparently at the top of the dominance hierarchy, performing well (Wohlfarth, 1977: the papers therein).

Further, size-grading or culling the bigger fish does not appear to eliminate the phenomenon, as then a few other, hitherto smaller fish, occupy the behavioural niche vacated by the culled fish, resulting in the same sort of skewed size distribution in course of time (Wohlfarth, 1977: the papers therein; Gunnes, 1976). Gunnes (*loc. cit.*), investigating this last phenomenon in Atlantic salmon (*Salmo salar*), size graded his fish into two categories - smaller than the modal size and larger than the modal size, and compared their growth rates with that of ungraded controls. He concluded that size grading salmon early in the fish's life might lead to fewer mortalities and more homogeneous growth rates. However, while the larger graded fish showed lower variation than the controls, the smaller fish showed comparable or much higher variation. Thus the gains due to size grading are dubious and the logistics formidable. Rather than culling fish, then, it seems that it would be a more meaningful exercise if the *behaviours* (excessive dominance and excessive sensitivity to dominance) that result in such stunting, could be eliminated from the fish stock by a process of genetic selection.

Some authors argue that since the behaviour patterns that enable monopolization of limited resources are likely to promote fitness (Fausch, 1984; Metcalfe, 1986; Grant, 1993; Nakano, 1995), these must be strongly favoured by natural selection, and thus difficult to eliminate (Ryer and Olla, 1995), suggesting instead that the behavioural mechanisms that lead to heterogeneous growth rates through differential access to food, be circumvented (Davis and Olla, 1987; Huntingford *et al.*, 1990; Noakes and Grant,

1992; Kadri *et al.*, 1996). However, an ideal aquaculture environment is not limiting in resource (food), and fitness is not merely growth related - it is decided by the culturist based on a number of factors. Indeed, an extremely large (fast-growing) fish might actually be eliminated from the broodstock because of the difficulty in handling, especially in species where manual stripping is done.

In any case, even if fast growth does lead to higher fitness in an aquaculture environment, the behaviours that lead to fast growth in a well managed culture pond are not necessarily the same as those in the wild. Resource monopolization is not a necessity (for feeding) in the culture pond; enough food should be spatially and temporally distributed so as to avoid any contest competition. Under such circumstances fish engaging in such behaviours are likely to forfeit some growth because of the diversion of energy towards "unnecessary" activities (Doyle and Talbot, 1986). Elimination of behaviours that promote such costly activities by means of genetic selection should then lead to stocks that exhibit not merely a high mean growth rate but also a greater homogeneity in their growth rates.

The present study is an attempt to find the feasibility of such an exercise, by estimating the heritability of agonistic behaviours as well as growth rate, and extent of genetic correlation, if any, between the two traits in a cultured tilapia (*Oreochromis niloticus*) population. Agonistic behaviours, and consequent dominance behaviour in cultured fish and its genetic relationship with growth rate, has been the subject of some controversy in the recent fish literature (*e.g.*, Ruzzante, 1994; Swain and Riddel, 1990; Doyle and Talbot, 1986). This chapter describes the estimation of the variance

components of different agonistic behaviours, and thus the heritability of the traits, in the young of the fish (*Oreochromis niloticus*).

Size-Aggression Feedback Loop

While it is commonly observed that larger fish are more aggressive and dominant, what has not quite been resolved is whether dominance behaviours are the result of larger body size or whether larger body size is the result of dominance behaviours, although there appears to be some evidence of an inherent "fierceness" in young fish that could confer a size advantage over time (Huntingford *et al.*, 1990). In any case, once size variation has been manifested, it becomes impossible to separate out the behaviours from the influence of relative size at the phenotypic level, as there is a positive feedback loop between behaviour and growth rate, thus making the study of either trait without the confounding effects of the other, very difficult.

This study circumvented the feedback loop in the following manner. On the one hand fish were tested for agonistic behaviours at or soon after swim-up, when the fish were, presumably, relatively "naive". More importantly, behavioural testing was done between pairs of fish chosen randomly from a pool of full- and half-sibs (the individuals chosen being later identified by DNA "fingerprinting"), such that each sire[1] had the behaviour of its offspring recorded in encounters with a random sample of the entire available range of size variation. Next, as per the design of the experiment, each full-sib

[1] In keeping with the practice in the animal genetics literature, from which this study has borrowed, as well as for simplicity, the terms "sire" and "dam" are used throughout, to refer to male and female parents, respectively.

family was split into three groups before being pooled with its half-sibs, one being used for behavioural observations and other two for the growth experiments (see Chapter 6 for details of the growth experiment). Thus, behavioural and growth experiments were not done on the same individuals, in which case behaviour and relative size would be confounded through the feedback loop which results from the phenotypic correlation of the traits at the level of the individual. Rather, the data for each sire family comprised behavioural observations and growth measurements on different full-sibs. Finally, genetic associations between traits were obtained by correlating the corresponding breeding values of the parents (sires) and the rest of the pedigree (*i.e.*, all the fish except those with measurements), for the different traits. (Breeding values for all the individuals in the pedigree, both with and without records, are obtained in an output file in MTDFREML, the set of computer programmes used in this study - for details, see Chapter 4) Since no measurements were taken on these individuals, the question of phenotypic confounding of behaviour and relative size does not arise.

Until recently the methodology for the estimation of the variance components depended largely on the analysis of variance (ANOVA) of records (measurements) on individuals that were related in certain ways (parent-offspring, half sibs, full sibs). These methods, although the mainstay of quantitative geneticists for long, are time-consuming and often involve the use of complex mating designs that are difficult to achieve. This ANOVA-based methodology has been well documented by Falconer (1989), Becker (1984) and Turner and Young (1969), among others.

Development of the mixed model (*e.g.*, the so-called animal model) along with the BLUP (Best Linear Unbiased Prediction) estimation of genetic values by Henderson (1950, 1963, 1975, 1984) and the REML / DFREML (Restricted Maximum Likelihood / Derivative-Free Restricted Maximum Likelihood) estimation of variance components (Cunningham and Henderson, 1968; Patterson and Thompson, 1971; Smith and Graser, 1986; Graser *et al.*, 1987) has revolutionized the study of quantitative genetics, particularly in its applications to genetic selection, making it now the routine method of choice, especially in livestock research.

This methodology eliminates most of the problems associated with ANOVA estimation of variance components, such as demands on the design or balance of data, and negative estimates of variance components. Further, it can be used for any arbitrary pedigree of individuals. These methods are now being incorporated into aquaculture genetics research as well (*e.g.*, Gall *et al.*, 1993).

The methodology employed in this study involves the use of DNA fingerprinting to establish parentage of fish reared together from swim-up. Variance component estimation of the behavioural traits is done using the ANOVA approach and a DFREML estimation using an animal model, employing a set of programs, MTDFREML, by Boldman *et al.*, (1995). The results of these methods are compared and interpreted in the light of the often rather subtle differences in the assumptions that underlie the methods.

5.2 MATERIAL AND METHODS

5.2.1 *Mating Design*

The fish used in this study was the tilapia *Oreochromis niloticus* at the tilapia culture facility of The Marine Gene Probe Laboratory (MGPL), Department of Biology, Dalhousie University. This fish was obtained by MGPL from the National Inland Fisheries Institute (NIFI), Thailand. The MGPL broodstock is tagged with Passive Induced Transponder (PIT) tags, and breeding events are easily recorded. Sixteen families (A - P) are being maintained in this line, and breeding is by manual stripping of both sexes. Rotational mating is used to minimize inbreeding.

For this study, crosses were made from these 16 families according to a nested design (1 female × 4 males), and ten half-sib families (A1, A2, B1, B2, C - H), henceforth referred to as 'dam groups', generated as shown in Table 5.2.1. Care was taken to ensure that each parent in a half-sib cross belonged to a different family (as maintained at MGPL), in order to minimize inbreeding. However, there was some sharing of parents among the sire and dam groups, although the design was not fully factorial. Thus A1 and A2 shared the same male parents (sires), as did B1 and B2. Further, A1 and C shared the same female parent (dam), as did B2 and E.

The actual breeding was done by stripping a ripe female into four different containers and fertilizing each with the milt from a chosen male. The four fertilized batches of eggs (*i.e.*, full-sib families, henceforth referred to as 'sire groups') in each dam group were hatched separately. Upon hatching, equal numbers (50 or 100, depending on

the number of eggs laid) from each batch were pooled and reared in an aquarium (labeled B), for the behavioural experiment.

The MGPL tilapia culture facility is under computer control that regulates the flow rate and temperature of the water entering the fish tanks. All the aquaria had a constant flow of pre-heated (28°C) and dechlorinated water. Metabolites and other chemicals were not allowed to accumulate due to the constant flow-through. The hatchlings were fed freshly hatched brine shrimp (*Artemia sp.*) larvae once daily, for 3-4 weeks. After about two weeks, they were also given finely powdered custom-made tilapia chow (Corey Foods). Food was given once daily, *ad libitum*.

5.2.2 *Measurement Of Behaviour*

Forty eight small Styrofoam cups were numbered (from 1 to 48, on the floor of the cup so as to be visible from above), and each glued into a 500 g. plastic yogurt container. These were placed in a long shallow tank, in 4 rows of 12 units each. A framework made of slotted angles was constructed around the tank, from which was suspended a video camera that could be moved back and forth as well as sideways. The video camera was connected to a video cassette recorder and a large TV monitor. The cups were filled with de-chlorinated, heated (28° C) and aerated water. (The outer yogurt container was also filled with water to provide stability.)

When the fish reached 3-4 weeks of age, a pair of fish was randomly chosen from the aquarium housing the fish slated for the behavioural experiments, and both members of the pair introduced simultaneously into a cup. It must be remembered that this aquarium

contained a dam group of fish, with equal numbers from each sire group. This meant that within each pair of fish, a member of one sire group (*i.e.*, the offspring of one male parent) was paired with a fish chosen at random from the half-sib pool, with equal probability of the second fish being chosen from any of the four full-sib families. Thus, the families of the paired members were randomized. As well, the relative size of each fish *vis-a-vis* its partner was also randomized, since any given male parent was likely to have offspring encountering fish larger than themselves as well as smaller than themselves, the proportions depending upon the size distribution of the brood.

Once introduced into the Styrofoam cup, the two fish were allowed to acclimatize for 15 minutes. During the period of acclimatization, the two members of each pair of fish invariably entered into a dominant-subordinate relationship, with the one fish attempting to stay at the bottom of the cup while chasing the other to the water surface. The fish at the bottom was therefore termed the winner of the encounter, and the one at the surface, the loser. Upon acclimatization, the number of aggressive behaviours, **AGR** (nips, chases, lateral displays, *etc.*) displayed by each of the two members of the pair was noted over a period of 5 minutes. Recording of the number of submissive behaviours (flights), **SUB**, displayed by each of the two members of the pair was initiated only at a later date and therefore only 5 dam groups had both kinds of behaviour recorded. These counts were made with the help of a manual laboratory counter. Since these counts were being made by watching the fish on a TV monitor, there was minimal disturbance to the fish and they were essentially unaware of the presence of the investigator during the observation period. Finally, the winner/loser status (**W/L**) of each fish was also noted at the end of the

observation period, by assigning the losers and winners a score of 1 and 2, respectively. Using the two measured behaviours **AGR** and **SUB**, where possible, three derived variables were constructed: "Net Aggression", **NET** (by subtracting **SUB** from **AGR**) - to give the extent to which a fish was more aggressive than submissive; "Total Agonistic Behaviours", **AGON** (by adding the **AGR** and **SUB**) - to give the extent of total activity; and "Difference in Aggression", **DIFF** (by taking the difference in **AGR** of the two fish) - to give the extent to which one fish was aggressive over the other.

At the end of the observation period, both fish were netted out (it was easy to separate the loser from the winner, since they occupied different areas), killed with an overdose of benzocaine and size measurements (length and weight) were made. The two fish were then preserved separately, in 100% ethanol for family identification with microsatellite DNA polymorphism. Table 5.2.2 summarizes the various measurements made on the fish in each half-sib family.

5.2.3 *Genotyping For Parental Identification*

Of the two members of each pair of fish used in the behavioural measurements, one was chosen randomly for genotyping. DNA extraction, PCR amplification and allele identification was done as described in Chapter 3. Alleles of offspring were matched with those of the parents to determine male parentage, as the inheritance of these loci follows a simple Mendelian pattern (Harris *et al.*, 1991; Queller *et al.*, 1993) . Use of microsatellites to determine female parentage was not necessary since the female parent

was the common parent, within half-sib families. However, each dam's DNA microsatellite profile at each locus was also recorded and had to be used for the unambiguous identification of the (male) parent. The pedigree of all the parents was available from MGPL records.

5.2.4 *Data Analysis*

After determining parentage of the fish studied the data were now amenable for statistical analysis to estimate genetic variances by the conventional ANOVA and the animal model/DFREML approach (using MTDFREML). These approaches are discussed in detail in Chapter 4.

The format of the data for ANOVA was a standard nested design with males nested within females.

The statistical model used in the ANOVA was:

$$y_{ijk} = \mu + d_i + s_{j(i)} + e_{ijk}$$

where,

y_{ijk} = the measurement on the k^{th} offspring of the j^{th} sire of the i^{th} dam,

μ = the overall mean,

d_i = the contribution of the i^{th} dam,

$s_{j(i)}$ = the contribution of the j^{th} sire of the i^{th} dam,

e_{ijk} = the random residual error.

Since the number of offspring unambiguously assigned parentage differed among families, the data used in the analysis were unbalanced. The analysis of variance was done

using the MGLH module of SYSTAT 7.0 for Windows (1997, SPSS Inc.), with the coefficients k_1, k_2 and k_3 determined using the formulae given by Becker (1984), and Sokal and Rohlf (1981, p. 297).

The animal model/DFREML approach was used by means of a set of computer programs collectively called MTDFREML (Multiple Trait Derivative-Free Restricted Maximum Likelihood), written by Boldman *et al.*, (1995), which was downloaded on to a PC from a ftp site in the World Wide Web (cgel.agsci.colostate.edu in pub/cvantass). While this program was primarily written with livestock quantitative geneticists in mind, it can be used with any data set with pedigree information. The data used in the analysis here contained pedigree information running back to four generations. As mentioned earlier the pedigree information beyond the immediate parents was obtained from MGPL records.

The method used by MTDFREML involves the use of MME (mixed model equations) for the simultaneous solution for fixed effects and random effects (breeding values) and estimation of (co)variance components, using a modification due to Kachman (Boldman *et al.*, 1995) to handle singularity of the MME. (For details see Chapter 4). (Co)variance component estimation by the program requires that starting values of the components be given. It then maximizes the log likelihood function (or minimizes -2 log of the likelihood function, termed the FVALUE) by a process of iteration. This is done by the Simplex (polytope) method described by Nelder and Mead (1965). Convergence occurs when the global maximum of the log likelihood function (or equivalently, the minimum of the FVALUE) is found, which is tested by restarting the program with the

estimates at apparent convergence as starting values, or with a fresh, "cold" restart with new priors.

Following is the animal model for which the FVALUE is sought to be minimized:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Zu} + \mathbf{e}$$

where,

$\mathbf{y}$ = an $n \times 1$ vector of observations, and n = number of animals observed.

β = a $p \times 1$ vector of unknown fixed effects associated with $\mathbf{y}$ by matrix $\mathbf{X}$,

$\mathbf{u}$ = a $q \times 1$ vector of random effects associated with $\mathbf{y}$ by matrix $\mathbf{Z}$, and

$\mathbf{e}$ = an $n \times 1$ vector of residuals.

$\mathbf{X}$ is a known, $n \times p$ incidence matrix, and $\mathbf{Z}$ is a known, $n \times q$ incidence matrix, that assign the various effects to $\mathbf{y}$.

Since $\mathbf{X}\beta$ is fixed, assuming that $\mathbf{u}$ and $\mathbf{e}$ are uncorrelated, $\mathbf{V}$, the $(n \times n)$ variance-covariance matrix for the vector of observations is given by the relationship, $\mathbf{V} = \mathbf{ZGZ}' + \mathbf{R}$, where $\mathbf{R}$ denotes the $(n \times n)$ variance-covariance matrix for the vector $\mathbf{e}$ of residual errors, and $\mathbf{G}$ the $(q \times q)$ variance-covariance matrix for the vector $\mathbf{u}$ of random effects (breeding values). In animal breeding applications, β is a vector of any fixed effects associated with records in $\mathbf{y}$ by $\mathbf{X}$, but $\mathbf{u}$ is a vector of breeding values, with $\mathrm{Var}(\mathbf{u}) = \mathbf{G} = \mathbf{A}\sigma_a^2$, where $\mathbf{A}$ is the numerator relationship matrix (NRM) and σ_a^2 is the additive genetic variance (variance of breeding values). Finally, $\mathrm{V}(\mathbf{e}) = \mathbf{R} = \mathbf{I}\sigma_e^2$, with σ_e^2 being the residual variance. The program requires that starting values for σ_a^2 and σ_e^2 be provided. Details regarding the animal model, DFREML estimation of variance

components and the MTDFREML set of computer programmes are provided in Chapter 4.

The set of programs MTDFREML comprise three main programs:

1) MTDFNRM, which forms the inverse of the relationship matrix using an ASCII free-formatted pedigree file, based on the rules of Quaas (1976),

2) MTDFPREP, which uses an ASCII free-formatted data file to form MME according to model specifications supplied to the program, and

3) MTDFRUN, which solves the MME for (co)variance components, using starting values supplied to the program.

5.3 RESULTS AND DISCUSSION

The phenotypic values of the observed variable **AGR** (number of aggressive behaviours exhibited by the genotyped fish in a 5 m. period) were investigated first, and the findings are given below.

A. PHENOTYPIC ANALYSIS

5.3.1 Relationship of aggression to other variables

The features of the observed aggression included a fairly high intensity (an average of 0.1 aggressive acts/second, with a high of 0.94 acts/second), and a very high amount of variation (CV = 133 per cent). To help understand this variation among the fish, the trait was investigated as below.

Resource-independent competition or territoriality?

During the behavioural observations, no specific resource (such as food) was placed in the cup. Therefore it can be assumed either that the fish were engaged in a competition that was resource-independent, or that they fought over territory. Since the number of attacks invariably decreased when the attacked fish occupied the surface of the water, it appears very likely that fish were being territorial. Therefore, the fish that stayed in the bottom after successfully chasing away the other to the surface was termed the *winner*, and the fish at the surface the *loser*. Tilapia are herbivores and detritivores (Bowen, 1982), and are likely to find food both in the water column as well as the bottom. However, staying at the bottom ensures that they are not preyed upon by birds. It is therefore an adaptive strategy to strive to stay at the bottom, and to reduce the competition for food there as well.

In a contest, a fish can attack, display or flee, the option chosen presumably being based on the cost-benefit ratio to the animal. As observed in this study, the costs of losing an aggressive encounter are possible injury and perhaps even death caused by the antagonist, which can be minimized by fleeing to the surface. The cost of staying at the surface is the hypothesized risk of predation. Perhaps the fish at the surface could be vigilant for predators, and submerge at the first sign of one. Indeed, it was seen that when the fish at the surface could sense my approach as I went to pick them out, it would immediately submerge to the very bottom of the cup, right next to the erstwhile aggressor. Both fish would then become completely immobile, and the aggressor would accept the other fish in its territory for some time even after the perceived threat (myself) was

removed, after which, however, it would re-establish its claim to the bottom and chase the other fish back to the surface. Therefore, for the fish that was certain to lose the fight between the two, the risk of paying a heavy cost by the staying at the top was evidently perceived to be smaller than staying at the bottom with a superior fighter.

Aggression and relative size

One of the obvious advantages to a fish in a conflict would be the greater strength conferred upon it by a bigger size. In order to verify this, **AGR** was plotted against relative size (the ratio of the total lengths[2] of the two fish), which can be seen in Fig. 5.3.1.1 (a). As expected, the bigger fish was the winner most of the time. The winners in the figure are mostly placed beyond the relative size of 1 (*i.e.*, equal size). It can be seen that the envelope of scores follows a more-or-less bell shaped curve that peaks sharply at around a relative size of 1. This is as one would intuitively expect, since a smaller fish cannot afford to be very aggressive, and a larger fish does not need to be excessively aggressive, in order to win a contest. On the other hand, each of two equal sized fish needs to establish dominance, as they have no size cue to help them; hence the greater aggression. Higher levels of aggression among size-matched animals has been observed in other animals too such as the domestic chicken (Rushen, 1985) and pigs (Tindsley and Lean, 1984; Rushen, 1987). It should be noted from the figure that although the envelope peaks at a relative size around 1.0, all levels of aggression are observed at that value. All

[2] Standard length was not measured for one dam group, and therefore, total length has been exclusively used in the behavioural study.

aggression values less than the maximum (envelope) value appear to be more or less equally likely between winners and losers.

During the observations, it was also seen that the costs of aggression depended upon the behaviour of the fish, given its relative size. A smaller fish that was initially aggressive usually appeared to receive many more aggressive acts directed at it, when compared to a fish that fled to begin with. These can be seen in the area marked 'A' in Fig. 5.3.1.1 (b). On the other hand, a smaller fish that did not reciprocate with aggression, but did not immediately flee to the surface either, tended to receive as much aggression as the one that was initially aggressive; they can be seen clustered (open circles) around the X axis in the figure.

