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**RAPID CHANGES IN SOCIAL BEHAVIOUR IN MEDAKA (*Oryzias latipes*) CAUSED
BY SELECTION FOR COMPETITIVE AND NON-COMPETITIVE GROWTH**

by

Daniel Eduardo Ruzzante

Submitted in partial fulfillment of the requirements

for the degree of Doctor of Philosophy

at

Dalhousie University

Halifax, Nova Scotia

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ABSTRACT

Behaviour is a major component of growth rate variation in fish, but the genetic relationship between behaviour and growth is largely unknown. In particular, it is not known how behaviour responds to natural or artificial selection on growth (as during domestication of aquaculture stocks). I selected medaka (*Oryzias latipes*) for high and low growth rate, in two environments that differed in the intensity of social interactions. In the high interaction environment food was provided to excess inside a floating cork ring, which limited access to the food, and allowed fish to attempt to monopolize the resource. In the low interaction environment, an equivalent amount of food was distributed over the container's surface. In each social regime fish were selected within family for fast and for slow growth rate during two generations (G_1 and G_2). Four selected lines were established after the first generation of selection: fast and slow growth rate lines in the high interaction environment, and fast and slow growth rate lines in the low interaction environment. In the third generation, half the number of broods in each line was raised in a high interaction, and half in a low interaction environment, bringing the number of sublines to eight. The response was measured in the third generation (G_3) on the directly selected trait (growth) and on several correlated behavioural traits: (a) agonistic behaviour; (b) social tolerance and locomotor activity; (c) schooling behaviour. The results were as follows: (1) Growth rate was modified as a consequence of selection, and the response was more pronounced in the high interaction than in the low interaction environment. (2) Selection for rapid growth in a high interaction environment, with food provided *ad lib.*, induced a decrease in the intensity of agonistic behaviour. These results show how agonistic behaviour changes during domestication selection, and confirm the predictions of Doyle and Talbot's (1986) game theoretic model. In particular, growth rate selection in medaka favours fish that are indifferent to the presence of conspecifics, and not those that are most aggressive. (3) The genetic correlation between growth and "social tolerance", and between growth and activity were rapidly modified as a result of selection for fast and slow growth in a high interaction environment. These results provide experimental support for the concern about the robustness of evolutionary inferences derived under the constant covariance assumption (e.g. Turelli 1988), and suggest at least some of the variability of these behavioural traits may be controlled by a few genes of major effect. (4) The school cohesiveness in the presence of a predator was also modified as a result of selection, indicating that schooling behaviour in medaka is influenced by genetic factors related to growth.

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CHAPTER 1

GENERAL INTRODUCTION

Individual differences in competitive abilities can be of paramount importance to the dynamics of populations (Begon 1982, Łomnicki 1982). Intraspecific competition can affect the overall abundance of individuals, their distribution, and their reproductive success. Despite the importance of individual differences, ecological modellers have traditionally treated populations as consisting of individuals that differ only in sex and age and are identical otherwise (Łomnicki 1978, 1988). Relatively little is known about the mechanisms by which individuals affect the performance of conspecifics (Rubenstein 1981a, Łomnicki 1988). Consideration of individual differences is, however, necessary for a more thorough understanding of the ecology of single populations. For instance, variation in population size can have significant non-linear effects on the potential reproductive success of individuals (Rubenstein 1981a). In addition, Pulliam (1988) recently argued that consideration of habitat-specific demographic rates may be more important for ecological and evolutionary considerations than the more widely considered age-specific demographic rates.

Whether the interactions among individuals are mediated through the establishment of dominance hierarchies and/or territoriality is likely to depend on four major factors and their interactions (Rubenstein 1981b, Davies and Houston 1984): first, the quality of the resource; second, its distribution in space; third, its distribution in time; and fourth, the intensity of competition (i.e. the quantity of the resource or the number of competitors). In general, these factors and their interactions will also affect the reproductive success of individuals. Other factors being equal, the more patchily distributed or clumped is a resource, the easier it is for

an individual to defend it, and thus, the more likely territoriality is to occur. Dominance hierarchies are common among group living female monkeys, yet dominant individuals exhibit a relatively high reproductive performance only when they are able to monopolize food resources to an extent that they disadvantage subordinates (Harcourt 1988). In addition, whether or not the resource will be economically defensible will also depend on the density of competitors. This density will in turn depend on the quality of the patch and on its size (Davies and Houston 1984).