Many fish displayed both aggressive as well as submissive behaviours. This is consistent with the game-theoretic idea that the initial agonistic exchanges represent an "information gathering" stage in the contest, which leads to decision that is adaptively advantageous to both because it avoids the risks of mortal combat (Maynard Smith, 1974). However, as mentioned earlier, scoring of both types of behaviours was initiated some time after the beginning of the experiment, and scores are available for only five dam groups (Table 5.2.2). Fig. 5.3.1.2 shows the plot of submissive scores against relative size. It can be seen that submissive acts (flights) were almost totally confined to the losers. In Fig. 5.3.1.1 (a) we see that aggressive acts (nips, bites, lateral displays and chases) are performed by potential losers as well as winners. However, Fig. 5.3.1.2 shows that submissive acts do not appear to be performed by the eventual winners at all, barring very few exceptions. Perhaps an animal that submits, even occasionally, forfeits any

chance of eventually winning the encounter. Thus, while it is commonplace to find both members in an encounter display aggressive behaviours, it appears that the likely winner can be behaviourally distinguished, not so much by its aggressive acts *per se*, but more by its lack of submission. We see in the figure that while there are some larger fish that show some submissive behaviours, they are mostly losers.

As mentioned earlier, for those groups where both aggressive as well as submissive scores were available, two new variables were constructed for each fish: "Net Aggression", **NET**, obtained by subtracting the submissive behaviours from the aggressive, and "Total Agonistic Behaviours", **AGON**, the sum of the aggressive and submissive acts (total agonistic activity). Further, for all the fish, the difference in aggression levels of the members of each pair, was obtained by subtracting the **AGR** of the opponent from that of the genotyped fish, to get the derived variable, "Difference in Aggression", **DIFF**.

NET and **AGON** can be seen plotted against relative size in Fig. 5.3.1.3 (a & b, respectively). Each graph in the figure gives a slightly different perspective on the relation between social behaviour, relative size, and the outcome of an encounter in terms of winner/loser status. In (a), we see that, unlike **AGR** in Fig. 5.3.1.1, winners and losers split up not only around the point of equal size (relative size = 1), but also around the point of equal aggression and submission (net aggression = 0). Thus, **NET** is a better determinant of eventual winner/loser status than **AGR**, which does not distinguish the two. It can also be seen from the figure that **NET** scores are low (around zero) for a relative size of one. This is what one would expect from the game-theoretic stand-point,

when the incidences of attacks and flights would be essentially equal and random in the absence of auxiliary information on the eventual outcome, which is provided by relative size (Maynard Smith, 1976). In fact, the figure shows high levels of net aggression (among the winners) at a relative size beyond one. In this context a closer look at Fig. 5.3.1.1 (a) reveals that the highest levels of aggression are not exactly at a relative size of one but slightly beyond it. In any case, Fig. 5.3.1.3 (a) quite clearly reveals the role of behaviour and/or relative size in determining or predicting winner/loser status. Fish that are larger also are more aggressive than submissive, and typically are winners.

In Fig. 5.3.1.3 (b) we see that the role of relative size is once again emphasized. Fairly high levels of agonistic behaviours, **AGON**, are seen across a wide range of relative sizes. However, the larger fish are winners and the smaller ones are losers. This graph appears to show comparable levels of agonistic behaviours among both winners and losers. However, from the graphs that we have already seen, it is clear that while many of the agonistic scores of the losers have large doses of submission in them, those of the winners are almost all pure aggression.

In order to further investigate what factors made a fish more, or less, aggressive than another in an encounter, **DIFF** was plotted against relative size, in Fig. 5.3.1.4. While the distinction between winners and losers is quite clear in all the graphs, it seems remarkable here in that there are very few "trespassing" points. Clearly, in order to win a contest, a fish that is equal in size to, or only slightly bigger than the fish it confronts, needs to be more aggressive than the other fish.

It is thus quite clear from the above graphs that there is a distinct relationship between aggression and relative size - fish are not nearly as aggressive towards fish far smaller than themselves, as they are towards fish that are almost equal in size. However, while this seems to be the general rule, there is a region of overlap, in almost every graph. This is a region where this general rule seems to be in question, and is therefore, of (genetic) interest. However, on checking to see if any particular dam/sire groups were represented in these regions, no specific trend was noticed.

In summary, among the behavioural variables, **NET** appears to be the variable that is ideally suited for further analysis, for theoretical as well as empirical reasons. By definition, a **NET** score tells us how much more (or less) aggressive a fish is than submissive. As seen in this study, submissiveness is a response to aggression by the antagonist; a submissive fish does not flee (criterion and measure of submissiveness in this study) unless attacked. Moreover, submissiveness is a safe response inasmuch as the well-being of the fish is concerned. In the confines of the container used to record behaviours in this study, the submissive fish was unable to flee out of sight of the aggressor. However, as long as it remained at the surface, the aggressor seemed appeased and generally did not attack as frequently or intensely as at the bottom of the cup. It was also observed that lack of submissiveness (when attacked) resulted in injury and/or escalated conflicts. A reasonable extrapolation to the pond environment can therefore be that a submissive fish fleeing from an attacker would soon go out of sight/territory and would be left alone (at least by that antagonist). On the other hand, a fish that does not flee appears to provoke the aggressor, thus leading to high energy

expenditure and risks of injury. Therefore, while an ideal aquaculture environment would be made up of no aggressors, certainly submission (fleeing to the minimum necessary distance) appears to be the "correct" strategy to adopt, as there is really nothing to fight over - food is available for everybody. This definition of submissiveness is akin to the "dove" strategy of Maynard Smith (1976).

However, it must be noted that adaptive strategies being considered in this study are not in terms of resource holding capacity (as there is no resource to hold) or in resolution of other conflicts among the fish. Rather, the strategies must lead to better growth, survival and attainment of sexual maturity. With these criteria, a fish with a high **NET** score should, in theory, have low fitness, as it would have a low probability of being chosen for breeding (since its energy is likely to be diverted from growth to agonistic behaviours). **NET** is seen to be a good predictor of the eventual outcome of encounters in terms of resource (or territory) holding ability (Fig. 5.3.1.3) - an energy demanding activity. Therefore, **NET** is an appropriate behavioural variable to investigate as a trait for selection. Further, there appears to be sufficient variation in the trait, thus rendering it suitable for genetic analysis. (While the trait **SUB** is the best behavioural predictor of eventual winner/loser status, there is little variation among the winners, Fig. 5.3.1.2.)

Aggression and age/size of fish

The different dam groups that were tested for aggression were of not quite the same age or size. Therefore, it is of interest to see if the level of aggression displayed in any way changed during ontogeny. However, since it has already been found that the

level of aggression displayed by a fish is strongly related to its relative size, this influence had to be first eliminated for understanding the effect of age and size *per se*. This was done by taking the residuals after fitting a 5th degree polynomial (best fit) to the **AGR** scores regressed on relative size, as in Fig. 5.3.1.1 (a).

The mean values of the trait, corrected for relative size as described above, was plotted (Fig. 5.3.1.5) against the age (in days) of the dam group (a) and the (total) length (cm) of the fish (b). No clear pattern is evident from either graph; the correlation coefficients are, respectively, 0.328 and 0.237 (both non-significant). These graphs, due to the lack of evidence of any significant influence, serve to again undescore the importance of *relative* size in determining the level of aggression.

Aggression and inbreeding

Inbreeding refers to mating between individuals that are related (*i.e.*, they have a common ancestor and therefore genes identical by descent). Traits connected with fitness are most subject to inbreeding depression - deterioration in a quantitative trait as a result of inbreeding (Su *et al.*, 1996; Falconer, 1989, p. 249), and therefore inbreeding is generally avoided in controlled mating systems such as in aquaculture. One of the output files of the MTDFREML programme gives the inbreeding coefficient (the probability that two alleles at a given locus are identical by descent) for all the fish with pedigree information. In order to see if the behavioural traits studied here were affected by the level of inbreeding, the fish were categorized based on the inbreeding coefficient, and the mean trait values of the fish at each inbreeding level were plotted against the inbreeding

coefficient (Fig. 5.3.1.6). As can be seen, there appears to be a rapid decline in the mean values of the traits with inbreeding (inbreeding depression); all correlations, except that with **NET**, were significant at $p < 0.05$. This decline in the mean values leads to the following speculations regarding the nature of genetic determination of these behavioural traits.

First, the strong negative association of the behavioural traits with inbreeding as seen in the figures indicates that there is a genetic basis to these traits. Secondly, as these behaviours appear crucial to ensure success in competition, it is reasonable to expect that they are closely tied in with the fitness of the animals. Perhaps the evidence of declining values with increasing levels of inbreeding also point to their role in determining the fitness of the fish, as traits closely connected with fitness are most vulnerable to inbreeding depression. Further, although not entirely clear from the figures (due to the small number of data points) it appears that the decline in the most traits is linear, implying that the loci affecting the traits combine additively, with low epistasis (Falconer, 1989, p. 251). (Lack of epistasis is very useful since it then means that dominance is the only other source of variation that adds to the estimate of additive genetic variance from full sibs in this study - see below.) Finally, since inbreeding invariably increases homozygosity in an unselected population (Falconer, 1989, p. 60), the figures indicate that heterozygotes (fish with lower inbreeding coefficients) are "superior" (have higher mean values), it can lead to one of two hypotheses regarding the genetic cause of inbreeding depression (Johnston and Schoen, 1995; Crow, 1952): the overdominance hypothesis or the partial dominance

the difference between the two corrected measures. However, since **SUB** does not render itself amenable for any straightforward correction (Fig. 5.3.1.2), such a transformation could not be attempted for **NET**. The scores, however, were corrected for the effect of inbreeding, to give **COR-NET**, just as in the case of **AGR**. Similarly, **SUB** and **AGON** scores were corrected for the influence of inbreeding, to give **COR-SUB** and **COR-AGON**, respectively.

Figure 5.3.2.1 shows the box plots for **COR-AGR** (a) and **COR-NET** (b), plotted for each set of half-sib families (*i.e.*, dam groups). Further, Table 5.3.2.1 shows the mean and standard deviation of the two traits for each dam group, and Figure 5.3.2.2 (a and b, respectively) shows a plot of the mean scores. The latter figure reveals considerable variation among the dam groups, for both traits. Further, the relative performance of each dam group is fairly consistent between the two traits, except perhaps for D and H. A one-way ANOVA (Table 5.3.2.2) confirmed that the dam groups do differ significantly ($p < 0.001$ and $p = 0.05$, respectively, for **COR-AGR** and **COR-NET**) in the average number of the behaviours. This result itself, of course, does not reveal much about the variation being necessarily due to differences among dams, much less about genetic differences. This is because effects of common environment (tank effect), if any, are confounded with any real differences among dams, since all the offspring of a given dam were housed in a single tank.

hypothesis. The overdominance hypothesis states that the interaction between the dominant and recessive alleles in the heterozygote confers on it a value superior to both the homozygous dominant and the homozygous recessive. On the other hand, the partial dominance hypothesis suggests that the decline (inbreeding depression) is a result of mutation giving rise to deleterious alleles that are exposed in the homozygous state, thus conferring an inferior value to the homozygotes even though the dominant allele is only partially dominant.

5.3.2 Comparisons among dam groups

All the following analysis is done for both **AGR** (since it is the original and the only complete behavioural measure of a given individual) as well as **NET** (for reasons given above). Before attempting any comparisons among dam groups using the trait **AGR**, however, it was necessary to remove the effects of relative size, which was done as mentioned above. Further, since the level of inbreeding was seen to have a strong influence on the trait (Fig. 5.3.1.6 (a)), inbreeding effects were also removed from these size-corrected aggression scores (to account for the effects on the phenotypic expression of the traits - Van Vleck, *pers. comm.*) by taking the residuals after a linear regression of aggression scores on the inbreeding coefficient. These aggression scores, corrected for both relative size as well as inbreeding effects, are henceforth termed **COR-AGR**. For **NET**, while the influence of relative size is very clear (Fig. 5.3.1.3 (a)), there is no obvious way in which this influence can be eliminated. If **SUB** could have been corrected for the effect of relative size, just as **AGR** was, then perhaps **NET** could have been obtained as

5.3.3 Comparisons among sires (within dams)

Table 5.3.3.1 shows, by half-sib group, the number of offspring unambiguously identified (using microsatellites) for each sire, their mean score and standard deviation for **COR-AGR** and **COR-NET**. The mean scores for both traits are plotted for each sire, for all the dam groups, in Figure 5.3.3.1. A few points of interest can be noted from the table and figures. First of all, variation among the sires-within-dams[3] appears quite high for some dam groups, for both traits. The overall variation, however, was found to be statistically non-significant ($p = 0.367$) for **COR-AGR** but significant for **COR-NET** ($p = 0.001$; Table 5.3.3.2). This means that the sires-within-dams, on an average, did not differ from each other in their aggression score, but did differ in the amount by which their aggression levels exceeded submission levels.

In the case of **COR-AGR** it is not surprising that there is lesser variation among sires-within-dams than among dams, since not only is there greater genetic divergence among half-sib groups than within, the dam groups in this study carry a possible common environment effect with them, and also perhaps differ due to differential maternal effects. As far as **COR-NET** is concerned, however, the table shows non-significant differences among dam groups. While the reasons for this can only be speculated, this fact has

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[3] All statements regarding comparisons among dams or sires actually refer to comparisons among the corresponding offspring. Thus, a statement such as "the dams differed significantly in their **COR-AGR** scores" will actually mean that the average performance of the offspring of the dams differed significantly, in the given trait. Such a usage will be retained henceforth only for semantic ease, and should not not construed to imply any conclusions regarding the parents themselves, unless mentioned specifically (as for example, while referring to breeding values).

amounted to each set of sire-groups being tested in what is essentially the same environment.

Next, it can be seen from the figure (Fig. 5.3.3.1) that the rank order of the sires of dam groups A1 and A2, which share the same set of sires, numbered 50-53 (Table 5.3.3.1), is not the same. Similarly the sires 54-57 do not display the same rank order in B1 and B2. For instance, the offspring of sire 52, which have the highest mean score in dam group A1, are actually the least aggressive in A2. Similarly, the offspring of sire 54 have the highest mean score in B2, and the lowest in B1. Thus, there appears to be an interaction effect between sires and dams. However, an ANOVA with interactions (Table 5.3.3.3), done separately for these two sets of dam groups, showed that the apparent interaction is not significant ($p > 0.30$).

From the above phenotypic analysis of the trait aggression, the following conclusions can be drawn:

1) Dam groups differ significantly from each other, on an average, as far as total aggression is concerned, but do not differ in their net aggression scores.
2) Sire-within-dam groups (half-sibs), however, show the converse behaviour, for the two traits - they do not differ significantly from each other in total aggression, but do differ in net aggression.
3) The single most influential environmental factor is the relative size of the fish.
4) Aggression levels are the highest when the adversaries are more or less equal in size.
5) Aggression levels are unrelated to the size and age of the fish, *per se*.

6) In an agonistic encounter, the likelihood of a fish winning, apart from being dependent on relative size, can be behaviourally determined by its lack of submissive acts.

7) All the behavioural traits show inbreeding depression.

B. GENETIC ANALYSIS

The presence of a large phenotypic variance in a trait is encouraging if one is interested in genetic selection on the trait. However, there can be little gain by selection unless a significant proportion of the observed variance is genetic in origin. Specifically, it must be additive genetic variation. This variance must therefore be calculated. One of the conventional statistical models used in the estimation of the variance components, and hence the heritability of a trait, follows from a sib analysis (where measurements obtained are from the full-sib and half-sib offspring derived from a nested mating design). Specifically, the model is written as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk},$$

where,

Y_{ijk} = measurement of the kth offspring of the jth dam of the ith sire

μ = overall mean

α_i = contribution of the ith sire

$\beta_{j(i)}$ = contribution of the jth dam within the ith sire

ε_{ijk} = residual error

The usual method of analyzing such a model is by the method of moments, commonly known as the ANOVA method. In this method, the phenotypic variance (of the trait) is partitioned into its various component mean squares, which in turn, are equated to their expectations, thus obtaining the observational components. The relationship between these observational components and the causal (genetic) components being known, the estimation of these causal components (particularly the additive genetic variance) is then straightforward (*e.g.*, Turner and Young, 1969, chapter 8; Becker, 1984, pp. 45-102; Falconer, 1989, chapter 10).

Other, more recent methods of variance component estimation are ML (Maximum Likelihood), REML (REstricted Maximum Likelihood), and MINQUE (MInimum Norm Quadratic Unbiased Estimation). For balanced data, ANOVA, REML and MINQUE should lead to identical estimates of the variance components (Anderson, 1979). For unbalanced data, however, a choice of the method of variance component estimation has to be made, keeping in mind that there is no uniformly best method, under the circumstances (Kennedy, 1981).

The set of programs MTDFREML (Multiple Trait Derivative Free REstricted Maximum Likelihood) by Boldman *et al.*, (1995), estimate variance components by maximizing the likelihood function using, as the name suggests, the derivative free method of Smith and Graser (1986) and Graser *et al.*, (1987). This set of programs was used for variance component estimation for the behaviour data in this study. These estimates are compared with the ANOVA estimates.

Variance component estimation by ANOVA

The analysis of variance for **COR-AGR** and **COR-NET** can be seen in Table 5.3.3.2. The variance components and the coefficients of the variance components in the two-level nested ANOVA with unequal sample sizes, were calculated following Sokal and Rohlf (1981, chapter 10) and Becker (1984, pp. 64-66), and the standard errors and confidence intervals were obtained by jacknifing the heritability, omitting one sire group at a time (Sokal and Rohlf, 1981, pp. 795-799).

The following is an illustration of the computation of the heritability estimates, using the trait **COR-AGR**. Consider Table 5.3.3.1 (for **COR-AGR**). The column 'N' contains the number of offspring unambiguously identified for each sire, the n_{ij}'s (where n refers to the number of offspring of the jth sire of the ith dam). The coefficients of the variance components are calculated as follows (using the df's from Table 5.3.3.2).

$$k_1 = \frac{\sum_{i}^{d}\left(\frac{\sum_{j}^{s_i} n_{ij}^2}{\sum_{j}^{s_i} n_{ij}}\right) - \left(\frac{\sum_{i}^{d}\sum_{j}^{s_i} n_{ij}^2}{\sum_{i}^{d}\sum_{j}^{s_i} n_{ij}}\right)}{df_{dams}},$$

$$k_2 = \frac{\left(\sum_{i}^{d}\sum_{j}^{s_i} n_{ij}\right) - \sum_{i}^{d}\left(\frac{\sum_{j}^{s_i} n_{ij}^2}{\sum_{j}^{s_i} n_{ij}}\right)}{df_{sires(dams)}}, \text{ and}$$

$$k_3 = \frac{\left(\sum_i^d \sum_j^{s_i} n_{ij}\right) - \frac{\sum_i^d \left(\sum_j^{s_i} n_{ij}\right)^2}{\sum_i^d \sum_j^{s_i} n_{ij}}}{df_{dams}},$$

where there are d dam groups and s_i sires in the ith dam group.

Using the above formulae, we get $k_1 = 15.16$, $k_2 = 12.78$, and $k_3 = 50.97$. Further, the three variance components are obtained by solving the following equations (where the MSS's refer to the corresponding mean sums of squares from Table 5.3.3.2):

$$MSS_{(DAMS)} = \sigma_W^2 + (k_1)\sigma_{S(D)}^2 + (k_3)\sigma_D^2$$

$$MSS_{SIRES(DAMS)} = \sigma_W^2 + (k_2)\sigma_{S(D)}^2$$

$$MSS_{WITHIN} = \sigma_W^2$$

Solving the above equations we obtain the following formulae for the variance components:

$$\sigma_W^2 = MSS_{WITHIN},$$

$$\sigma_{S(D)}^2 = (MSS_{SIRES(DAMS)} - MSS_{WITHIN}) / k_2$$

$$\sigma_D^2 = (MSS_{DAMS} - MSS_{WITHIN} - (k_1)\,\sigma_{S(D)}^2) / k_3$$

Using the values from Table 5.3.3.2 and the coefficients obtained above, we obtain:

σ^2_{DAMS} = 78.033

$\sigma^2_{SIRE(DAM)}$ = 6.038, and

σ^2_{WITHIN} = 1073.632

Therefore, the total phenotypic variance, V_P = 1157.703.

Since $\sigma^2_{DAMS} = Cov_{(half\ sibs)}$ (as the mating design used in this study is a maternal half-sib design), it estimates $\frac{1}{4} V_A$. Estimating the additive genetic variance V_A from the dam component, we therefore get:

$V_A = 4\ (78.033) = 312.132$

Therefore, since the additive genetic variance, as a proportion of the total phenotypic variance gives the heritability (narrow-sense), we get

$h^2 = 312.132 / 1157.703 = 0.269$ (from the dam component)

It must, however, be noted that this value is probably an over-estimate, since the dam component has been used in the estimation, and therefore, perhaps contains some maternal effects, not to mention tank effects (as there were no replicates).

The jacknifed estimate of the above heritability is 0.23219, and the 95% confidence interval is 0.00715 to 0.4572.

Using the sire component, we get (considering only the additive genetic variance)

$V_A = 4\ (6.038) = 24.152$

Therefore, from the sire component, we get the following estimate of heritability:

$h^2 = 24.152 / 1157.703 = 0.021$

The jacknifed estimate of the sire component of heritability is 0.00514 and the 95% confidence interval is -0.1748 to 0.18508.

The heritability estimates using the ANOVA approach and the corresponding confidence intervals of the other behavioural traits were also obtained as detailed above, and can be seen in Table 5.3.4. It can be seen that, except in the case of **COR-AGR**, sire components of heritability are higher than dam components. Also with the exception of **COR-AGR**, the sire-component heritabilities are fairly high for all the traits, with **COR-NET** having the highest point estimate (0.391). However, the confidence intervals for all the estimates (with the exception of the dam component of **COR-AGR**) include zero. This latter fact is the result of large standard errors, perhaps due to small sample sizes.

Variance component estimation using the ANIMAL MODEL

The set of computer programs MTDFREML by Boldman *et al.*, (1995) has been developed to estimate (co)variance components using animal models and derivative free REML. In this study MTDFREML has been used to first form the inverse of the relationship matrix using pedigree information obtained by genotyping with DNA microsatellites for immediate parent identification, and the breeding records maintained at MGPL for pedigree information going back three generations, beyond the parent generation.

Some of the many advantages of MTDFREML over ANOVA is that it can incorporate fixed effects and covariates in the analysis. Further, it can also estimate a correlated random effect such as the maternal genetic effect, as well as an uncorrelated random effect such as maternal permanent environment effect. Yet another advantage of a DFREML based program is that since it makes use of the relationships specified in the pedigree, it is unaffected by imperfections in the data set generated by the inability, at times, to exactly follow a specified mating design. For instance, in the present study the same sires have been used to generate dam groups A1 and A2. Similarly, another set of sires was used to generate both B1 and B2. Also, two dams generated two half-sib families each. Such liberties with data generation are not tolerated very well by ANOVA, whereas DFREML remains completely unaffected, as long as the exact relationships are specified.

Accordingly, the trait **AGR** was analyzed, with tank and age as fixed effects, relative size (5th degree polynomial) and the inbreeding coefficient (linear) as covariates, and with maternal genetic effects and an maternal permanent environmental effects incorporated in the model. The trait **NET** was analyzed in an identical fashion, except that it had only the inbreeding coefficient as a covariate.

Inbreeding enters the analysis in two ways. One is that it influences the relationships among animals, which with an animal model and full pedigree is taken into account by MTDFREML. The second is because inbreeding has effects on the phenotypic expression of a given trait (inbreeding depression, etc.). Thus with the animal model and

full pedigree, and with inbreeding as a covariate, the effects of inbreeding are fully accounted for in the analysis (Van Vleck, *pers. comm.*).

The general representation of the animal model used in this study is as follows:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z_u u} + \mathbf{Z_m m} + \mathbf{Z_c c} + \mathbf{e}$$

where,

$\mathbf{y}$ = an $n \times 1$ vector of observations,

β = a vector of fixed effects containing the population mean, the effect of tank, sex and age, and the regressions on relative size and inbreeding coefficients. β is associated with $\mathbf{y}$ by matrix $\mathbf{X}$,

$\mathbf{u}$ = a vector of random effects (additive or direct genetic effects) associated with $\mathbf{y}$ by matrix $\mathbf{Z_u}$

$\mathbf{m}$ = a vector of maternal genetic effects associated with $\mathbf{y}$ by matrix $\mathbf{Z_m}$

$\mathbf{c}$ = a vector of permanent maternal environmental effects associated with y by matrix $\mathbf{Z_c}$, and

$\mathbf{e}$ = an $n \times 1$ vector of residuals.

All the behavioural traits used in this study were analyzed using the above model, with the exception that relative size was used as a covariate only in the analysis for **AGR**.

MTDFREML requires that starting values be input, which are then used in the iterations to finally obtain the variance components. The starting values used were taken from the results of the ANOVA, and the following results obtained (Table 5.3.5). Fresh starts were made using values at apparent convergence as starting values. This process of iteration was continued until the FVALUE did not change until the sixth decimal. The

convergence criteria (the variance of the simplex) for each run was set at six decimal places to begin with and later increased to nine decimal places. The results for **AGR** can be seen to be:

$h_A^2 = 5.49 \times 10^{-6}$ (Direct)

$h_M^2 = 0.09564$ (Maternal)

$r_{A.M} = 0.63925$ (Genetic correlation)

From these results it appears that the fixed effects and perhaps more importantly, the covariates relative size and inbreeding coefficient, account for almost all of the variability in the trait aggression. Figure 5.3.1.1 (a) shows the overwhelming influence of relative size. Relative size depends upon the size of the individual it encounters, and is therefore, an environmentally determined cause of variation. Considering the strong influence that relative size seems to exert upon the aggression displayed by an individual, as shown in Fig. 5.3.1.1 (a), it is perhaps not surprising that the heritability of the trait is almost zero. Further, as Fausch (1984), Metcalfe (1986), Grant (1993), Nakano (1995), and Kadri *et al.* (1996) suggest, it is likely that the behaviour patterns (such as aggression) that enable monopolization of limited resources are likely to be strongly linked to fitness, and hence the low heritability.

Blanckenhorn and Perner (1994), studying the genetics of various behavioural attributes (including aggressiveness) that affect foraging success and fitness in water strides, found that the heritabilities of most of these traits were not significantly different

from zero. Repeatabilities, however, showed significant, even high values. Unfortunately, the data in the present study do not permit the calculation of repeatability.

This possible evidence of a linkage between aggression and fitness in this study perhaps shows that the method of feeding, even in this laboratory population, still promotes resource mobilization and competition among the fish. If this behaviour is to be eliminated by genetic selection, it is important, as Kadri *et al.*, (1996) suggest, that it not be allowed to manifest, by means of appropriate feeding management. It is important that the social behaviour during feeding is understood thoroughly for each of the commercially important food-fish species, in order that the feeding method minimizes aggressive interactions among the fish. For example, Kadri *et al.*, (1996), studying the feeding behaviour of the Atlantic salmon, *Salmo salar*, have found that food presented to the fish that is unpredictable in time and space, decreases the chances of food monopolization by some fish.

Although there is no reason, *a priori*, to expect any maternal genetic effect on the aggressive behaviour (**AGR**) of the fish, the heritability of the maternal effects, while low, was found to be much higher than the heritability of the direct effects ($h^2 = 0.09654$; Table 5.3.5). There is also a positive and highly significant ($p < 0.01$) genetic correlation between the direct and maternal genetic effects, as can be seen from the table. In other words, maternal genetic effects appear to be strongly tied in with the additive genetic effects, and influence the trait in the same direction, albeit at a very low level.

Interestingly, there is also a much higher (compared to the additive effects) uncorrelated random effect (maternal permanent environment effect). This variance

component, as a proportion of the total phenotypic variance, turns out to be about 5 percent, as can be seen from the table. This effect is similar to a common environment effect, but is due to the mother, and is not genetic in origin. It can perhaps be surmised that this effect is due to differences in nutrition and general well-being of the female parents, which result in a chemically induced difference in the behaviour of the fish. As the fish were young when tested for aggression, any such influence could conceivably still be present. Thus the maternal effect (genetic and non-genetic) together account for about 15 percent of the total variation in the trait.

The trait **SUB**, although analyzed only for 5 dam groups, also shows very similar genetic characteristics to **AGR** - a very low heritability for direct effects, a high maternal genetic as well as permanent environment effect, and a highly significant correlation between the direct and maternal genetic effects. Just as aggression in a limited resource environment helps in resource monopolization, and hence better growth and fitness, it can be surmised that submission during hostile encounters simply aids survival, and is therefore, closely related to fitness.

It can be seen from Table 5.3.5 that while the two primary or original variables, **AGR** and **SUB**, both show low heritabilities, and perhaps strong relationships with fitness, the derived variables, **AGON (AGR + SUB)** and **NET (AGR - SUB)** show much higher values for the heritability of direct genetic effects, although the confidence interval obtained by jacknifing all the statistics for **NET** show that none of the estimates, including heritability of direct effects, is significantly away from zero, for this variable (because of high standard errors as a result, perhaps, of low sample sizes, just as in the case of the

ANOVA estimation). (Incidentally, it can be seen from Table 5.3.4 and Table 5.3.5 that the jacknifed estimates, as well as the confidence interval, of the heritability of direct effects (for **NET**) obtained using MTDFREML (Table 5.3.5) are quite close to the jacknifed estimate of the sire component of heritability, obtained using the ANOVA approach (Table 5.3.4), perhaps validating the sire component estimates rather than the dam component estimates from the ANOVA). Table 5.3.5 also shows that while both **AGON** as well as **NET** show low and non-significant genetic correlations between the direct and maternal genetic effects, there is a higher maternal genetic than direct effect, for **AGON**; **NET** has a low maternal genetic effect.

While **AGON** reflects the total agonistic activity of the fish, **NET** refers to how much more aggressive a fish is than submissive. Ideally, low levels of both these behaviours should lead to increased fitness in a well managed aquaculture system, with abundant food. Although the confidence intervals appear to suggest that the heritabilities are not away from zero, this is likely due to the small sample sizes in this study; these variables can perhaps be used in a selection programme to reduce agonistic behaviours.

Apart from the extent of aggression itself, each fish was also scored as winner or loser of the encounter, **W/L**, as mentioned earlier. It is apparent from Fig. 5.3.1.1 (a), which also distinguishes winners from losers, that in general, aggression is used to determine winner/loser status only when relative size cannot be used as a cue for the resolution of the conflict. Thus aggression seems to a means to an end (winning), conditional upon the relative size being close to unity. Otherwise, winner/loser status as an outcome, appears to be independent of aggression. This outcome of the encounter,

therefore, was considered a separate trait, and analyzed for estimation of the variance components (Table 5.3.5).

The winner/loser status in this study is an outcome of what apparently is a contest. It is thus not a trait in itself in the conventional sense of the term. Nevertheless, it clearly appears to be a consequence of the ability of a fish to utilize its behavioural repertoire to achieve success in the contest, given that the opponent fish is attempting to reach the same goal using the same means. It is in this sense that Dewsbury (1990) and Moore (1990) viewed the term dominance and demonstrated the presence of a heritable component to it in male deer mice, *Peromyscus maniculatus* and the cockroach, *Nauphoeta cinerea*, respectively. The winner/loser status **W/L** in the present study has been perceived in the same light, and analyzed for estimation of the variance components (Table 5.3.4).

This trait also appears to show a far greater proportion of additive genetic variance ($h^2 = 0.105$) when compared to aggression itself. Maternal genetic effects are low, but, just as in the case of aggression, there is a highly significant ($p < 0.01$) genetic correlation between direct and maternal genetic effects. Further, a fairly high proportion (20 percent) of the total phenotypic variance is explained by the maternal permanent environment effect.

It is difficult to speculate about the differential contribution of the dams in helping/ inhibiting their offspring from winning an encounter, or in the case of any other behavioural variables either, since all behavioural testing was done within dams. It is possible, at least in the case of **W/L** that since some dam groups had 48 pairs tested while

others had 96, these differences in sample size were construed by MTDFREML as differential levels of success in winning, among dam groups.

5.4 CONCLUSIONS

In conclusion, it appears from the present study that a genetic selection programme can be initiated for the given population, with at least one behavioural trait, namely **NET** (Net Aggression). However, while intuition suggests that there must be positive correlated gains in growth associated with low levels of **NET** (due to the diversion of energy towards growth), it would be necessary, before embarking on a selection programme, to determine the association between this variable and growth rate. This is the subject matter of the next two chapters.

Table 5.2.1 Family (A to P) of parents used to generate the half-sib groups (dam groups) used in the study. Note that dam groups A1 and A2 share the same sires, as do B1 and B2. Similarly, dam groups A1 and C share the same female parent, as do B2 and E. All other sharing of letters among dam groups in the table below only means that the parents have been drawn from the same families (as maintained by MGPL), not that they are the same individuals.

Dam Group	Family of Parent				
	Male 1	*Male 2*	*Male 3*	*Male 4*	*Female*
HS A1	A	B	C	D	F
HS A2	A	B	C	D	G
HS B1	E	F	G	H	B
HS B2	E	F	G	H	C
HS C	C	G	J	P	F
HS D	B	F	J	P	G
HS E	A	G	J	K	C
HS F	B	C	I	K	F
HS G	D	H	K	P	F
HS H	D	E	H	K	G

Table 5.2.2 Summary of behavioural observations and body measurements collected for the various half-sib families tested. (* These columns show if aggressive/submissive behaviours were measured for both members of the pair, or for neither. The x marks in the subsequent columns show if the particular measurement was taken for the fish of that family.)

Dam group	No. of pairs	Agonistic behav.		Dom/ Sub	Length (cm.)		Wt. (g)
		*Agr.**	*Sub.**		*Std.*	*Total*	
HS A1	96	Both	None	x	x	x	x
HS A2	48	Both	None	x	x	x	x
HS B1	48	Both	None	x		x	x
HS B2	48	Both	None	x		x	x
HS C	96	Both	None	x	x	x	x
HS D	48	Both	Both	x	x	x	x
HS E	96	Both	Both	x	x	x	x
HS F	96	Both	Both	x	x	x	x
HS G	96	Both	Both	x	x	x	x
HS H	96	Both	Both	x	x	x	x

Table 5.3.2.1 Table showing the total number of pairs of offspring (N) tested, and the mean and standard deviation for **COR-AGR** and **COR-NET** scores, for each dam group. (Dam groups A2, B1 and B2 were some of the first groups to be tested, and only 48 pairs were used from these; 96 pairs were tested from all the other dam groups. Of these, only 23 pairs could be tested in B1, due to mortality in the rest even before testing. Groups A1, D and G contained one extreme value each, which are omitted for the calculations here.)

Dam Group	N	COR-AGR		COR-NET	
		Mean	SD	Mean	SD
A1	95	64.85	33.36	-	-
A2	48	58.99	40.29	-	-
B1	23	38.49	13.15	-	-
B2	48	64.61	43.38	-	-
C	96	75.91	45.28	-	-
D	47	55.72	18.59	-5.70	55.34
E	96	44.74	17.80	-0.76	39.34
F	96	43.40	19.97	0.09	38.91
G	95	65.48	32.10	16.83	64.46
H	96	67.12	31.05	10.63	63.88

Table 5.3.2.2 Analysis of variance showing significant differences in **COR-AGR** scores and **COR-NET** among the dam groups.

Trait	Source	Sum of squares	DF	Mean Sum of Squares	F- Ratio	P
COR-AGR	**Dam Groups**	95436.90	9	10604.10	10.456	0.000
	Error	740358.43	730	1014.19		
COR-NET	**Dam Groups**	27962.75	4	6990.69	2.458	0.045
	Error	1208899.31	425	2844.47		

Table 5.3.3.1 Table showing the total number of pairs of offspring (N) tested, and the mean and standard deviation for **COR-AGR** and **COR-NET** scores, for the sire-within-dam groups.

Dam Group	Sire ID (within-dam)	N	COR-AGR		COR-NET	
			Mean	SD	Mean	SD
A1	50	15	66.20	42.04	-	-
	51	12	63.97	26.07	-	-
	52	10	75.83	53.50	-	-
	53	16	58.66	23.27	-	-
A2	50	12	72.20	38.53	-	-
	51	15	57.69	33.39	-	-
	52	7	54.93	61.98	-	-
	53	6	65.86	47.77	-	-
B1	54	7	32.00	12.68	-	-
	55	2	48.13	6.09	-	-
	56	8	40.33	14.49	-	-
	57	6	40.39	12.68	-	-
B2	54	6	96.97	69.05	-	-
	55	15	57.05	37.57	-	-
	56	13	63.47	47.74	-	-
	57	3	62.51	34.93	-	-
C	58	9	58.52	43.63	-	-
	59	28	77.50	45.91	-	-
	60	18	80.23	43.63	-	-
	61	11	58.88	37.45	-	-
D	62	7	62.38	14.96	29.57	45.28
	63	5	64.16	10.43	-28.40	62.00
	64	11	51.96	27.67	-19.55	75.09
	65	2	54.90	7.21	-88.00	19.80
E	66	15	46.69	15.49	-13.00	42.29
	67	25	46.41	15.82	-2.28	28.79
	68	29	47.74	22.29	17.52	39.41
F	69	10	46.91	27.87	30.80	31.10
	70	21	42.40	18.89	-4.62	56.98
	71	26	39.72	18.24	-8.62	24.01
	72	22	51.42	17.41	1.95	35.04
G	73	7	48.80	17.34	8.71	33.79
	74	16	51.52	21.93	1.31	58.54
	75	29	74.04	39.01	46.10	60.47
	76	13	81.94	29.25	4.31	86.13
H	77	16	73.02	45.82	11.31	77.19
	78	8	76.54	29.75	53.00	41.59
	79	17	56.18	25.27	-13.59	52.67
	80	19	68.99	27.99	3.95	72.90

Table 5.3.3.2 A nested analysis of variance showing significant differences in **COR-AGR** among half-sib family groups (*i.e.*, due to dams) but not among half-sib families (due to sires). For **COR-NET**, dams are comparable while sires-within-dams are significantly different. This analysis was carried out only for offspring whose male parent was identified by DNA fingerprinting.

Trait	Source	Sum of squares	DF	Mean Sum of Squares	F - Ratio	P
COR-AGR	Dam Groups	46282.77	9	5142.53	4.468	0.000
	Sires(Dams)	33373.09	29	1150.80	1.072	0.367
	Error	513196.09	478	1073.63		
COR-NET	Dam Groups	27487.72	4	6871.93	0.941	NS
	Sires(Dams)	102249.17	14	7303.51	2.620	0.001
	Error	777868.41	279	2788.06		

Table 5.3.3.3 Non-significant interaction (for the trait **COR-AGR**) between sires and dams in the two sets of dam groups A1,A2 and B1,B2, that each share the same sires.

Dam Group	Source	Sum of squares	DF	Mean Sum of Squares	F - Ratio	P
A1 & A2	Dams	253.36	1	253.36	0.163	0.688
	Sires	1048.04	3	349.35	0.224	0.879
	Sire * Dam	2382.8	3	794.27	0.509	0.677
	Error	132500.71	85	1558.83		
B1 & B2	Dams	8995.43	1	8995.43	6.101	0.017
	Sires	1490.29	3	496.76	0.337	0.799
	Sire * Dam	5035.251	3	1678.42	1.138	0.342
	Error	76665.11	52	1474.33		

Table 5.3.4 Variance components of different traits, as estimated using ANOVA. The last column contains, for each trait, estimates of the heritability for the full sample, the jacknifed estimates (obtained by omitting one sire group at a time), and the 95 percent confidence interval for the jacknifed estimates. (Superscript 1: data available for 5 dam groups only)

TRAIT	VARIANCE COMPONENTS		HERITABILITY
	Direct (Additive)	Residual	
COR-AGR			
1. Dam: *Full sample*	312.132	1073.63	*0.269*
Jacknife			0.232
95% CI			*0.007 to 0.457*
2. Sire: *Full sample*	24.152	1073.63	*0.021*
Jacknife			0.005
95% CI			*-0.175 to 0.185*
COR-SUB[1]			
1. Dam: *Full sample*	neg.	1098.199	*neg.*
Jacknife			0.043
95% CI			*-0.289 to 0.375*
2. Sire: *Full sample*	280.628	1098.199	*0.240*
Jacknife			0.174
95% CI			*-0.316 to 0.663*
COR-NET[1]			
1. Dam: *Full sample*	neg.	2788.059	*neg.*
Jacknife			0.043
95% CI			*-0.276 to 0.361*
2. Sire: *Full sample*	1200.184	2788.059	*0.391*
Jacknife			0.309
95% CI			*-0.206 to 0.825*
COR-AGON[1]			
1. Dam: *Full sample*	190.224	1316.676	*0.127*
Jacknife			0.137
95% CI			*-0.408 to 0.683*
2. Sire: *Full sample*	526.364	1316.676	*0.352*
Jacknife			0.308
95% CI			*-0.179 to 0.795*

Table 5.3.5 Variance components of various traits, as estimated using the Animal model and MTDFREML. (Explanation of superscripts: 1 - Aggression scores with fixed (tank and age) effects and influence of relative size and inbreeding removed; 2 - data available for 5 dam groups only; 3 - The only variable where the heritability has been jacknifed and a confidence interval obtained; 4 - The categorical "winner/loser" variable; 5 - Covariance and Correlation between direct and maternal genetic effects; 6 - The maternal permanent environment effect as a proportion of the total phenotypic variance.)