Variation in breeding systems provides further evidence that the distribution of resources influences the social organization of animal populations. For instance, in cervid species where females congregate around patchily distributed resources and form large parties during the breeding season, male-male competition for females is intense, sexual dimorphism is pronounced, and polygyny is the most common breeding system. In contrast, in species where females remain solitary or in small scattered parties all year round (because resources are scattered), male-male competition for females, sexual dimorphism, and polygynous breeding systems are less common (Clutton-Brock et al. 1982). A similar relationship between social structure and resource distribution has been described for primates (Clutton Brock et al. 1977).

Distribution and abundance of resources can affect the dynamics of fish populations as well. For example, growth rate differences among individuals are known to increase under competition (Brown 1946, Allee et al. 1948, Yamagishi 1962, Rubenstein 1981a, Koebele 1985, and others). This increase in variance of growth with time when fish are reared in a group (growth depensation)(Magnuson 1962) results from the establishment of dominance hierarchies, which persist even in the presence of excess food (Abbott et al. 1985, Jobling 1985). There are several mechanisms by which the social structure of a population can

influence the growth rate of individuals. Dominant fish may grow at a relatively fast rate because they prevent subdominant individuals from gaining access to the food (Yamagishi 1962, Symons 1971, Li and Brocksen 1977, Koebele 1985) or because their presence induces a "physiological stress" on the subdominant individuals, which therefore grow at less than the optimal rate (Yamagishi 1962, Fenner et al. 1968, Ejicke and Schreck 1980, Jobling and Wandvisk 1983, Koebele 1985, Metcalfe 1986, Abbott and Dill 1989). The costs of being a dominant individual can, however, offset the benefits, depending on an array of environmental circumstances (Houston and Davis 1984). Excessive energetic demands on the dominant individuals appear to explain why in some cases they are not the largest in body size (Li and Brocksen 1977, Rubenstein 1981b). In addition, excessive time devoted to territory defense and consequently not enough to feeding could also explain these results (Yamagishi 1962, Yamagishi et al. 1974, Rubenstein 1981b, Jobling and Wandvisk 1983).

The various mechanisms used by the pygmy sunfish (*Elassoma evergladei*) to compete for food are also influenced by the distribution of resources and the density of competitors (Rubenstein 1981b). For instance, dominant fish defend territories when food is predictably located in clumps. However, whether territorial aggression persists when food is randomly distributed depends on population densities: at low and high densities dominant fish stop displaying territorial behaviour, whereas at intermediate densities they remain territorial regardless of the pattern of distribution of food. These results suggest that population density and resource patterning interact to influence the cost-benefit ratio of different competitive tactics (Rubenstein 1981b).

Because of the relatively common occurrence of dominance hierarchies in fish populations, aquaculture geneticists have expressed the concern that selection for enhanced growth under competitive conditions may favour fish that are more aggressive (Purdum 1974,

Kinghorn 1983). If this view is correct, then selection for fast growth would be mostly ineffective, since it would increase overall aggressiveness in the population, and thus, not productivity. Moyle (1969) reported limited evidence suggesting domestic strains of juvenile brook trout (*Salvelinus fontinalis*) were more aggressive than the laboratory reared offspring of wild parents, but attributed this result to an increase in the overall swimming activity of the domestic strain. More recently, Swain and Riddell (1990) compared agonistic behaviour of newly emerged coho salmon (*Oncorhynchus kisutch*) between hatchery and wild populations using mirror image stimulation tests. They concluded that hatchery coho salmon showed higher levels of aggressiveness than wild strains (Swain and Riddell 1990).

Doyle and Talbot (1986), using a game theoretic argument, modelled the consequences on aggressive behaviour of selection for enhanced growth in a competitive environment. They concluded, in direct opposition to the suggestions by the previous authors, that selection for fast growth in a moderately rich environment was likely to favour fish that are "uninvolved" in aggressive interactions, and that tameness would increase, not aggressiveness (Doyle and Talbot 1986). They argued that selection for enhanced growth was likely to result in a higher level of aggressiveness only if aggressiveness was independent of relative size, and the intensity of competition was very high.