TRAIT	(CO)VARIANCE COMPONENTS					HERITABILITY		MAT.	GEN.
	Direct	Maternal genetic	Cov.[5]	Mat. perm. env.	Residual	Direct	Mat. gen.	PERM. ENV.[6]	CORR.[5]
AGR[1]	0.00653	113.609	0.5506	57.9699	1015.736	5.49×10^{-6}	0.096	0.0488	0.639
SUB[2]	0.00329	171.130	0.15906	60.3766	1050.123	2.56×10^{-6}	0.134	0.0471	0.212
AGON[2]	210.235	260.986	3.162	12.6867	1117.506	0.131	0.163	7.906×10^{-3}	0.014
NET[2,3]	889.941	195.795	0.31909	116.163	2246.210	0.258	0.057	0.0337	0.764×10^{-4}
Jack.						*0.358*	*-0.148*	*-0.0914*	*-0.0004*
C I						-0.132 to 0.849	-0.263 to 0.234	-0.214 to 0.031	-.005 to 0.004
W/L[4]	0.03496	0.00157	0.00151	0.065149	0.22865	0.105	4.72×10^{-3}	0.1963	0.204

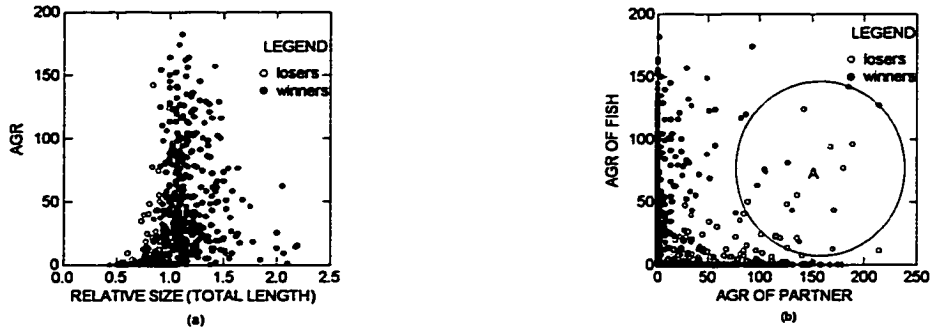

Figure 5.3.1.1 Aggression (**AGR**) plotted against two variables - relative size (total) of the fish *vis-a-vis* its partner (a), and aggression of the partner (b). Note clear demarcation of winners and losers in both figures. The region marked "A" in (b) refers to those fish that received high levels of aggression from their partners apparently because they displayed some initial aggressive activity; as can be seen, these fish are mostly losers (details in text).

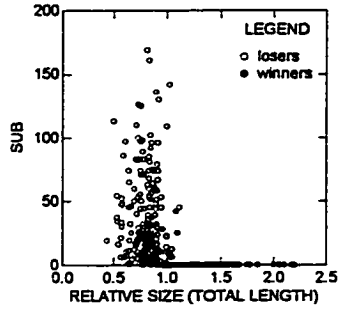

Figure 5.3.1.2 Number of submissive acts (**SUB**) plotted against relative size (total length). Note the clear demarcation of winners and losers, and the few submissive acts displayed by the winners.

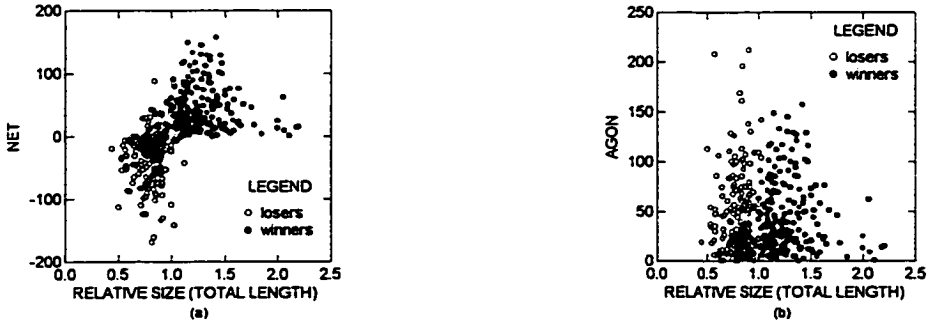

Figure 5.3.1.3 a) Net Aggression (**NET**) plotted against relative size (total length), and b) Total Agonistic acts (**AGON**) plotted against relative size (total length). Note the clear demarcation of winners and losers in both graphs.

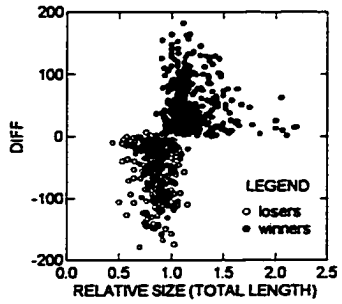

Figure 5.3.1.4 Difference in aggression (**DIFF**) plotted against relative size. Note clear demarcation of winners and losers.

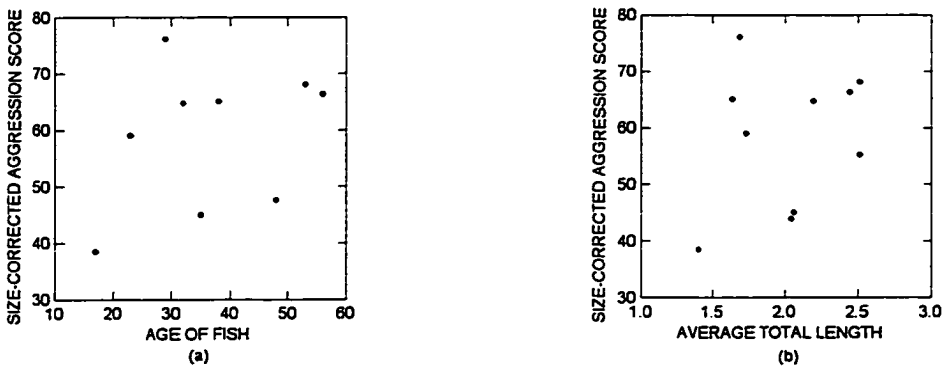

Figure 5.3.1.5 Mean aggression (corrected for relative size) scores plotted against age in days (a) and the average (total) length (cm)of the fish.

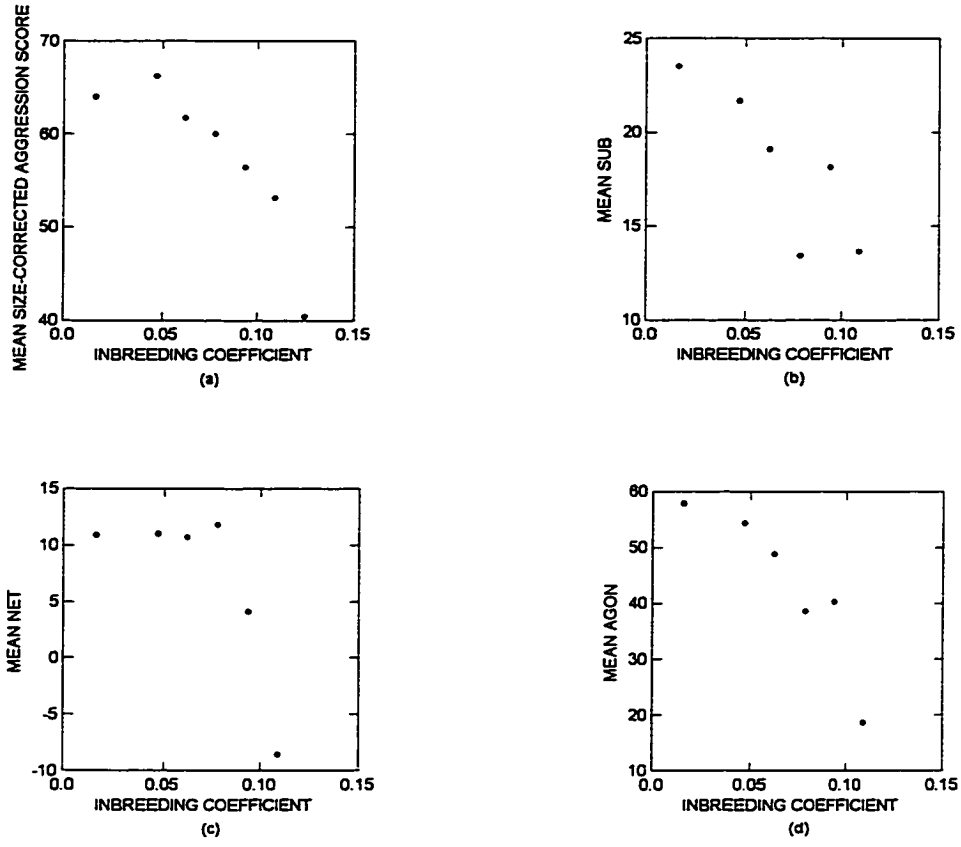

Figure 5.3.1.6 Scatter graphs showing mean values for **AGR** - corrected for relative size effects (a); **SUB** (b); **NET** (c); and **AGON** (d), each plotted against the inbreeding coefficient. Note the rapid loss in the mean values of all the behavioural traits, with increase in inbreeding.

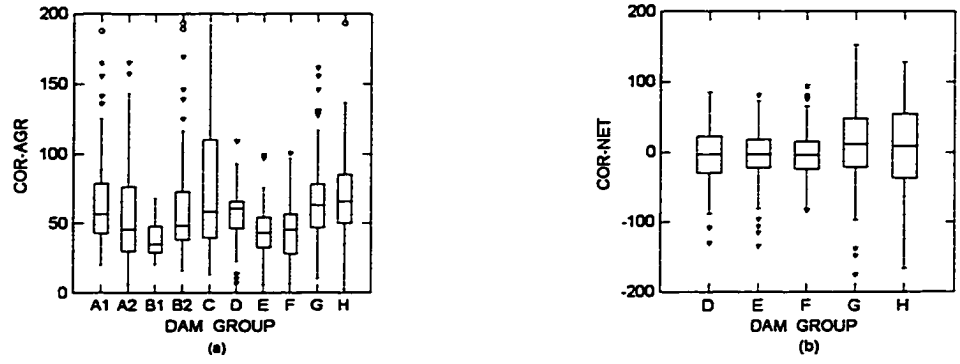

Figure 5.3.2.1 Box plot of a) **COR-AGR** (Aggression scores corrected for relative size and inbreeding effects), and b) **COR-NET** (Net Aggression scores corrected for inbreeding effects), for the offspring of each dam group.

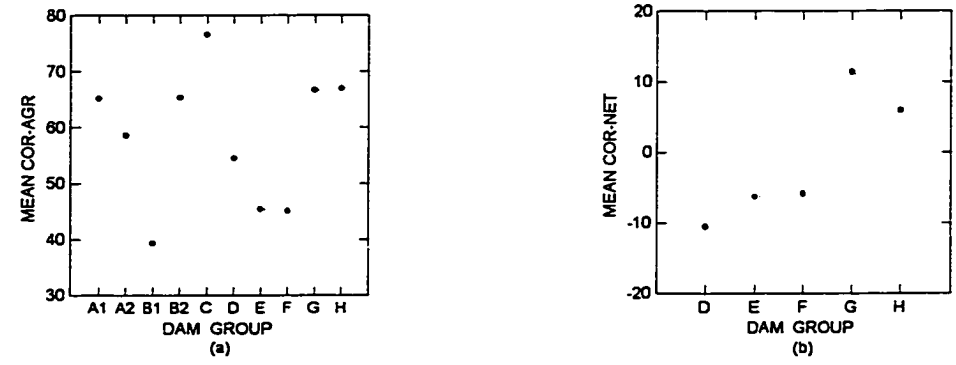

Figure 5.3.2.2 Scatter plot of a) mean **COR-AGR** (aggression score corrected for relative size and inbreeding effects), and b) mean **COR-NET** (net aggression score corrected for inbreeding effects), for each dam group. The data points are connected with dotted lines only to highlight the relative magnitude of the different dam groups.

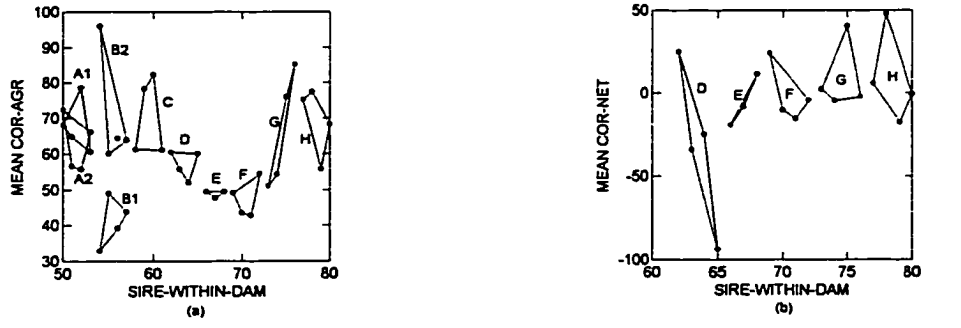

Figure 5.3.3.1 Scatter plot of a) mean **COR-AGR** (aggression score corrected for relative size and inbreeding effects), and b) mean **COR-NET** (net aggression score corrected for inbreeding effects), for the sire-within-dam groups. The dam group that each cluster of sire groups belongs to is denoted next to the cluster.

Chapter 6

Estimation of genetic parameters in cultivated tilapia (*Oreochromis niloticus*) using DNA fingerprinting. II. Growth in competitive and non-competitive environments.

ABSTRACT

The competitive regime that a group of fish is subjected to often determines the role of agonistic behaviour in achieving success. This chapter deals with the growth performance, and the quantitative genetics thereof, of fish reared in each of two competitive environments. In one tank, a high interaction (HI) environment was created by point feeding, while in the other, a low interaction environment (LI) was achieved by broadcast feeding. The fish used in these growth experiments were the full sibs of the fish used for the behavioural observations. As in the case of the behavioural experiments, fish from each of the ten half-sib crosses were pooled together to give ten half-sib groups. Each half-sib pool was reared in each of two tanks that differed in the type of competition. The observations made on each fish included length, weight, sex, maturity status, and the extent of a disease attack

The family membership of each fish was assigned using DNA fingerprinting with microsatellites, just as in the case of the fish used for behavioural observations. The variance components, as before, were determined using the conventional ANOVA as well as DFREML. Sire component heritabilities from ANOVA as well as DFREML estimates gave high to very high values for all the traits from the HI environment (0.843 to >1.0, and 0.652 to 0.962, respectively, as estimated by the two methods) while the estimates from the LI environment ranged from 0.218 to 0.345 (sire component from ANOVA) and 0.290 to 0.544 (DFREML). The extremely high values from the HI environment can

perhaps be attributed to the effect of the higher level of genotype-by-social microenvironment interaction in the HI environment.

6.1 INTRODUCTION

Individuals that compete for and succeed in obtaining and exploiting a limited resource such as food, mates or nesting sites, are thus favoured by natural selection to fare better than the less successful individuals, leading to the propagation and maintenance of the physiological, morphological and behavioural traits that ensured their success, inasmuch as these traits have a genetic, heritable component. There are two basic ways in which such a competition takes place, namely *interference* competition and *exploitation* competition (also known as *contest* and *scramble* competition). Interference competition happens when some individuals obtain a resource by restricting the access of other individuals to it, while exploitation competition occurs when all individuals have equal access to the resource but differ in their capacity for its efficient exploitation (Starr and Taggart, 1992).

The behavioural status (dominant/subordinate) of an individual is generally reflected in the outcome of a contest. Dominants usually manage to obtain more of the resource when compared to subordinates (*e.g.*, by defending it and aggressively driving off competitors), and hence grow faster, which in turn ensures the maintenance of their dominant status. For example, Metcalfe (1986), using published data, found that while there was a positive correlation between metabolic expenditure and food intake in both dominant as well as subordinate rainbow trout, the dominants were more efficient because their food intake was higher as a proportion of the energy expended, when compared to the subordinates. In fact, subordinates that adopted a high energy expenditure - high

returns strategy actually lost out, expending more energy than they acquired, thus actually showing a negative correlation of growth rate with food intake. Such an effect on the growth of subordinates has also been reported by Blankenhorn (1992) for the pumpkinseed sunfish (*Lepomis gibbosus*).

Since there are costs (*e.g.*, energy expenditure, risk of injury) involved in defending any resource from competitors, it is reasonable to expect that a contest takes place only when the benefits from utilizing that resource are greater than the costs of fighting for it - the "economic defendability" of Brown (1964), as shown, for example in birds (Gill and Wolf, 1975). In this context it appears that the costs of being dominant are generally high in endotherms such as birds (*e.g.*, Røskaft *et al.*, 1986; Hogstad, 1987), and are relatively much lesser in the case of ectotherms (*e.g.*, fish, Blankenhorn, 1992; spiders, Riechert, 1988). Blanckenhorn (1992), however, speculates that while dominance may not involve heavy costs (relative to the benefits derived from the coveted resource such as food) in the short run, it could be expensive in the long run in terms of reduced body mass (inasmuch as fitness is related to body mass) due to loss of time and missed opportunities. Nevertheless, even in the long run it is only absolute fitness that is likely to be reduced; relative fitness should still be high. In an aquaculture setting, since the broodstock is typically derived from among the offspring of the existing broodstock, it is the relative and not absolute fitness that matters. Thus, at least in the case of frequent spawners such as tilapia, even if only the short term costs of dominance are low, agonistic behaviours leading to dominance should be a good strategy to employ in a environment where food is contested.

On the other hand, in exploitative or scramble competition the best strategy for an individual depends on what the others are doing. For instance, in the absence of territoriality and resource defence, animals are known to distribute themselves among different resources such that the *per capita* returns are the same for all the animals - the "ideal free distribution" of Fretwell and Lucas (1969). For example, Milinski (1979) found that when six fish (stickleback) were put in a tank with two sources of food (*Daphnia*), one twice as much as the other, the fish distributed themselves between the good and the poor resources in a ratio of 2:1, thus ensuring that each fish obtained the same rations.

The predictions of the ideal free distribution, however, are usually not so straightforward (*e.g.*, Kacelnik *et al.*, 1992) since resources are typically not so predictable and consistent in time and space and animals tend to be territorial, or defend resources. Further, in an environment such as an aquaculture pond with broadcast feeding, there are no discrete food patches for the fish to be able to distribute themselves in any corresponding pattern. Food provided to the fish in aquaculture ponds is typically in pellet form. While pellets cannot be defended, some fish are better competitors and obtain a greater share than others (*e.g.*, Kadri *et al.*, 1996). What behavioural factors aid success in such a situation? Does an aggressive fish obtain more food under the circumstances or would it end up wasting too much time in vain attempts at defending an undefendable resource? Further, do fish that are successful in obtaining more food in such a situation actually end up growing faster?

The obvious way of obtaining answers to such questions is by actual observation in a realistic simulation of pond conditions in the laboratory (*e.g.*, Kadri *et al.*, 1996). However, not only are the logistics of such a study quite formidable (as it involves observing the behaviour of individual fish over the time it takes for growth depensation to take place), the size-dependent feedback loop sets in between agnostic behaviour and growth, thus rendering them inseparable in any phenotypic study of behaviour and growth.

As mentioned earlier, the present study is an alternative method of understanding the role of behaviour in affecting growth rate among fish in confined waters. Behaviour and growth are not measured on the same fish. Rather, if behaviour is measured on one group of fish, growth is measured on their full sibs. Chapter 5 dealt with the behavioural aspect of the study, while the present chapter describes the results of the growth experiments, where one group of full sibs (of each dam group used in the behaviour study) was grown in a high interaction (HI) environment (contest competition) while another group was grown in a low interaction (LI) environment (scramble competition).

6.2 MATERIAL AND METHODS

6.2.1 *Mating Design*

The material, mating design, DNA fingerprinting to identify parentage, and the statistical models used in this study have been described in earlier chapters (Chapters 3-5). As mentioned there, fish were mated according to a maternal half-sib design, with four

males crossed with one female to produce each set of half-sib families. A total of ten such half-sib groups (dam groups) were generated.

Eggs from each full-sib brood in each dam group were hatched separately. Upon hatching, 50 to 100 hatchlings from each of the four full-sib broods were pooled and raised together for the behaviour experiments, the experiments conducted and the results analyzed as described in Chapter 5.

The remaining hatchlings in the smallest full-sib family were divided equally into two lots, and each lot transferred to an aquarium labeled LI (for "Low Interaction") or HI ("High Interaction") - for the growth experiments. Equal numbers were then transferred from each of the three remaining broods to the LI and HI tanks so that the number of hatchlings from all four broods were identical. The hatchlings in these aquaria were fed freshly hatched brine shrimp (*Artemia sp.*) larvae once daily. After about two weeks, they were also given finely powdered custom-made tilapia chow (Corey Foods). Food was given *ad libitum*.

6.2.2 *Growth In LI And HI Environments*

The fish from the above aquaria were transferred to round 100-litre FRP tanks (also marked LI or HI) after 3-4 weeks, when the fish were large enough to be able to feed entirely on artificial food. The two types of tanks LI and HI were characterized by the method of feeding - LI tanks had food broadcast (to induce a low level of interaction among the fish - scramble competition), while fish in HI tanks had the food point-fed to them (to induce a high level of interaction - contest competition). Point-feeding was

achieved by placing the food in a plastic funnel whose outlet was secured with a piece of mosquito netting tied to it with a slight overhang, forming a small bag. The food from the funnel would fall into this small bag and the fish had to compete with each other to gain access to the food through the netting. Both LI and HI tanks for a given dam group received equal amounts of food - approximately 3 percent of the body weight per day. Food was provided once daily, seven days a week.

The growth experiment in each tank was terminated after some fish in it reached sexual maturity. All the fish in that tank were then harvested. The time required to reach sexual maturity appeared to depend upon the size of the fish, which in turn depended upon the density (number surviving) of the tank. The growth experiment was therefore terminated at different ages for the different tanks. The following details were gathered from each fish: length (standard, **SLT** and total, **TLT**), weight (**WGT**), maturity status (**MST**) - determined by dissection (codes: 1 - immature, 2 - maturing, 3 - mature, 4 - ripe), sex, **SEX** (1 - male, 2 - female, 3 - unknown), and the extent of external damage caused by an unknown disease, **DIS** (0 - no damage, 1 - some damage, 2- moderate damage, 3 - heavy damage), the external symptom of which was gouged-out flesh on either side of the head. The damage caused by this disease was confined to the head of the fish. There was no evidence during routine observations of the fish, that these lacerations could be caused by aggressive acts directed by other fish, because no aggressive acts directed towards the parts of the head that were damaged, were ever observed. It was quite severe at times, especially in large fish. Histo-pathological studies of the affected fish, however, revealed no evidence of any known pathogen.

From the length and weight data, the relative condition factor (**RCF**) of each fish was determined using the following formula due to Ricker, which gives the best index when there is variation in the length among the groups being compared (Le Cren, 1951; Bolger and Connolly, 1989):

Relative condition factor, $K = \frac{100W}{L^b}$, where

W is the weight in g., L is the length in cm., and b is the slope of the regression of log weight on log length.

6.2.3 *DNA Fingerprinting To Assign Parentage*

Fin clips from each fish were preserved in 100% ethanol for genotyping using microsatellites. Development of microsatellite primers and protocols for PCR amplification, gel electrophoresis, and subsequent scoring of alleles for pedigree identification, has been described in Chapter 3. As mentioned in Chapter 5, alleles of offspring were matched with those of the parents to determine male parentage, as the inheritance of alleles at these loci follows a simple Mendelian pattern (Harris *et al.*, 1991; Queller *et al.*, 1993). Use of microsatellites to determine female parentage was not necessary since the female parent was the common parent, within half-sib families. However, the female parent also had to be genotyped as this information was necessary for unambiguous identification of the male parent.

6.2.4 *Data Analysis*

After determining the parentage of the fish the data were now amenable for statistical analysis to compare the various dam and sire-within-dam groups within and between the LI and HI environments, and to estimate genetic variances in each of the two macroenvironments. The genetic analysis was done using two methods: the conventional ANOVA and the animal model/DFREML approach. These approaches are discussed in detail and compared with each other in Chapter 4.

The following variables were analyzed: **SLT** (standard length), **WGT** (weight), **RCF** (relative condition factor), **MST** (maturity status) and **DIS** (extent of damage caused by disease).

The format of the data for ANOVA was a standard nested design with males nested within females.

The statistical model used in the ANOVA was:

$$y_{ijk} = \mu + d_i + s_{j(i)} + e_{ijk}$$

where,

y_{ijk} = the measurement on the k^{th} offspring of the j^{th} sire of the i^{th} dam,

μ = the overall mean,

d_i = the contribution of the i^{th} dam,

$s_{j(i)}$ = the contribution of the j^{th} sire of the i^{th} dam,

e_{ijk} = the random residual error.

As mentioned in Chapter 5 for the behaviour data, the growth data used in the analysis was also unbalanced since the number of offspring unambiguously assigned parentage varied among families. The analysis of variance was done using the MGLH module of SYSTAT 6.0 for Windows (1996, SPSS Inc.), with the coefficients k_1, k_2 and k_3 determined using the formulae given by Becker (1984), and Sokal and Rohlf (1981, p. 297). Standard errors and confidence intervals were determined using the jacknife procedure (Sokal and Rohlf, 1981, pp. 795-799), eliminating one sire group at a time.

The data were also analysed using the animal model/DFREML approach by means of a set of computer programs collectively called MTDFREML (Multiple Trait Derivative-Free Restricted Maximum Likelihood), written by Boldman *et al.*, (1995), which was downloaded on to a PC from a ftp site in the World Wide Web (cgel.agsci.colostate.edu in pub/cvantass). The analysis was done in the UNIX environment. MTDFREML is oriented towards and used mainly by livestock quantitative geneticists. However, it can be used with any data set with pedigree information, such as the one used in this study. (It can also, of course, be used for data sets with no pedigree information - one simply enters zeros for parental IDs. Since the programme utilizes the relationships among the animals in obtaining the solutions, the absence of any pedigree information renders it equivalent, in this respect, to obtaining variance component estimates from ANOVA.) The data used in the analysis here contained pedigree information running back to four generations. As mentioned earlier the pedigree information beyond the immediate parents was obtained from MGPL records.

The method used by MTDFREML involves the use of MME (mixed model equations) for the simultaneous solution for fixed effects and random effects (breeding values) and estimation of (co)variance components (with a modification due to Kachman (Boldman *et al.*, 1995) to handle singularity of the MME). (For details see Chapter 4). (Co)variance component estimation by the program requires that starting values of the components be given. It then maximizes the log likelihood function (or minimizes -2 log of the likelihood function, termed the FVALUE) by a process of iteration. This is done by the Simplex (polytope) method described by Nelder and Mead (1965). Convergence occurs when the global maximum of the log likelihood function (or equivalently, the minimum of the FVALUE) is found, which is tested by restarting the program with the estimates at apparent convergence as starting values, or with a fresh, "cold" restart with new priors.

The general representation of the animal model used in this study is as follows:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z}_u\mathbf{u} + \mathbf{Z}_m\mathbf{m} + \mathbf{Z}_c\mathbf{c} + \mathbf{e}$$

where,

$\mathbf{y}$ = an $n \times 1$ vector of observations,

β = a vector of fixed effects containing the population mean, the effect of tank and age, and the regressions on tank density and inbreeding coefficients. β is associated with $\mathbf{y}$ by matrix $\mathbf{X}$,

$\mathbf{u}$ = a vector of random effects (additive or direct genetic effects) associated with $\mathbf{y}$ by matrix $\mathbf{Z}_u$

$\mathbf{m}$ = a vector of maternal genetic effects associated with $\mathbf{y}$ by matrix $\mathbf{Z}_m$

$\mathbf{c}$ = a vector of permanent maternal environmental effects associated with y by matrix $\mathbf{Z}_c$, and

$\mathbf{e}$ = an $n \times 1$ vector of residuals.

As in the case of the ANOVA estimation, standard errors and confidence intervals were determined by the jacknife method (Sokal and Rohlf, 1981, pp. 795-799), leaving out one sire group at a time. For this procedure, however, a simpler model than the one given above was used, with the maternal genetic and maternal permanent environment components omitted.

6.3 RESULTS AND DISCUSSION

6.3.1 Phenotypic Examination

Size (SLT)

The population density of fish and crustaceans in enclosed water bodies such as cages or culture ponds is known to affect growth rates (*e.g.*, Sehgal and Toor, 1995; Yi *et al.*, 1996). In the present study the number of fish surviving was found to vary among dam groups within environments (Table 6.3.1.1 (a)), and the standard length was found to have a significant negative correlation ($p < 0.01$) with density in both environments. In order to analyze standard length, therefore, the influence of this important covariate, tank density, was removed by regression, and the residuals used in further analysis.

The influence of another possible covariate, age of the fish, was also examined, but was found to be of little consequence, in either environment. This result, while apparently incongruous, can be explained by the fact that there is a high correlation (0.697, $p < 0.05$,

for LI; 0.527, p = 0.118, for HI) between age at termination of the experiment and the number surviving (tank density) at termination. This correlation is an artifact of the study since the experiment was terminated for each tank only after some fish reached a size of approximately 15 cm (the size at which healthy females begin to show sexual maturity in the MGPL stock). Since size is negatively correlated with tank density, obviously the fish in tanks with greater density took longer to reach the target size, and *vice-versa*; hence the high correlation between age and density. Therefore, it is to be expected that standard length would also be negatively correlated with age, as indeed it is (- 0.478 and - 0.294 for LI and HI environments, respectively; both non significant, however). The standard length corrected for density effects, when tested against age yielded correlations close to zero (0.089 and 0.084 for LI and HI, respectively; both non significant).

Just as in the case of the behavioural variables, MTDFREML yielded inbreeding coefficients for the growth variables too. **SLT** and all the other variables were checked to determine the effect of inbreeding on them, in the two environments. The plots can be seen (Fig. 6.3.1.1). As can be seen the there is a uniformly negative relationship between all the growth variables and inbreeding, except for **RCF** in the LI environment, which shows no relationship, and **DIS** in the HI environment, which shows a positive relationship. The graphs seem to show a strong influence of inbreeding in most cases, although the correlations are non-significant. This influence was also removed by regression, and the residuals used in further analysis.

Table 6.3.1.2 shows the mean standard length corrected for density and inbreeding (henceforth termed **COR-SLT**) and the corresponding standard deviation, for the ten dam

groups in the two environments, for the pooled data as well as separately for the two sexes. Figure 6.3.1.2 shows a scatter plot of the mean standard length (a) as well as that of mean **COR-SLT**, for the ten dam groups, in each of the two environments. (This graph was drawn after adding a constant to all the values to avoid negative values in the graph.) The remarkable similarity in the performance of each dam group in the two environments can be easily seen. This observation was confirmed by examining the correlation ($r = 0.688$; $p < 0.05$) and an analysis of variance (Table 6.3.1.3; 'Dam Groups'). The ANOVA revealed significant overall differences among dam groups within environments ($p < 0.001$) as well as a significant interaction ($p < 0.05$) between environment and dam group, but no differences of mean growth between environments. Each dam group mean was also compared between environments using the Student's t test - only dam groups C and D were found to differ significantly ($p < 0.05$) between environments; every other group had comparable growth (**COR-SLT**) performances. This can perhaps be explained by the fact that both LI and HI environments for each dam group received the same amount of food, and therefore, what is seen in the figure is essentially food-limited growth rate, which, when corrected for density, was found to be comparable for each dam group (that is, for each tank), between environments.

This comparable performance of each dam group between environments permits straightforward comparisons of sire performances between environments. The sample sizes for the various sire-within-dam groups, that is, the number of offspring unambiguously identified using microsatellites, can be seen in Table 6.3.1.1 (b). Table 6.3.1.4 shows the mean **COR-SLT** for the sire groups within dam, for the HI and LI

environments, for the overall data as well as separately for the two sexes. Figure 6.3.1.3 shows the scatter plot of these means for each sire group within dam, for the overall data, in each of the two environments. (As in the case of the dam means in Fig. 6.3.1.2, the sire means were now all made positive by adding a constant.) It is apparent from the figure that while some of the sire-within-dam groups are clustered around each other, most others seem to have considerable differences, in both environments. Analysis revealed a highly significant correlation ($r = 0.588$; $p < 0.00001$) between the environments. To check if sire groups differed within dams, an analysis of variance was done (Table 6.3.1.3; 'Sires within Dam group'). As can be seen, the sire-within-dam groups differ significantly ($p < 0.001$). Figure 6.3.1.2 also shows that, while the relative performances of some sire groups seem consistent between environments (*e.g.*, B1, C and D), others (*e.g.*, A2) appear to differ in their relative performance between the two environments. Analysis of variance done separately for each dam group revealed significant interaction ($p < 0.05$) between sire and environment in two groups - A2 and F. (A1 could not be analysed due to missing values in LI environment). Significant differences ($p < 0.05$) between sires-within-dams were seen in the case of B1 and F only. Significance at $p < 0.1$ was seen in A2 and C also.

Investigating the within environment differences among dam groups further, a one-way ANOVA (Table 6.3.1.5) with Bonferroni-corrected post-hoc test was done separately for the two environments. It was found that, in both environments, while there was a significant overall difference among the dam groups as a whole and among many pairwise combinations, there was no single group that was different from all the others. However,

in the high interaction environment, F, which also had the least mean residuals, was significantly different from all the groups except B1, C and E. (While dam group F had the least mean residual in the HI environment, it can be seen from Fig. 6.3.1.2 that it also contained a sire group which had the highest mean residual among all sire groups of all dam groups; thus exhibiting much variation among the sires of this dam group.) There were other differences too, between specific pairs of groups. In the low interaction environment, the differences among groups were less drastic, with H being different from B1, C and F, and A2 being different from C. All other pairs were comparable.

As mentioned earlier, the similarity in performance of dam groups between environments can be explained by the fact that the growth rate of each dam group in the two environments was restricted by the amount of food available, and since each dam group received the same amount of food in the two environments, they showed similar mean growths. This fact made it convenient to compare the growth rates of the different sires-within-dam groups. As well, it allowed a straightforward comparison of the sire groups between environments.

Next, as competition differentially affects growth responses of fish, the variation among both dam and sire-within-dam groups was compared between the two environments. This was done by comparing the corresponding mean sums of squares from Table 6.3.1.6. While the variation among both the dam and the sire-within-dam groups seem to have been exaggerated in the HI environment when compared to the LI environment, these differences, however, were not statistically significant ($p > 0.2$ and $p > 0.1$, for the dam and sire-within-dam groups, respectively), for the overall data. However,

when the data are categorized by sex, in the case of males, both dam groups as well as sires within dams exhibited significant differences ($p < 0.05$ and $p < 0.01$, respectively) in variation between environments, with greater variation being seen in the HI environment.. A nested ANOVA could not be done for females due to insufficient data in some sire groups. However, as the overall data showed a non-significant variation between environments, and a subset of this data (the males) has already been seen to show a significantly higher variation in one of environments (HI), it is unlikely that the complementary subset (females) would also show significant differences.

The data categorized by sex yielded other comparisons (Table 6.3.1.7). Similar to the overall data (Table 6.3.1.3), ANOVA for males (M) revealed significant differences ($p < 0.001$) within environments (*i.e.*, among dam groups) and a weakly significant interaction between dam groups and environments ($p < 0.1$), but no differences between environments. Females (F) on the other hand, revealed weakly significant differences between environments ($p < 0.1$) and a highly significant interaction term ($p < 0.001$) between dam group and environment. The non significant or weakly significant differences between environments could perhaps again be traced to the identical amounts of food provided between environments for each dam group. These results appear to indicate that males do equally well in contest or scramble environment, while the mean performance of females is slightly better in the HI environment.

Examining the mean **COR-SLT** of the data categorized by sex, significant correlations were seen for males between environments ($p < 0.001$), for females between environments ($p = 0.038$), and between the sexes in the LI environment ($p = 0.004$), for

sire groups. There were no significant correlations seen for the sex-categorized data for the dam groups, even though the overall data revealed a significant correlation between the environments, as reported earlier.

A comparison between the sexes (Table 6.3.1.8) revealed that while both males and females performed equally well in the HI environment, there were significant differences ($p < 0.005$) between them in the LI environment, with the males showing faster growth. The ANOVA also revealed that in both environments, there was a significant interaction ($p < 0.001$) between the sex of the fish and the dam group it belonged to.

Relative condition factor

Analysis of variance revealed significant differences among dam groups ($p <$ 0..001) as well as sire-groups ($p < 0.05$), in both environments (Table 6.3.1.9). Since **RCF** is a function of both the length and weight of the animal, a nested ANOVA was also done for weight, and it was found that dam groups did not differ in either environment, while sire-within-dam groups differed only in the HI environment. It has already been reported that as far as standard length is concerned, dam groups did not differ significantly while sire-within-dam groups did.

Maturity Status

As mentioned earlier fish were given scores based on their maturity status (with a minimum of 1 for an immature fish and a maximum of 4 for a ripe/spawning fish). These scores were then averaged for each dam and sire group for the overall data as well as

separately for each sex, for each of the two environments. Friedman's two way analysis of variance was used in order to compare these averaged scores between environments for the overall data and for each sex separately, as well as between the sexes within environments. While all comparisons among dam groups yielded non-significant results ($p > 0.05$), two came close. One was a comparison, in the dam groups, between the sexes in the low interaction environment ($p = 0.058$), with the males, on an average, maturing faster. The other was when males were compared, again among dam groups ($p = 0.078$) - B2 had the highest rank sum, with D, E and F also scoring high, while A2 showing the lowest rank sum.

As far as the sire groups were concerned, males, on an average, matured significantly faster than the females in the LI environment ($p < 0.001$) as well as in the HI environment ($p < 0.05$). The fish in general, irrespective of their sex, matured significantly faster in the HI environment than the LI environment ($p = 0.055$). However, the test could not detect the differences individually for the two sexes.

The average maturity status of the dam or sire groups was found to have little dependence on the age of the fish ($p > 0.20$), for the overall data as well as the individual sexes, irrespective of the environment.

6.3.2 Genetic Analysis

Estimation of heritability from sib analysis requires that the variance components of the trait be first estimated. This has been done, for both environments, first using the conventional ANOVA approach, and then by means of the animal model/dfreml approach.

The ANOVA, as most of the preliminary analysis in this study, was done using SYSTAT VERSION 6.0 (Wilkinson, 1996), while the animal model/dfreml analysis was done, as mentioned earlier, using MTDFREML (Boldman *et al.*, 1995) in the UNIX environment.

Analysis of Variance

Table 6.3.2.1 shows the mean squares for the corrected standard length in each of the two environments (HI and LI). Since, in both environments, the data contained unequal sample sizes, coefficients of the variance components needed to be calculated. This, and the subsequent calculations for obtaining estimates of the three variance components were done following the procedure given by Sokal and Rohlf (1981) and Becker (1984), as detailed in Chapter 5.

Thus, after equating observed mean squares to the expected mean squares, the variance components were found to be as follows:

	HI Environment	*LI Environment*
Dams:	$\sigma^2_D = 0.401$	$\sigma^2_D = 0.181$
Sires(dams):	$\sigma^2_{S(D)} = 1.426$	$\sigma^2_{S(D)} = 0.528$
Progenies:	$\sigma^2_W = 4.938$	$\sigma^2_W = 5.413$
Total:	$\sigma^2_T = 6.765$	$\sigma^2_T = 6.122$

Since all the half-sib families for each dam group were grown in one tank for each environment, a common-environment effect was expected. However, as can be seen from the variance components above, the dam component is smaller than the sire component. Therefore, an inference can be made that neither common environment nor maternal effects are at any significant level. However, such an inference contradicts with the analysis already done wherein significant differences were found between dam groups within environments. Using the above values of the variance components, therefore, heritability was obtained as a combined estimate as well as from the individual components, that is, the dam component and the sire-within-dam component. These estimates are shown below:

Heritability estimates

	HI Environment	*LI Environment*
Dam-component: $h^2_D = \frac{4\sigma^2_D}{\sigma^2_T}$		
	$h^2_D = \mathbf{0.237}$	$h^2_D = \mathbf{0.118}$
Sire-component: $h^2_{S(D)} = \frac{4\sigma^2_{S(D)}}{\sigma^2_T}$		
	$h^2_{S(D)} = \mathbf{0.843}$	$h^2_{S(D)} = \mathbf{0.345}$
Sire + Dam: $h^2_{D+S(D)} = \frac{2(\sigma^2_D + \sigma^2_{S(D)})}{\sigma^2_T}$		
	$h^2_{D+S(D)} = \mathbf{0.540}$	$h^2_{D+S(D)} = \mathbf{0.232}$

The variance components and heritability estimates for **COR-SLT, COR-WT** and **RCF**, for each of the two environments, as estimated by ANOVA can be seen in Table 6.3.2.1.

The variance components for these traits as well as the categorical traits **MST** and **DIS**, as estimated using MTDFREML can be seen in Table 6.3.2.2. Maternal effects and genetic correlations are shown for **SLT** (using the tank the fish were reared in and their sex as fixed variables, and the density of the tank and the inbreeding coefficient as linearly related covariates). **RCF, MST** and **DIS** have been analyzed using the same fixed effects as above, but with no covariate. MTDFREML calls for feeding the program with starting values for the (co)variances - the variances were obtained by running the program for the traits separately and guessing the covariances. Each time the values from the output, at apparent convergence, were used again as starting values for a fresh, "cold" start of the program. This was done until two successive FVALUEs (-2 log likelihood of the estimation function) did not differ up to 6 digits. During the final run of the program the convergence criterion (the variance of the simplex) was kept at less than or equal to 10^{-9}.

Perhaps the most striking feature of Tables 6.3.2.1 and 6.3.2.2 is the high heritabilities of growth and growth-related traits in the HI environment. The sire estimates from the analysis of variance for **COR-WT** and **RCF** actually exceed 1. The estimates for **COR-SL** and **COR-WT** from the dfreml analysis are also very high. Nested mating designs to obtain heritability estimates from sib analysis are typically paternal half-sib designs, where each of several males is mated with several females. In such cases, inferences regarding the potential for selection are usually drawn from the

heritability estimates obtained from half-sibs. This is because the full-sib component can have non-genetic effects such as common environment effects, and non-additive genetic effects such as dominance effects, confounding the estimate of the additive genetic effects. The full-sib component can also contain maternal effects, since groups of full sibs within a half-sib family have different female parents. However, the mating design used in this study was the maternal half-sib design, as mentioned earlier, for the reasons given below.