In the following paragraphs I will provide a brief summary, by no means exhaustive, of examples describing the adaptive flexibility of foraging, territorial, and aggressive behaviour in general, with particular reference to fish. With these examples, I hope to be able to convince the reader that a scenario whereby aggressive behaviour in fish would have evolved to be independent of relative size is extremely unlikely. The available evidence is conclusive: foraging, agonistic, and territorial behaviours in fish are flexible and can be adjusted to changing environmental circumstances.

Territorial behaviour depends on resource availability and distribution

Territorial behaviour is widespread among foraging, juvenile salmonids living in streams. There is considerable evidence suggesting the size of their territories is often inversely proportional to the availability of food resources (Slaney and Northcote 1974, Hixon 1981, Dill et al. 1981, Dill 1983a). These experiments provided evidence that salmonids adjust the size of their territory according to cost-benefit considerations (Dill et al. 1981), defending smaller territories when food is abundant, and larger territories when food is scarce (Slaney and Northcote 1976). In addition, the distance at which territorial coho salmon react to attacking intruders increases asymptotically with hunger level (Dill et al. 1981), and the intensity of the aggressive defense of the territory by resident brook charr (*Salvelinus fontinalis*) is inversely related to the distance between the resident's station and the intruder (McNicol et al. 1985).

In the three-spot damselfish (*Eupomacentrus planifrons*), a territorial species that feeds on benthic algae, the aggressiveness of a resident individual is often proportional to the degree of threat posed by the intruder: residents defend the largest territories against intruder species with whom they have the largest diet overlap (Thresher 1976).

Size asymmetry in fish is frequently a reliable predictor of dominance hierarchies (Symons 1968, Gorlick 1976, Abbott et al. 1985, Parmigiani et al. 1988, cf. Huntingford et al. 1990). Experimental evidence (Symons 1968, Frey and Miller 1972, Wankowski and Thorpe 1979, Torricelli et al. 1988) confirms the prediction of game theory that the level of escalation in aggressive encounters is inversely related to the relative difference in size among contenders (Parker 1974, Maynard Smith and Parker 1976, Maynard Smith 1982).

Many other species are also known to adjust foraging territory sizes in response to the availability of food resources. For instance, observations and experiments with immature, migratory rufous hummingbirds (*Selasphorus rufus*) have demonstrated that these birds adjust the size of their foraging territories to maintain approximately constant reserves of energy per individual (e.g. nectar content) (Gass et al. 1976, Kodric-Brown and Brown 1978).

Foraging behaviour depends on predation risk

Fish are also known to adjust their foraging behaviour in relation to predation risk. For instance, after exposure to a model avian predator, foraging three-spined sticklebacks (*Gasterosteus aculeatus*) prefer the relatively low density regions of *Daphnia* swarms, instead of the more profitable, high density central regions. The immediate consequence of this behaviour is that feeding rate decreases, but the probability of detecting a predator nearby increases (Milinski and Heller 1978). In addition, consideration of individual differences in behaviour has demonstrated that sticklebacks also adjust their foraging tactics in relation to their competitive abilities and to patch profitabilities (Milinski 1988).

Godin and Smith (1988) have demonstrated the existence of a fitness-associated cost to foraging in the guppy, *Poecilia reticulata*. Individual decisions on where and when to forage, when foraging poses a risk of being eaten are dependent on the forager's current feeding rate, its food deficit, and local food density (Godin and Smith 1988).

Thus, there is little doubt that foraging, aggressive and territorial behaviour of fish and other groups is adaptively flexible, and environmental conditions can and do influence the level of aggression and territoriality exhibited by individual fish. The question remains,

however, whether an individual becomes dominant and behaves aggressively because it is relatively large, or whether it grows at a relatively fast rate because it is inherently more aggressive than other individuals in the population, thus being able to monopolize or obtain a disproportionate amount of food.

In this dissertation I will show what behavioural changes actually occurred in response to selection on growth in fish. I will then discuss why these changes should have been expected, despite arguments to the contrary in the literature. I conducted a divergent selection program on the basis of growth, using the japanese medaka (*Oryzias latipes*). Selection was conducted with fish raised in two environments that differed in the intensity of social interactions.