This is a study of the genetics of competition, and ideally, all broods should be of the same age and reared in the same container. This would not only have eliminated common-environment effects altogether, but would also have exposed offspring from each family to the offspring from every other family. Such an experimental design was, however, not feasible because of the difficulty in obtaining all the crosses simultaneously. Further, even if such a spawning were possible, segregating the various full and half-sib families using the technique of DNA fingerprinting would have been impossible with the available microsatellite primers owing to the low level of polymorphism in the given population.

This meant that each half-sib had to be obtained separately, and therefore, housed in separate containers, thus admitting common environment effects. A paternal half-sib design would then have common environment effects adding to the variation among half-sib groups, and maternal effects confounding the variation among full-sibs. A maternal half-sib design, on the other hand, would add both these sources of extraneous variation to the half-sib component, leaving the full-sib component with only the dominance deviation confounded with the additive genetic variation. Further, the full-sib component was of

interest since competitive effects would obviously affect only this component. The final consideration in opting for maternal rather than paternal half-sibs was the logistics - it is much easier to simultaneously obtain milt from several males than to obtain eggs from several females at the same time.

The estimates of heritability from the LI environment appear to be similar to those reported in the literature for tilapia. Thien (1971), in what is perhaps one of the earliest published quantitative genetic work on tilapia (*Tilapia mossambica*), reported realized heritabilities for weight ranging from almost zero to 0.36, with one estimate as high as 0.76. Tave and Smitherman (1980), working with the Nile tilapia (the Ivory Coast Strain of the Auburn stock), did a half-sib analysis (paternal half-sibs) for length and weight at 45 and 90 days, and obtained sire component estimates of 0.04 to 0.10 and dam component estimates of -0.02 to 0.54, with the only estimate differing significantly from zero being the dam component at 45 days. Later, mass selection was applied to fish from the same stock by Teichert-Coddington and Smitherman (1988), who found a realized heritability for rapid growth in the same range as Tave and Smitherman (1980), but higher heritabilities for slow early growth.

Estimates of heritability for growth traits in fish range from low - *e.g.*, Moav and Wohlfarth (1968,1976), and Kirpichnikov (1971), for carp; Aulstad *et al.*, (1972), and Møller *et al.*, (1979), for rainbow trout; Ryman (1972) and Refstie and Steine (1978), for Atlantic salmon; Nilsson (1994), for the Arctic charr; Huang and Liao (1990), for the Nile tilapia; to the moderately large - *e.g.*, Reagan *et al.*, (1976), for channel catfish; Gunnes and Gjedrem (1978), for Atlantic salmon; Silverstein and Hershberger (1995), for coho

salmon; Winkelman and Peterson (1994), for chinook salmon; and Jarimopas (1990), for the Thai red tilapia.

The estimates of heritability in the present study range from low to moderately high in the LI environment, and high to very high in the HI environment. However, in almost every case where a 95 % confidence interval has been constructed (using the jacknife procedure), the estimates appear not to differ significantly from zero - this is very likely the result of high standard errors, which in turn, are a consequence of small sample sizes.

While the estimates from the LI environment are more or less in agreement with earlier reports, as mentioned earlier, the extremely high estimates from the HI environment deserve some explanation. The HI environment was expressly designed to create a high level of competition and interaction among the fish, and as seen in this as well as many other studies, competitive interactions both depend upon and effect size differences among fish. Since all the fish within a tank belong to the same batch of eggs, and essentially hatched at the same time, size differences among them represent growth rate differences. It is the additive genetic component of these growth rate differences that determines the heritability of the trait. Competition among fish is known to inflate the variance through amplification of the genetic variability in the trait, and could be caused by social interactions, among other things (Moav and Wohlfarth, 1974).

Social interactions among fish typically depend upon the relative size of the fish. This relative size, being different for different fish, thus causes the social microenvironment to vary among fish. This in turn, introduces a genotype-by-

environment interaction (the environment being the social microenvironment) that adds to the variation among fish. The methods used in the estimation of the variance components depend upon the resemblance among relatives. Since GE interaction, by definition, means that genotypes behave differently in different environments, this effectively reduces the variation (*i.e.*, increases the resemblance) within genotypes since it increases the variation among genotypes. Such a distribution of the variation within and between genotypes inflates the estimate of the additive genetic variation. This manifestation of competitive effects can be seen to be only in the HI environment, and is independent of the method used in the variance component estimation.

As far as the two methods, ANOVA and DFREML are concerned, as mentioned earlier, the DFREML estimates can be considered to be more accurate estimates, since they are based on a larger sample size and also utilize pedigree information.

6.4 CONCLUSIONS

In conclusion, the results show that there is a moderate amount of variation in all the growth traits, as evidenced by the estimates in the LI environment, and selection for these traits may perhaps be effective in this population. Further, there appears to have been a magnification of the estimated additive genetic variation in the HI environment, presumably because of the higher genotype-by-social-microenvironment interaction caused by higher levels of competition.

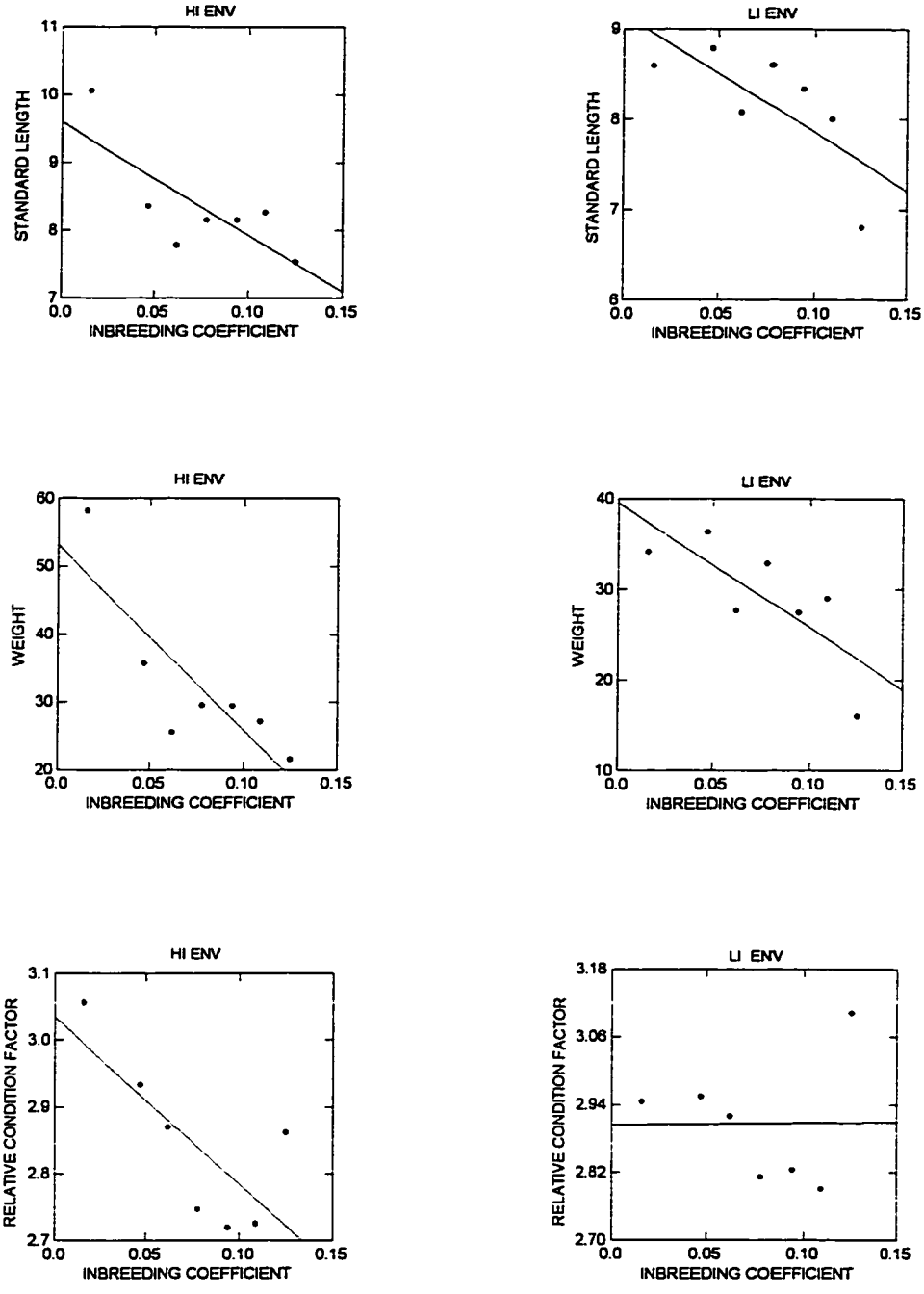

Fig. 6.3.1.1 Scatter plots of mean values of various traits (**SLT, WGT** and **RCF**) against the inbreeding coefficient, shown for both environments.

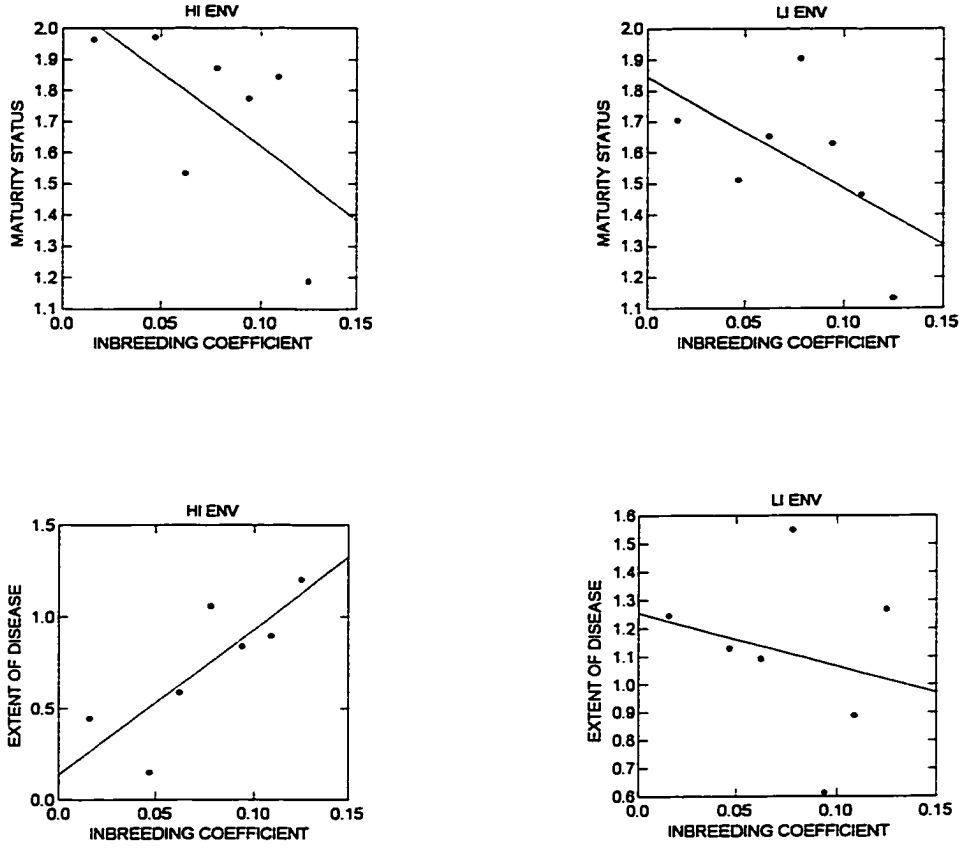

Fig. 6.3.1.1 (contd.) Scatter plots of mean values of the remaining traits (**MST** and **DIS**) against the inbreeding coefficient, shown for both environments.

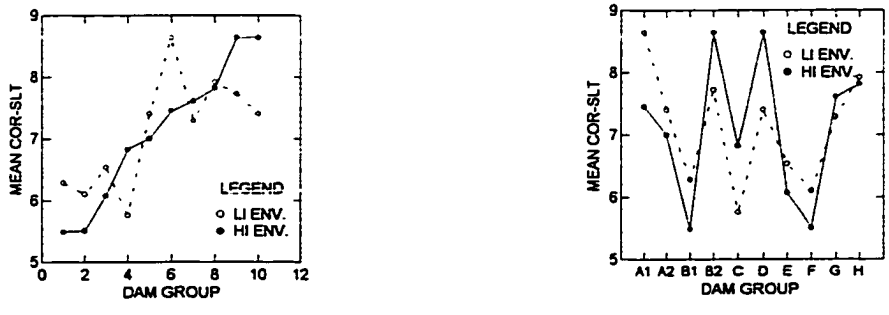

Fig. 6.3.1.2 Scatter plot of mean standard length corrected for density and inbreeding (**COR-SLT**) showing the remarkable similarity in the dam group means between environments (HI - competitive and LI - non-competitive). The graph on the left has the data points ordered according to the magnitude in the HI environment, whereas the graph on the right has the data points ordered by dam group identification. The data points have been connected only to highlight the similarity in relative magnitude between the two environments.

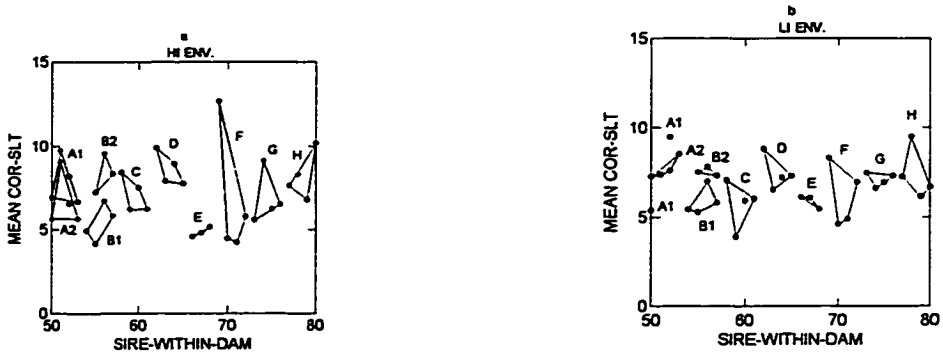

Fig. 6.3.1.3 Scatter graphs showing the residuals (standard length corrected for density and inbreeding) for each sire group within dam group, in each of the two environments (HI - competitive and LI - non-competitive).

Table 6.3.1.1 (a) Age and survival (number and percentage) at termination of growth experiment

H.S. FAMILY	MACRO-ENV. (LI / HI)	AGE AT TERMINATION (weeks)	NUMBER SURVIVING	% SURVIVAL
HS A1	LI	25	11	2.75
	HI	33	100	25.00
HS A2	LI	31	108	38.57
	HI	28	102	36.43
HS B1	LI	31	79	19.75
	HI	30	57	14.75
HS B2	LI	26	26	13.98
	HI	26	21	11.23
HS C	LI	25	33	8.25
	HI	29	63	15.75
HS D	LI	29	59	29.50
	HI	28	36	18.00
HS E	LI	26	75	50.00
	HI	29	51	34.00
HS F	LI	27	78	44.32
	HI	25	48	27.43
HS G	LI	30	71	35.50
	HI	30	63	31.50
HS H	LI	31	59	29.50
	HI	31	47	23.50

Table 6.3.1.1 (b) Sample sizes (number of offspring identified for the various sire groups using microsatellites) upon termination of growth experiment

H.S. FAMILY	SIRE WITHIN DAM	NO. OF FISH IDENTIFIED HI	NO. OF FISH IDENTIFIED LI
HS A1	50	9	-
	51	2	1
	52	15	-
	53	17	3
HS A2	50	12	14
	51	12	6
	52	18	9
	53	5	7
HS B1	54	17	15
	55	10	12
	56	5	16
	57	17	15
HS B2	54	-	-
	55	6	2
	56	2	11
	57	8	8
HS C	58	6	5
	59	10	2
	60	5	1
	61	14	9
HS D	62	4	8
	63	12	8
	64	13	17
	65	3	12
HS E	66	5	21
	67	4	10
	68	8	7
HS F	69	3	11
	70	6	4
	71	11	18
	72	9	11
HS G	73	4	1
	74	7	14
	75	13	20
	76	9	10
HS H	77	10	7
	78	4	9
	79	2	4
	80	6	7

Table 6.3.1.2 Mean and standard deviation of **COR-SLT** (standard length corrected for tank density and inbreeding) of dam groups in HI and LI environments; 'M' and 'F' denote males and females.

	A1	A2	B1	B2	C	D	E	F	G	H
HI (Mean)	0.438	-0.013	-1.201	1.589	-0.204	1.598	-0.96	-1.528	0.574	0.777
HI (SD)	2.394	2.333	2.975	2.083	2.174	2.698	2.959	2.864	2.600	2.576
LI (Mean)	1.058	0.467	-0.718	0.724	-1.238	0.403	-0.379	-0.683	0.289	0.917
LI (SD)	2.687	1.909	2.717	0.995	1.966	2.936	2.269	2.800	2.676	2.610
HI (M) (Mn)	0.567	-0.431	-0.622	0.877	0.111	1.468	-0.887	-1.586	0.715	0.673
HI (M) (SD)	2.610	2.099	2.822	2.210	2.181	2.658	2.941	2.961	2.553	2.493
HI (F) (Mn)	-0.147	0.786	7.047	1.932	-0.407	0.444	-2.697	-3.332	-0.795	0.525
HI (F) (SD)	1.780	2.690	3.889	2.077	1.990	2.761	2.437	0.615	2.349	2.726
LI (M) (Mn)	1.202	0.256	0.750	0.381	-1.278	0.242	-0.249	-1.252	0.201	0.901
LI (M) (SD)	2.057	1.960	2.575	0.789	2.112	30.84	2.328	2.505	2.644	2.585
LI (F) (Mn)	-0.187	0.504	0.523	1.295	-0.944	-0.192	-2.184	0.104	-0.847	-0.719
LI (F) (SD)	2.811	1.450	2.210	1.095	1.456	2.257	0.804	3.398	2.709	2.620

Table 6.3.1.3 Analysis of variance of **COR-SLT** for the overall growth data. Dam groups vary significantly ($p < 0.001$) within environments but not between. Interaction between dam groups and environment is significant at $p < 0.05$. Sires within dam groups also differ significantly ($P < 0.001$).

	Source	Sum of squares	DF	Mean Sum of Squares	F- Ratio	P
Dam groups	**Environment**	0.031	1	0.031	0.005	0.943
	Dam group	696.553	9	77.395	12.591	0.000
	Env.*Dam Grp.	132.331	9	14.703	2.392	0.011
	Error	7173.288	1167	6.147		
Sires within Dam group	**Environment**	0.457	1	0.457	0.087	0.768
	Dam group	452.113	9	50.235	9.527	0.000
	Env.*Dam Grp.	91.389	9	10.154	1.926	0.046
	Sire (Dam Grp.)	525.504	28	18.768	3.559	0.000
	Error	3211.175	609	5.273		

Table 6.3.1.4 Mean (Mn) and standard deviation (SD) of **COR-SLT** (standard length corrected for tank density) of sire groups in HI and LI environments; 'M' and 'F' denote males and females. Hyphens within cells denote missing data: there were no offspring identified for the sire or sex-sire in question.

DAM GRP	SIRE	Mn (HI)	SD (HI	Mn (LI	SD (LI	Mn (HI) (M)	SD (HI (M)	Mn (HI) (F)	SD (HI (F)	Mn (LI (M)	SD (LI (M)	Mn (LI (F)	SD (LI (F)
A1	50	-0.072	1.502	-1.624	-	-0.117	1.602	-0.457	-	-2.435	-	-	-
A1	51	2.722	5.02	-	-	6.196	-	-0.957	-	-	-	-	-
A1	52	1.172	2.19	2.476	1.375	1.523	2.381	-0.132	0.991	1.665	1.375	-	-
A1	53	-0.369	1.929	-	-	-0.913	1.571	0.623	2.423	-	-	-	-
A2	50	-1.372	1.37	0.248	2.067	-1.666	1.253	-0.367	1.838	-0.179	2.146	0.523	1.471
A2	51	2.037	3.148	0.363	1.197	1.454	3.915	2.416	2.414	0.851	1.212	-1.427	0.566
A2	52	-0.474	1.932	0.585	1.461	-0.37	1.998	-1.467	1.572	0.588	1.558	-0.027	-
A2	53	-1.37	0.896	1.52	2.082	-1.83	0.351	0.033	-	0.926	2.002	1.473	2.265
B1	54	-2.109	2.429	-1.568	1.958	-1.327	2.624	-	-	-0.827	1.338	-0.362	1.03
B1	55	-2.878	1.316	-1.736	1.631	-	-	-	-	-1.144	2.302	-0.262	-
B1	56	-0.318	2.332	0.003	2.685	-0.593	2.332			1.123	2.084	0.778	2.851
B1	57	-0.556	3.469	-1.221	1.965	-2.033	1.028	7.047	3.889	-0.164	2.286	-	-
B2	55	0.165	2.108	0.497	0.354	-1.56	0.99	0.985	2.34	0.067	-	1.212	-
B2	56	2.498	0.566	0.802	0.935	-	-	2.685	0.566	0.187	0.769	1.712	1.126
B2	57	1.286	1.427	0.297	1.125	0.89	1.794	1.41	1.234	0.067	0.931	0.812	1.244
C	58	1.391	2.111	0.047	0.513	3.603	1.131	0.177	0.956	-0.437	0.479	0.313	
C	59	-0.822	2.039	-3.093	1.98	-0.747	2.461	-1.023	1.926	-	-	-0.887	-
C	60	0.458	3.226	-1.093	-	-2.097	-	1.052	3.417	-1.712	-	-	-
C	61	-0.814	2.025	-0.982	2.391	-0.481	1.594	-1.236	2.297	-1.512	4.521	-0.22	0.981
D	62	2.834	3.134	1.806	2.531	2.081	3.731	4.061	-	1.696	2.596	0.062	-
D	63	0.826	2.735	-0.481	2.083	0.798	2.978	0.236	2.389	-1.164	1.303	0.612	4.455
D	64	1.865	2.624	0.203	2.631	1.84	2.38	-2.339	-	0.107	2.981	-0.121	1.959
D	65	0.701	2.701	0.294	3.469	0.315	2.701	-	-	0.273	3.58	-1.338	2.97
E	66	-2.451	1.441	-0.9	2.363	-2.144	1.345	-3.314	1.344	-0.549	2.711	-2.248	0.39
E	67	-2.206	1.242	-0.264	2.654	-2.519	1.242	-	-	-0.515	2.654	-	-
E	68	-1.881	2.639	-1.577	1.997	-1.977	2.371	-1.944	3.053	-0.751	2.888	-2.527	0.619
F	69	5.622	0.361	1.702	2.41	5.295	0.361	-	-	0.696	2.344	2.429	2.374
F	70	-2.561	1.27	-2.404	2.24	-2.685	1.307	-3.499	-	-1.971	2.219	-4.591	-
F	71	-2.796	1.559	-2.124	1.901	-3.124	1.559	-	-	-2.349	1.901	-	-
F	72	-1.278	2.31	-0.079	2.509	-1.063	2.348	-3.099	0.566	-0.049	2.731	-1.441	0.212
G	73	-1.442	2.448	0.461	-	-0.997	2.121	-2.123	3.394	0.175	-	-	-
G	74	2.072	2.602	-0.411	3.21	2.286	2.505	-0.723	-	-0.833	3.299	1.308	-
G	75	-0.781	1.382	-0.074	2.869	-0.572	1.455	-1.503	0.952	0.406	2.693	-3.192	0.469
G	76	-0.52	2.937	0.311	2.377	-0.54	3.33	-1.323	0.849	-0.011	2.463	0.341	2.686
H	77	0.573	2.993	0.258	1.893	0.241	2.993	-	-	-0.133	1.893	-	-
H	78	1.248	1.179	2.51	1.976	1.341	1	0.056	-	2.403	2.034	2.502	2.136
H	79	-0.277	3.606	-0.881	2.234	-3.159	-	2.356	-	-1.447	2.828	-0.488	2.616
H	80	3.123	2.799	-0.328	3.449	2.307	4.102	3.69	1.436	0.353	3.53	-2.788	1.202

Table 6.3.1.5 Analysis of variance done separately for the two (HI and LI) environments, showing significant differences in **COR-SLT** among dam groups within both environments.

Source	Sum of squares	DF	Mean Sum of Squares	F- Ratio	P
Dam groups (HI)	511.372	9	56.819	9.109	0.000
Error (HI)	3605.566	578	6.238		
Dam groups (LI)	290.083	9	32.231	5.697	0.000
Error (LI)	3303.980	584	5.658		

Table 6.3.1.6 Mean sum of squares from a nested ANOVA for **COR-SLT** , in each of the two environments (HI and LI), for the overall data as well as for the male offspring. (A nested ANOVA could not be done for female offspring due to insufficient data in some sire groups).

	Source	Sum of squares	DF	Mean Sum of Squares	F Ratio	P Valu
OVERALL DATA	**Dams (HI)**	286.035	9	31.782	1.957	NS
	Sires within Dams (HI)	454.697	28	16.239	3.289	< 0.001
	Within progenies (HI)	1402.372	284	4.938		
	Dams (LI)	156.359	9	17.373	1.951	NS
	Sires within Dams (LI)	231.541	26	8.905	1.697	< 0.025
	Within progenies (LI)	1558.406	297	5.247		
MALE OFFSPRING	**Dams (HI)**	150.984	9	16.776	1.066	NS
	Sires within Dams (HI)	409.106	26	15.735	3.136	< 0.001
	Within progenies (HI)	843.090	168	5.018		
	Dams (LI)	42.383	9	4.709	0.739	NS
	Sires within Dams (LI)	159.200	25	6.368	1.032	NS
	Within progenies (LI)	1147.360	186	6.169		

Table 6.3.1.7 Analysis of variance for **COR-SLT** corrected standard length of dam groups showing differences in performance between males (M) and females (F).

Source	Sum of squares	DF	Mean Sum of Squares	F-Ratio	P
Environments (M)	2.355	1	2.355	0.378	0.539
Dam groups (M)	434.966	9	48.330	7.751	0.000
Env. * Dam Grp (M)	99.684	9	11.076	1.776	0.069
Error (M)	4820.151	773	6.236		
Environments (F)	17.671	1	17.671	3.475	0.063
Dam groups (F)	451.198	9	50.133	9.859	0.000
Env. * Dam Grp (F)	173.942	9	19.327	3.801	0.000
Error (F)	1520.391	299	5.085		

Table 6.3.1.8 Analysis of variance for corrected standard length revealing differences between the sexes in the non-competitive (LI) environment but no differences in the competitive (HI) environment. Note the significant interaction term in both environments.

Source	Sum of squares	DF	Mean Sum of Squares	F- Ratio	P
Sex (HI)	0.241	1	0.241	0.039	0.844
Dam groups (HI)	378.154	9	42.017	6.797	0.000
Dam Grp. * Sex (HI)	243.909	9	27.101	4.384	0.000
Error (HI)	3276.504	530	6.182		
Sex (LI)	50.091	1	50.091	8.861	0.003
Dam groups (LI)	228.854	9	25.428	4.498	0.000
Dam Grp. * Sex (LI)	160.186	9	17.798	3.148	0.001
Error (LI)	3064.038	542	5.653		

Table 6.3.1.9 Nested ANOVA done separately for the two (HI and LI) environments, showing significant differences in **RCF** among dam and sire groups in both environments.

Source	Sum of squares	DF	Mean Sum of Squares	F- Ratio	P
Dams (HI)	5.847	9	0.650	9.420	0.000
Sires within Dams (HI)	1.933	28	0.069	1.568	0.035
Within progenies (HI)	12.414	284	0.044		
Dams (LI)	3.783	9	0.420	6.667	0.000
Sires within Dams (LI)	1.631	26	0.063	1.658	0.026
Within progenies (LI)	11.244	297	0.038		

Table 6.3.2.1 Variance components of different traits, as estimated using ANOVA. The results for **COR-SLT** contain estimates of the heritability for the full sample, the jacknifed estimates (obtained by omitting one sire group at a time), and the 95 percent confidence interval for the jacknifed estimates.

TRAIT	HI ENVIRONMENT			LI ENVIRONMENT		
	VARIANCE COMPONENTS		HERITABILITY	VARIANCE COMPONENTS		HERITABILITY
	Additive	Residual		Additive	Residual	
COR-SLT						
1. Dam: Full Sample	1.604	4.938	0.237	0.724	5.413	0.118
Jacknife	-	-	*0.223*	-	-	*0.079*
95 % CI	-	-	*-0.631 to 1.004*	-	-	*-0.213 to 0.498*
2. Sire: Full Sample	5.704	4.938	0.843	2.112	5.413	0.345
Jacknife	-	-	*0.834*	-	-	*0.291*
95 % CI	-	-	*-0.193 to 1.689*	-	-	*-0.349 to 0.802*
3. Sire + Dam			0.540			0.232
COR- WGT						
1. Dam	65.000	710.582	0.066	23.568	704.038	0.031
2. Sire	1060.976	710.582	> 1.00	163.728	704.038	0.218
RCF						
1. Dam	0.068	0.044	> 1.00	0.040	0.038	0.780
2. Sire	0.0075	0.044	0.120	0.015	0.038	0.284

Table 6.3.2.2 Variance components of various traits, as estimated using the Animal model and MTDFREML. (Explanation of superscripts: 1 - The only variable where the heritability has been jacknifed and a confidence interval obtained; 2 - Covariance and Correlation between direct and maternal genetic effects; 3 - The maternal permanent environment effect as a proportion of the total phenotypic variance.)

TRAIT	(CO)VARIANCE COMPONENTS					HERITABILITY		MAT. PERM. ENV.[3]	GEN. CORR.[2]
	Direct	Maternal genetic	Cov.[2]	Mat. perm. env.	Residual	Direct	Mat. gen.		
COR-SLT[1]									
1. HI ENV.									
F.S	6.644	0.283	-0.019	0.018	1.782	0.763	0.033	0.002	-0.014
Jack	-	-	-	-	-	1.017	-	-	-
95% CI	-	-	-	-	-	0.520 to 1.514	-	-	-
2. LI ENV.									
F.S	2.125	0.674	0.087	0.233	40205	0.290	0.092	0.032	0.073
Jack	-	-	-	-	-	0.306	-	-	-
95% CI	-	-	-	-	-	-0.205 t 0.816	-	-	-
COR-WGT									
1. HI ENV.	1332.292	-	-	-	51.957	0.962	-	-	-
2. LI ENV.	541.845	-	-	-	453.717	0.544	-	-	-
RCF									
1. HI ENV.	0.036	-	-	-	0.025	0.588	-	-	-
2. LI ENV.	0.018	-	-	-	0.033	0.351	-	-	-
MST									
1. HI ENV.	0.860	-	-	-	0.401	0.682	-	-	-
2. LI ENV.	0.231	-	-	-	0.546	0.298	-	-	-
DIS									
1. HI ENV.	0.345	-	-	-	0.185	0.652	-	-	-
2. LI ENV.	0.307	-	-	-	0.257	0.544	-	-	-

Chapter 7

Estimation of genetic parameters in cultivated tilapia (*Oreochromis niloticus*) using DNA fingerprinting. III. Genetic correlations between behaviour and growth variables.

ABSTRACT

The social microenvironment (the behaviour elicited by a fish as a consequence of its relative size) in a fish pond exerts a feedback loop in which growth rate (and hence relative size) affects agonistic behaviour, and *vice-versa*, thus making it difficult to study either trait individually. The present study breaks this feedback loop by considering family means and breeding values of behaviour and growth, rather than phenotypic values, in *Oreochromis niloticus*.

Fish were mated according to a maternal nested design to give 10 half-sib families. Equal numbers from each full-sib cross (within half-sib families) were pooled into each of three tanks (B, HI and LI). Fish from the first tank (B) were tested for their behaviour, while the other two tanks were used to study growth under contest, or high interaction (HI) and scramble, or low interaction (LI) competition, respectively. Upon termination of the experiments, parentage of the fish was established by DNA fingerprinting. An animal model, with pedigree information, was used to estimate breeding values for the growth and behavioural traits.

Genetic correlations were estimated in two ways: 1. correlation of family mean trait values between B and HI & LI tanks, and 2. correlation of breeding values. The correlations between some early agonistic behaviours (net aggression and winner/loser status) and subsequent growth were positive and significant in both competitive environments, thus making these behaviours good predictors of growth. Submission was

negatively correlated with subsequent growth in both environments. A path analysis revealed very strong dependence of growth on net aggression, and also that aggressive behaviour was associated with poor growth - more so in the LI environment than HI. Submission, on the other hand, was associated with good growth, more in the LI environment than in the HI environment. Finally, it is speculated that net aggression represents a behaviour that is under inadvertent selection during domestication.

7.1 INTRODUCTION

A harvest of uniform sized fish increases production efficiency and is therefore cited as one of the primary goals in aquaculture (Noakes and Grant, 1992). However, this goal is rarely achieved, especially in confined waters. Typically, size variation among the fish sets in even as they are growing, leading to quite disparate sizes over time. Agonistic behaviours among the fish are now known to be largely responsible for the differential growth rates commonly seen among fish farmed in confined waters (Magnuson, 1962; Purdom, 1974; Jobling and Wandsvik, 1983; Koebele, 1985; Jobling and Reinsnes, 1986; Abbott and Dill, 1989; Blanckenhorn, 1992; Carter *et al.*, 1992; McCarthy *et al.*, 1992; Jobling and Baardvik, 1994).

Differential growth rates lead to a phenomenon known as *growth depensation* (Magnuson, 1962), where there is an increase in the variance of sizes of the fish over time due to differences in growth rates, resulting in a size distribution that is typically skewed positively, towards the larger fish (Nakamura and Kasahara, 1955; 1956; 1957; 1961 - all in Wohlfarth, 1977). Such an increase in variance cannot merely be explained as an effect of the increase in mean size over time, as the above studies show. Individuals in a confined water body, such as a culture pond, that have but minute size differences (expressed as coefficient of variation) when young and small, exhibit a magnification of the differences in relative size as they grow, resulting in a whole range of sizes at harvest. Large fish show higher growth rates than small fish (*e.g.*, Jobling and Wandsvik, 1983). Though particularly well known in captive fish populations (Magnuson, 1962; Fagerlund

et al., 1981; Koebele, 1985; Metcalfe 1986; Metcalfe *et al.*, 1990; Jobling *et al.*, 1993), growth depensation is also known to occur in crustaceans (Ra'anan and Cohen, 1984; Karplus *et al.*, 1989, 1991, 1992), amphibians (Rose, 1959), and birds (Nelson, 1978).

Size-Aggression Feedback Loop

It is commonly observed that larger fish are behaviourally dominant and usually manage to either consume more of the available food, or somehow inhibit other smaller, more submissive, fish from feeding (Newman, 1956; Jenkins, 1969; Wankowski and Thorpe, 1979; Jobling and Wandsvik, 1983; Koebele, 1985; Davis and Olla, 1987; Grant, 1990). But it is not easy to decide whether such despotic behaviour gives these fish a size advantage with which to maintain their supremacy (which serves to increase their size advantage, and thus further reinforcing their dominant role), or whether the fish take advantage of a fortuitous size difference to assume a dominant role (thus initiating the feedback loop just mentioned). Research has not yet resolved this question, although there appears to be some evidence of an inherent "fierceness" in young fish that could confer a size advantage over time (Huntingford *et al.*, 1990). In any case, once size variation has been manifested, it becomes impossible to separate out the behaviours from the influence of relative size at the phenotypic level, (because of the feedback loop between behaviour and growth rate), thus making the study of either trait without the confounding effects of the other, very difficult.

This study circumvented the feedback loop by 1) testing for agonistic behaviours in fish that were still relatively "naive", 2) pair-wise testing of offspring from full- and

half-sib families to expose them to the whole range of relative sizes, and 3) correlating family means and parental breeding values of growth and behavioural traits, rather than correlating phenotypic values.

Competition and growth

Ecological theory distinguishes two common ways by which animals compete for limited resources, a high interaction situation known as *interference competition* and a low interaction state known as *exploitation competition* (Starr and Taggart, 1992), also known as *contest competition* and *scramble competition*, respectively. A contest competition involves high levels of interaction because the resource is limited in time and/or space, and the animals, in order to be successful, must win a contest by fighting for the resource. While contest competition is not the norm in an aquaculture setting where abundant food is broadcast (while scramble competition is), there is, however, potential for this form of competition at the micro level, particularly if the food is limiting or broadcast as discrete pellets.

The relationship between the agonistic behaviour in fish (or the lack thereof) and their growth performance in these two kinds of competitive environments (high and low interaction), is far from clear. Food provided to the fish in aquaculture ponds is typically in pellet form. While pellets cannot be easily defended, some fish do manage to be better competitors and obtain a greater share than others. Competitive success in feeding appears to be determined by different factors in different fish. For example, while body size was found to be important in the case of rainbow trout (Jenkins *et al.*, 1969) and

brown trout (Abbott and Dill, 1989), size or sex were found to be unrelated to success in food acquisition in the Atlantic salmon (Kadri *et al.*, 1996). In the pond environment, where entire fistfuls of food pellets are dropped simultaneously, there is a clear scramble among the fish. It is not entirely clear what behavioural factors aid success in such a situation (Adams and Huntingford, 1996). An overtly quarrelsome fish will probably not obtain more food under such circumstances as it is likely to end up wasting too much time in vain attempts at defending an un-defendable resource. On the other hand, dominant fish could intimidate others into limiting their food intake (Koebele, 1985; Kadri *et al.*, 1996). Furthermore, the snatching of food particles by fish does not necessarily indicate ingestion (Carrieri and Volpato, 1991).

As mentioned in Chapter 6, the obvious way of obtaining answers to such speculations is by actual observation in a realistic simulation of pond conditions in the laboratory (as attempted by Kadri *et al.*, 1996; however there was no growth component in their study). Unfortunately, not only are the logistics of such a study quite formidable (as it involves observing the behaviour of individual fish over the time it takes for growth depensation to take place), but perhaps more importantly, the size-dependant feedback loop sets in between agonistic behaviour and growth, thus rendering them inseparable in any phenotypic study of behaviour and growth.

The present study is an alternative method of understanding the role of agonistic behaviour in affecting growth rate among fish in confined waters. Behaviour and growth were not measured on the same fish. Rather, growth and behaviour were measured on different members of full-sib families. The product moment correlation of traits from

these sibships then provides an estimate of the genetic correlation among the traits. A second estimate of the genetic correlation was obtained by correlating the breeding values for the traits (as determined from pedigree records extending over 3 generations).

7.2 MATERIAL AND METHODS

7.2.1 *Mating and Experimental Design*

The material, mating design, molecular techniques used to identify family affiliation, and the statistical models/methods used in estimation of variance components in this study, have been described earlier (Chapters 3-6). Briefly, each of ten maternal half-sib groups (dam groups) was produced by mating one female with four males. For each dam group, eggs from each brood (full-sib family or sire group) were hatched separately, and equal numbers of hatchlings from each sire group were pooled and reared in three different containers, B, HI and LI. Behavioural observations were made on naive young fish (at swim-up) from tank B, by pairing fish randomly chosen from the half-sib pool. The behavioural measurements made were the number of aggressive acts (**AGR**) by each member of the pair. The number of submissive acts (**SUB**) by the two fish were recorded only for five dam groups. Using these two variables, three derived variables, **NET** (**AGR** minus **SUB**), **AGON** (**AGR** + **SUB**) and **DIFF** (**AGR** of one fish minus **AGR** of opponent), were constructed. Growth studies were done on fish in tanks HI and LI, which were manipulated to differ in the type of competition (contest and scramble, respectively) to elicit high and low levels of agonistic interactions. The measurements

made on termination of the growth experiments were standard length (**SLT**), weight (**WGT**), maturity status (**MST**), and the extent of a disease attack (**DIS**).

At the termination of the behavioural observations and growth experiments, the sire group that each fish belonged to was determined by genotyping each fish at up to 9 loci using DNA microsatellite primers. These genotypes were compared with the genotypes of the parents, and the male parent thus determined, as there is Mendelian inheritance at microsatellite loci. The female parent did not have to be identified since it was the common parent in each dam group.

7.2.2 *Data Analysis*

After determining the parentage of the fish studied, the data were analyzed to obtain variance components using the conventional ANOVA technique. Variance components were also determined using DFREML, a recently developed technique that is more commonly used in livestock genetics research. The analysis of variance was done on a PC using the statistical package SYSTAT version 6 for Windows (SPSS Inc., 1996), while the DFREML analysis was done in the UNIX environment using a set of computer programs termed MTDFREML (Boldman *et al.*, 1995), downloaded from an ftp site. Details are given in Chapters 3-6. The program can be used with any data set with pedigree information. The data used in the analysis here contained pedigree information running back four generations, the pedigree beyond the immediate parents obtained from MGPL records.

The present chapter deals with the calculation of genetic correlations among the behaviour and growth variables. Genetic correlations were calculated by the following two methods:

1. Correlating mean full-sib family values obtained for the behavioural and growth traits (sib-correlation), and
2. Correlating breeding values obtained for the traits, as explained below.

One of the output files of MTDFREML contains breeding values for all the fish in the pedigree file. This includes fish on which measurements have been made as well as the parents and the rest of the pedigree. The method used by MTDFREML involves the use of MME (mixed model equations) for obtaining simultaneous solutions for fixed effects and random effects (breeding values) and estimation of (co)variance components.

7.3 RESULTS

Figure 5.3.1.1(a) in Chapter 5 (p. 125) shows **AGR** plotted against relative size (ratio of the length of the fish genotyped and that of its opponent), while Fig. 5.3.1.2 and Fig. 5.3.1.3 (a) and (b) show **SUB**, **NET** and **AGON** plotted against relative size (all diagrams on p. 125). In each case there is a demarcation of winner/loser status (**W/L**). It is clear from the figures that relative size is an important determinant of the level of aggression or submission elicited. It also determines the winner/loser status - larger fish win and smaller fish lose, in general. However, there were exceptions to this general rule: 7.71 percent of the fish did not follow this norm; they were either winners when smaller or losers when larger. Further, it can be seen from the figures that in the absence of a size

cue (*i.e.*, when both fish are of similar size), both aggression (**AGR**) as well as total agonistic activity (**AGON**) show high values.