Genetic correlations among traits usually arise by pleiotropic effects, whereby a single gene affects two or more characters simultaneously (Hedrick 1983, Falconer 1989). Linkage disequilibrium, the non-random association of alleles at different loci, can, however, be a temporary cause of correlations between traits. Genetic correlations are genotype dependent. Thus, the changes in genomic composition that occur as a result of selection on one trait are responsible for changes in the magnitude of the genetic correlations involving that trait. Hence, if growth and behaviour are genetically correlated, it is reasonable to expect that selection on growth would induce changes in the value of these correlations. In addition, if there is genotype-environment interaction in the expression of this correlation, then the sign and magnitude of the changes occurring during selection will depend on the environment in which selection took place. Based on the game theoretic analysis of Doyle and Talbot (1986) I predicted that selection for enhanced growth in an environment where access to the resource depended on social interactions would induce a decrease in the intensity of aggressiveness, so

long as food was not limited in quantity. In contrast, selection for enhanced growth in an environment where equivalent amounts of food were evenly distributed, and thus where fish did not have to interact with each other to gain access to it, would induce no changes in the phenotypic expression of aggression. I therefore expected genotype-environment interaction in the behavioural changes that occurred as a result of selection on growth. Both, the direct response to selection (growth rate) and the correlated response on agonistic behaviour are reported in chapter two.

In chapter three, I describe experiments conducted with unselected fish raised in high and low interaction environments. My objective was to determine the flexibility of behaviour in response to environments that differed in the intensity of enforced social interactions. I analyzed the relationship between growth and two behavioural traits, which I defined as locomotor activity and tolerance for a crowd of conspecifics. The phenotypic correlation between growth and locomotor activity, and between growth and tolerance for a crowd of conspecifics are expected to be environment dependent. If resources are clumped, high levels of activity in the presence of conspecifics are expected to be associated with fast growth, since activity under such conditions can be interpreted as a manifestation of territorial or aggressive behaviour. In contrast, when resources are evenly distributed, and equally accessible to all individuals in a population, any increase in the level of activity will lead to an increase in metabolic cost that does not necessarily translate into an increase in food acquisition.

Similarly, I expected the correlations between growth and tolerance for a crowd of conspecifics to differ across environments. When food is restricted in quantity or, more importantly, in distribution (Harcourt 1988), fish often have to interact with each other to acquire food (Magnuson 1962). In such an environment, fish that exhibit a tolerance to be among a crowd of conspecifics may grow faster than fish that exhibit no such tolerance. In

contrast, in an environment where food is evenly distributed, fish with a high tolerance for a crowd of conspecifics have no advantage over the ones lacking this trait. Engagement in social interactions is energetically costly, and in this environment is not rewarded with better access to food. Since these experiments were conducted with unselected individuals, differences in the correlations of growth and the behaviours tested are the result of environmental effects.

In chapter four, I examine the relationship between growth and the same 2 behavioural variables for individuals of the different selected lines in the third generation (G_3). The objective was to determine if selection for growth in opposite directions affected the genetic correlation of these 2 behavioural variables and growth differently, and if the changes were dependent on the environment in which selection had been conducted.

Chapter five describes the results of schooling tests performed with selected individuals of the third generation (G_3). The decision by an individual fish whether or not to join a school of conspecifics depends, to a large extent, on the balance between its cooperative and competitive tendencies. These, in turn, are dictated by environmental cues (Reimers 1968, Magurran and Bendelow 1990, Christiansen and Jobling 1990), by the individual's physiological state (e.g. hunger, Morgan 1988), and also to a certain extent by its genetic composition (Seghers 1974, Magurran and Seghers 1990). In chapter two, I demonstrated that selection for enhanced growth in a high interaction environment induced a correlated decrease in the level of agonistic behaviour. This decrease in agonistic behaviour implies that fish in this line may have experienced an increase in the tolerance for a crowd, i.e. a decrease in the nearest distance between individuals that they can support without recurring to agonistic interactions. Thus, I predicted that individuals in the selected line exhibiting a relatively low level of agonistic interactions would also tend to show a relatively high level of school cohesiveness.