Of the behavioural variables in the figure, **SUB** and **NET** are good determinants of **W/L**. Aggression *per se* does not necessarily win an encounter; a fish must be more aggressive than submissive. Indeed, the figure clearly indicates that winning is decided not so much by the level of aggression, as by the lack of submission. This point is mentioned again in the discussion.

Further, the figures mentioned above indicate that of the behavioural variables used, only **AGR** and **NET** are suitable for further analysis (as **SUB** does not show sufficient variation and **AGON** does not convey any new information when compared to **AGR**, and **AGR** is the only complete variable). Before any further analysis could be done however, the influence of relative size on **AGR** had to be removed - this was done by taking the residuals from a 5th degree polynomial fitted to the plot, to give a "corrected" measure for aggression.

As far as the growth experiments were concerned, the single most influential variable affecting **SL** and **WT** was the number of fish in each tank (the tank density). The correlation of these variables with tank density was negative and significant in both environments ($r = -0.808$ and -0.807 respectively, for the two traits in the HI environment, and $r = -0.908$ and -0.846 respectively, in the LI environment; $p < 0.01$ in all cases). Hence this influence was removed by regression, and the residuals used in further analysis.

Genetic relationship between behaviour and growth

In order to obtain breeding values, both behavioural and growth traits were subjected to analysis using MTDFREML, with tank and age of fish as fixed effects. This set of programmes also gives the inbreeding coefficient for each animal on which measurements were taken, as well as for the entire pedigree. Owing to the mating design used in this study, the inbreeding coefficients fell into a discrete set of values. Therefore, it was easy to categorize the fish based on these values, and then study the influence of inbreeding on any trait, by plotting mean trait values against the corresponding inbreeding coefficient. The plots of all the behavioural and growth variables with the inbreeding coefficient were uniformly negative, with statistically significant correlations. While the effect of inbreeding on behaviour has not been as widely studied as that on morphological traits and fitness, there is evidence that inbreeding reduces aggression levels and lowers the chances of winning staged encounters in some animals such as mice (Barnyard and Fitzsimons, 1989; Eklund, 1996).

Inbreeding enters the REML analysis in two ways. One is that it influences the relationships among animals, which with an animal model and full pedigree is taken into account by MTDFREML. The second is because inbreeding has effects on the phenotypic expression of a given trait (inbreeding depression, *etc.*). Thus, with the animal model and full pedigree, and with inbreeding as a covariate, the effects of inbreeding were fully accounted for in the analysis (Van Vleck, *pers. comm.*).

Inbreeding effects were removed by regression (using the inbreeding coefficients obtained from MTDFREML) before genetic correlations using Method 1 were estimated, also.

The entire protocol for the measurement of behaviour in this study was geared towards characterizing families (sire groups) according to the level of hostility of the fish towards each other. The same sire groups were also characterized according to their performance in growth and growth-related traits in the two macroenvironments, thus making it possible to match family performances across behavioural and growth traits.

Table 7.3.1 shows the genetic correlations between the behavioural traits, **AGR** and **SUB**, and the growth traits **SL** and **WT**, while Table 7.3.2 shows the genetic correlations between the behavioural variables **NET** and the outcome **W/L** (which is also treated as a behavioural trait), and the two growth traits. **AGR** and the growth traits were first corrected for the influence of relative size and density effects, respectively. All the behavioural and growth traits, with the exception of **W/L**, were then corrected for inbreeding effects. (**W/L**, being a binary variable, did not lend itself for easy correction.) The tables show the correlations for the overall data, as well as separately for the two sexes. The columns named SIBS comprises the genetic correlations obtained using Method 1, while column EBV contains genetic correlations estimated by correlating breeding values (Method 2) of the parents (and the rest of the pedigree) obtained using MTDFREML, for the various traits.

7.4 DISCUSSION

Most of the genetic correlations between the behavioural and growth traits are positive in both environments, except those that involve **SUB**, which are negative. This implies that higher aggression / lower submission at swim-up is genetically correlated with better growth in both environments. Significant correlations are seen for **SUB**, **NET** and **W/L**, but not for **AGR**, and only under the EBV column. The stronger correlations from the breeding values are perhaps because of the additional information provided by the relationship matrix (explained further below).

The genetic correlations presented above are not influenced by a proximal cause-and-effect relationship, and thus are independent of the phenotypic feedback loop. In this study, the behavioural measurements were obtained from "naive" fish, and to that extent can be considered innate, akin to the "fierceness" of Huntingford *et al.*, (1990). It is important to note that behaviour was measured soon after swim-up and growth measured later on. Therefore, the genetic component of behaviour (measured soon after swim-up) should be considered to be a genetic component of subsequent growth, rather than *vice versa*. The important point is that such an inference can be made while still keeping safely out of the feedback loop by which individual animals which are more aggressive may become larger, and *vice-versa*.

As to why the derived variable **NET** is more strongly correlated with growth than **AGR**, the answer perhaps lies in Fig. 5.3.1.1 (a) and 5.3.1.3 (both on page 125), where it was seen that **NET** was a better predictor of winner/loser status than **AGR** itself. It can perhaps even be surmised that it is not aggression *per se* that is in play in determining

growth rate, but a different, un-measured behavioural trait, that is better reflected by the net aggression, or better still, by the winner/loser status, in this study. As mentioned earlier in connection with Fig. 1, it appears to be lack of submission rather than aggression *per se* that is the determining factor. However, the genetic correlations between **SUB** and the growth variables seen in Table 7.3.1 are inconclusive, as two correlations are significant and two are not (EBV column).

There is some uncertainty in the literature regarding the fate of aggression during domestication (reviewed by Ruzzante, 1994). The present study indicates that early aggression itself is poorly correlated with later growth performance, whereas net aggression and winner/loser status show high correlations. It can therefore be speculated that the behaviour that is under selection in the domestication process is not aggression or submission *per se* but a more comprehensive, latent (in the statistical sense) behaviour that could perhaps be termed "tameness" or "fearlessness". Such behaviours, that represent successful adaptation to the domestication environment by the fish, have been shown to manifest under domestication selection in a number of ways. For example, Vincent (1960) found that domestic brook trout (*Salvelinus fontinalis*) were tamer and exhibited less fright than their wild counterparts. Domestic brook trout were also found to grow faster, have a more robust body, produce more eggs, and were "easier to rear" than their wild counterparts (Moyle, 1969; the references therein). Moyle (1969) himself found greater overall activity and fearlessness among domestic brook trout.

It is reasonable to expect that during the process of domestication fish are adapting to the various conditions and disturbances of the culture environment. Due to the high

densities commonly seen in culture ponds, fish are also having to adapt to dealing with greater contact with other fish. The behaviours exhibited by the fish during these encounters undoubtedly influence the pattern of food intake and assimilation, and hence growth. Perhaps the typically measured behaviours, aggression and submission, are too simplistic and do not realistically depict the behavioural changes taking place in the fish. It is in this context that the higher correlation seen between **NET** and the growth traits takes on greater significance. Perhaps **NET** better reflects these changes than the individual behaviours.

The results of the present study in relation to those reported in the literature

On the one hand, there is a general consensus that tameness is an integral part of the process of domestication, and there is evidence that tameness seems to be indeed increasing under domestication (*e.g.*, Vincent, 1960; Price, 1978; Barnett *et al.*, 1979; Holm and Fernö, 1986; Doyle and Talbot, 1986; Robinson and Doyle, 1990; Ruzzante and Doyle, 1991, 1993). Also, Huntingford *et al.*, (1990) have shown that aggression appears to be an "innate" property in young Atlantic salmon, and may not be size-related. On the other hand, other studies suggest that aggression appears to be increasing during domestication selection (*e.g.*, Swain and Riddell, 1990; and Mesa, 1991).

The present study shows positive, but low genetic correlations between aggression and growth, whether in high or low competition environments. The genetic correlations between submission and growth are negative but inconclusive as far as the type of

environment is concerned. On the other hand, net aggression shows significant and positive genetic correlations with length and weight in both environments.

In order to understand this further, a path analysis was done, using SYSTAT 7.0 for Windows (SPSS Inc., 1997), for the following three models:

1. Paths leading from **AGR** and **SUB** to **NET** and to **W/L**, and a path leading from **NET** to **W/L**; covariance between **AGR** and **SUB**.
2. Same path as above but replacing **W/L** by **SL** (HI environment).
3. Same path as (1) but replacing **W/L** by **SL** (LI environment).

All three models fit well (Sample Discrepancy Function Value < 0.0001 in all three cases). The GLS point estimates of the parameters in the dependence relationships can be seen in table 7.3.3. Model 1 treats W/L as an outcome dependent directly upon AGR and SUB, as well as indirectly through NET, while Models 2 and 3 consider the dependent variable SL (in HI and LI environments, respectively) with direct paths from AGR and SUB and an indirect path through NET.

As can be seen the path through NET is the strongest in all cases. This path is so strong that it apparently affects the direct paths, thus actually giving a negative coefficient for AGR and a positive coefficient fro SUB, in all cases. The negative dependency between AGR and SL, is however, stronger in the LI environment than in the HI environment. Similarly, the positive relationship between SUB and SL is stronger in the LI environment than in the HI environment. These results agree with intuition as well with the theoretical arguments of Doyle and Talbot (1986). It pays less to be aggressive in the LI environment than in the HI environment. Similarly, it pays more to be submissive

in the LI environment than in the HI environment. The results also seem to suggest that it pays to be more "net" in the LI environment than in the HI environment. Perhaps, as suggested earlier, there is an un-measured variable that is best reflected by NET in this study, that is akin to the "uninvolved" strategy of Doyle and Talbot (1986). The pay-off is greater for this variable in the LI environment than in the HI environment. A LI environment is similar to what an ideal aquaculture environment should be, with enough food being made available to all the fish, by broadcasting.

When tested at swim-up, this behaviour was manifested as the ability by the fish to protect itself against possible predation by occupying the bottom of the container. In the growth experiments this behaviour helped fish grow better in both environments, but more so in the LI environment.

Now some attention needs to be given to the correlations obtained using the family means. These have turned out to be uniformly small (and non-significant) when compared with those obtained from the breeding values (Tables 1 and 2). The SIBS estimates are much less reliable than the EBV estimates for the following reason. Family means in this study could be obtained only after unambiguous identification of male parents. This was invariably impossible for all the offspring, given the low polymorphism of this fairly inbred population. Because of this about 30 percent (overall) of the offspring could not be assigned a male parent, and information from these offspring could therefore not be used in computing full-sib family means. This reduced the sample size on which these means were based, thus decreasing the precision of the sample means (because of large standard errors). This in turn, meant that correlation of these means would also be less precise.

The genetic correlations based on the breeding values did not suffer from non-identification of male parents. Female parents were already known since they were the common parents. MTDFREML makes use of this partial information in the relationship matrix which is used in the estimation of variance components and breeding values. Further, the pedigree beyond the immediate parents is also used in the relationship matrix, thus making this methodology more informative.

7.5 CONCLUSIONS

In conclusion, we find that **NET** is a better measure of the ability to succeed in a staged, pairwise, resource-free contest than **AGR**. We also find that **NET** and **W/L** (winner/loser status in the contest) are good predictors of subsequent growth performance in both HI and LI environments. The correlation of family means shows positive genetic correlations between early behaviours and subsequent growth. Correlation of breeding values shows positive genetic correlations much more strongly, especially for **W/L** and **NET**. A path analysis revealed that low levels of aggression in the LI environment had a better pay-off in terms of growth. The analysis also revealed a strong path between NET and SL, more so in the LI environment than in the HI environment. From these results it can perhaps be concluded that there is an un-measured behavioural variable at play here, higher levels of which ensure better growth rates in the LI environment. This measure is best reflected by **NET**.

Table 7.3.1 Genetic correlations between behaviour variables (aggression, **AGR** and submission, **SUB**,) and growth traits (standard length, **SL** and weight, **WT**) in each of the two environments (HI and LI). SIBS and EBV refer to whether the genetic correlations were obtained by sib-correlations or correlations of estimated breeding values, respectively. All traits were corrected for inbreeding effects, with the addition that **AGR** was also corrected for relative size effects and **SL** and **WT** were also corrected for density effects, respectively. Statistical significance at $p < 0.05$ and $p < 0.01$ is denoted, respectively, by * and **, and correlations not computed are denoted by dashes)

	AGR				SUB			
	SIBS		EBV		SIBS		EBV	
	HI	LI	HI	LI	HI	LI	HI	LI
Overall dat								
SL	0.165	0.273	0.160	-0.018	0.064	-0.091	-0.238*	-0.048
WT	0.013	0.426	0.140	0.143	0.142	0.008	-0.109	-0.330*
Males								
SL	0.201	0.335	-	-	0.006	-0.120	-	-
WT	0.036	0.429	-	-	-0.039	0.011	-	-
Females								
SL	0.247	0.027	-	-	-0.146	-0.074	-	-
WT	0.02	0.281	-	-	-0.175	-0.089	-	-

Table 7.3.2 Genetic correlations between behaviour variables (net aggression, **NET**, and winner/loser status, **W/L**) and growth traits (standard length, **SL** and weight, **WT**) in each of the two environments (HI and LI). SIBS and EBV refer to whether the genetic correlations were obtained by sib-correlations or correlations of estimated breeding values, respectively. All the variables (except **W/L**) were corrected for inbreeding effects, with the addition that **SL** and **WT** were also corrected for density effects, respectively. Statistical significance at $p < 0.05$ and $p < 0.01$ is denoted, respectively, by * and **, and correlations not computed are denoted by dashes)

	NET				W/L	
	SIBS		EBV		EBV	
	HI	LI	HI	LI	HI	LI
Overall data						
SL	-0.031	0.374	0.395**	0.320**	0.438**	0.317**
WT	-0.065	0.295	0.340**	0.421**	0.413**	0.333**
Males						
SL	0.216	0.425	-	-	-	-
WT	0.192	0.291	-	-	-	-
Females						
SL	0.263	0.211	-	-	-	-
WT	0.164	0.265	-	-	-	-

Table 7.3.3 Path coefficients for 3 models, with **W/L, SL** in HI environment, and **SL** in LI environment as the dependent variable, respectively, and **AGR, SUB,** and **NET** as the manifest explanatory variables. Note the extremely strong path from **NET** to all the three dependent variables.

Model 1		Model 2		Model 3	
Path	**Coeffs.**	**Path**	**Coeffs.**	**Path**	**Coeffs.**
W/L <- AGR	- 0.242	SL(HI) <- AGR	- 0.149	SL(LI) <- AGR	- 0.376
W/L <- SUB	0.136	SL(HI) <- SUB	0.192	SL(LI) <- SUB	0.546
W/L <- NET	1.032	SL(HI) <- NET	0.639	SL(LI) <- NET	0.985
NET <- AGR	0.329	NET <- AGR	0.329	NET <- AGR	0.329
NET <- SUB	- 0.629	NET <- SUB	- 0.629	NET <- SUB	- 0.629

Chapter 8

General Conclusions

Growth rate variation in cultivated fish can take on alarming proportions, leading to lowered profits for the fish farmer. This phenomenon has been widely recognized and studies have shown that it is the differential responses to agonistic behaviours from other fish in the culture pond, that lead to different growth rates and consequently, different sizes at harvest. This study was undertaken to understand the relationship between agonistic behaviours and growth rates in two environments differing in the type of competition among the fish.

The technique of pooling fish to eliminate replicate variance and expose the fish to a wide range of social microenvironments, and to later establish parentage by using DNA microsatellite markers, was found to be an efficient experimental procedure.

Heritability estimates by DFREML were in general agreement with estimates obtained from the full sib component (sire estimates) of the ANOVA procedure. Estimates of heritability for behaviour traits showed low values, except for the derived variable **NET** (**AGR** minus **SUB**). Heritability estimates for growth traits in the HI environment were extremely high, with either procedure, presumably because of higher levels of genotype-by-social microenvironment in the HI environment. The estimates from LI environment were in general agreement with reports in the literature.

Genetic correlation between behaviour and growth traits exhibited positive, but low correlations between **AGR** and growth, in both environments. **SUB** showed a negative genetic correlation with growth in both environments. **NET** showed significant and positive correlations with growth traits in both environments.

A path analysis done with different models also indicated that **NET** is an important explanatory variable for the dependent variable **SL** in both environments, as well as **W/L**. The path analysis also indicated that the growth pay-offs were lower for aggressive fish in the LI environment and higher in the HI environment. Similarly it paid more for a fish to be more submissive in the LI environment than in the HI environment. Higher levels of **NET** were better displayed in the LI environment than the HI environment, for good growth.

It is speculated that **NET** perhaps represents a variable that is under selection during the process of domestication selection for rapid growth.

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IMAGE EVALUATION
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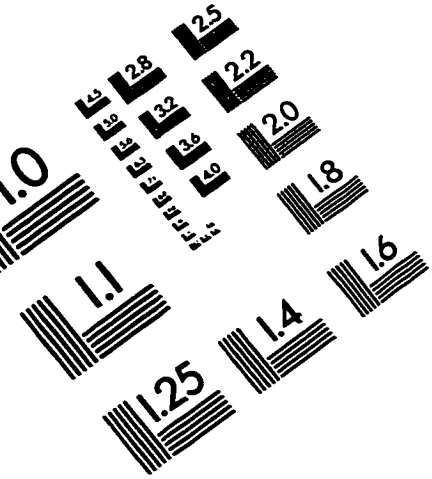

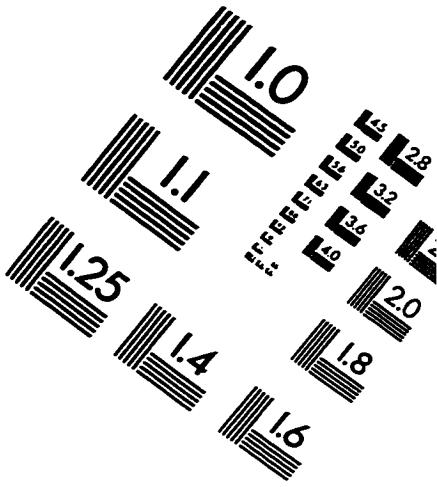

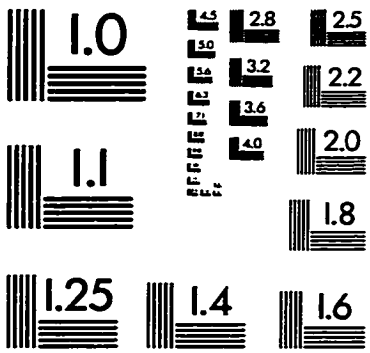

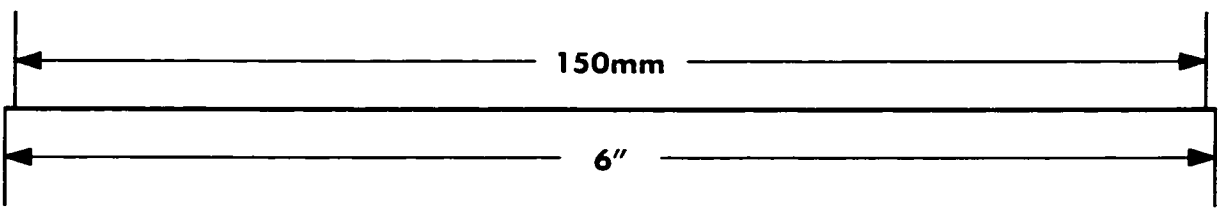

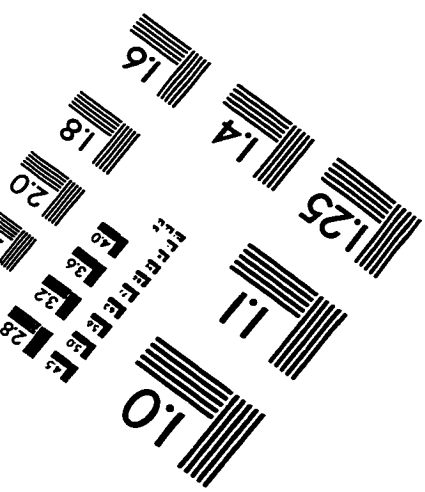

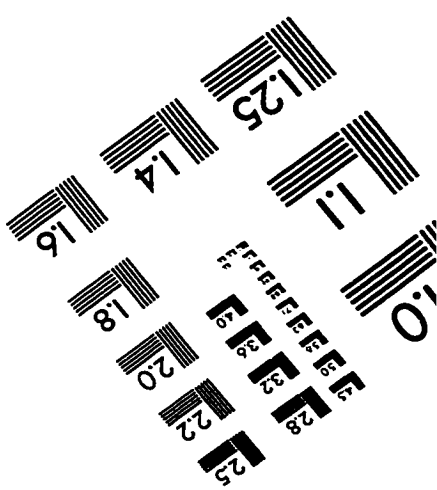

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