In this study I claim to show the following: (1) There was a direct response to selection on growth rate. Fish in the lines selected for fast growth grew faster than fish in the lines selected for slow growth, but this response was more pronounced when selection was carried out in the high interaction environment. (2) Selection for enhanced growth induced a decrease in the intensity of agonistic behaviour when it was conducted in the high interaction environment, so long as food was not limited in quantity. In contrast, no significant changes in agonistic behaviour were observed when selection was conducted in a low interaction environment. (3) The phenotypic correlation between growth and 2 behavioural variables (locomotor activity and tolerance for crowds) in unselected individuals is dependent on the environment in which fish have been raised. (4) The genetic correlations between those same variables is rapidly modified as a consequence of divergent selection on growth in a high interaction environment, but not in a low interaction environment. (5) Schooling tendencies under predation threat were relatively more pronounced in the line selected for fast growth in a high interaction environment, presumably due to the decrease in agonistic behaviour that resulted from selection for fast growth.

These results show how behaviour changes during the domestication process and provide experimental support for the predictions of Doyle and Talbot's (1986) game theoretic model. The application of quantitative genetics theory to the study of the evolution of natural populations has for the most part relied on the simplifying assumption that genetic correlations and covariances remain relatively constant (Lande 1979, 1982, Arnold 1981). My results also provide experimental support for the concern (Turelli 1988) that this assumption may not be correct as a basis for general theory.

CHAPTER 2

RAPID BEHAVIOURAL CHANGES CAUSED BY SELECTION FOR COMPETITIVE AND NON-COMPETITIVE GROWTH

ABSTRACT

Several mechanisms have been described to explain the relationship between social hierarchies and growth in fish. It has been argued that artificial selection for fast growth in competitive environments might lead to higher levels of overall aggression, and therefore there would be no net gain in assimilation efficiency or growth (Kinghorn 1983). It is not immediately obvious, however, whether fish that avoid interacting with conspecifics should grow faster, or whether more aggressive, dominant individuals should grow faster. In the present study I selected fish on the basis of growth in two environments that differed in the intensity of social interactions. In the high interaction regime (HI) food was provided to excess inside a floating cork ring, which limited access to the food and allowed fish to attempt to monopolize the food supply. In the low interaction regime (LI) an equivalent amount of food was spread over the container's surface. In each social regime fish were selected within family for fast and for slow growth rate during two generations (G_1 and G_2). The response was measured in the third generation (G_3) on the directly selected trait (growth) and on a correlated trait (agonistic behaviour). Agonistic behaviour in medaka was inversely related to growth when broods were raised and selected in an environment where enforced social interaction took place, so long as food was not limited in quantity. Selection for enhanced growth during "domestication" should favour fish that are relatively indifferent to the presence of conspecifics.

INTRODUCTION

Individual growth rates in fish populations are often influenced by behavioural interaction. Growth depensation, the increase in the variance of size of individuals reared in a group, is usually considered to be evidence of social hierarchies (Brown 1946, Allee et al. 1948, Magnuson 1962, Yamagishi 1962, Purdom 1974, 1979, Peters 1982, Fausch 1984). Several non-exclusive mechanisms have been proposed to explain the relationship between social hierarchies and growth (or size) in fish. Subdominant individuals may grow at less than their maximal rate due to prevention of sustained access to the resource by aggression of the dominant fish which therefore become larger even in the presence of excess food (Fenderson et al. 1968, Symons 1971, Li and Brocksen 1977, Koebele 1985, Metcalfe 1986, reviewed in Doyle and Talbot 1986). Physiological "stress" induced by the mere presence of the dominant fish may cause loss of appetite and/or low efficiency of food conversion by subdominants (Fenderson et al. 1968, Ejike and Schreck 1980, Fagerlund et al. 1981, Jobling and Wandsvik 1983, Koebele 1985). Alternatively, consideration of energetic demands on dominant, more aggressive individuals provides a plausible explanation for those cases in which the dominant fish are not the largest in body size. Aggressive or territorial behaviour leads to higher metabolic expenditure by the dominant individual (Yamagishi 1962, Yamagishi et al. 1974, Li and Brocksen 1977), and it also diverts the dominant fish's attention from food, resulting in lowered consumption (Yamagishi 1962, Li and Brocksen 1977, Jobling and Wandvisek 1983).

These scenarios indicate that it is not immediately obvious whether fish that avoid interacting with conspecifics should grow faster or whether aggressive fish should grow faster. It has been argued that in competitive environments, artificial selection for fast growth might lead to higher levels of overall aggression, and therefore there would be no net gain in

assimilation efficiency or growth (Kinghorn 1983). This argument, however, does not fit with available evidence from domesticated terrestrial and aquatic vertebrates (Hale 1969, Price 1984, Kohane and Parsons 1988). For example, laboratory reared Norway rats (*Rattus norvegicus*) are significantly less aggressive than wild strains (Barnett and Stoddart 1969, Price 1978, Barnett et al. 1979). In addition, Holm and Fernö (1986) suggested that there is a negative genetic correlation between growth and aggression in Atlantic salmon parr, and Robinson and Doyle (1990) reported negative phenotypic correlations among aggressive behaviour and growth variables in tilapia (*Oreochromis mossambicus x hornorum*). The common carp (*Cyprinus carpio*) has been cultivated for more than 2000 yr and is extremely docile and tolerant of crowding (Weatherley 1976). Populations of Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) bred in captivity appear to be tamer and less easily frightened than their wild counterparts (Vincent 1960, Holm and Fernö 1986, Gjedrem, et al. 1987). By contrast, nipping behaviour was reported to be more frequent among domesticated brook trout than among wild strains (Moyle 1969), but this effect was attributed to a relatively high swimming activity in the domestic strain. Mirror image stimulation tests on coho salmon (*Oncorhynchus kisutch*) suggested that populations of hatchery origin displayed higher levels of aggressiveness than wild populations (Swain and Riddell 1990). However, Swain and Riddell's conclusions were based on tests performed with individuals raised under just one environment, the hatchery. Thus, it is not clear if the individuals of "wild" origin are inherently less aggressive, or whether it is the novel environment, for which they have not been adapted, that inhibits the expression of agonistic behaviour in these strains. Huntingford et al. (1990) reported that a behavioural trait which they defined as "fierceness" may be the cause, rather than the consequence of growth advantages in Atlantic salmon parr. The implications of such results are that aggressive

behaviour in Atlantic salmon might be independent of relative size in the initial stages but would lead to size polymorphism by a positive feedback between growth and behaviour in a competitive environment. Doyle and Talbot (1986) modeled the consequences of artificial selection on growth on competitive behaviour in fish using a game theoretic approach. They concluded that in experimental or aquaculture environments in which food is supplied non-competitively, growth rate selection will indirectly select tamer, not more aggressive fish. The "indifference" in the Doyle and Talbot (1986) model is the inverse of the "fierceness" in Huntingford et al. (1990); whether it is selected for or against depends on the environment.

Despite the conflicting evidence derived from different experiments and theoretical models, there have been very few studies specifically designed to document the rate of genetic changes in behaviour during domestication (Kohane and Parsons 1988). The application of quantitative genetics theory to the study of the evolution of natural populations has for the most part relied on the simplifying assumption of relative constancy of genetic correlations under weak selection (Lande 1979, 1982, Arnold 1981, see also Price and Grant 1985, Lofsvold 1986, 1988). It now appears this assumption may frequently be unrealistic as a basis for general theory (Turelli 1988), because genetic covariances are inherently less stable than genetic variances (Bohren et al. 1966). Comparative studies with migratory and non-migratory populations of the milkweed bug (*Oncopeltus fasciatus*) revealed significant differences in genetic variance-covariance structure across populations (Palmer and Dingle 1986, Dingle et al. 1988). In chapter four I report rapid changes in the genetic correlation of social behaviour and growth after just two generations of artificial selection in medaka.

In the present study I selected fish on the basis of growth in two environments that differed in the intensity of social interactions required for food acquisition in the presence of adequate or excess food. I then analyzed the direct (growth) and correlated (agonistic

behaviour) response to selection in each environment. If agonistic behaviour is related to the growth of individuals (fitness under artificial selection), then I should expect genotype-environment interaction in the relationship between growth and the level of phenotypic expression of aggression. Based on the game theoretic analysis of Doyle and Talbot (1986) I predicted a negative association between agonistic behaviour and growth under conditions of spatially localized (but not quantitatively limited) food supply, and no association when food was evenly distributed (i.e. when opportunities for aggressive/submissive behaviour were reduced).

MATERIALS AND METHODS

Base population:

The initial population of fish was provided by Carolina Biological Supplies. The fish originated from 3 ponds, 2 had an area of 1500 m² and the third of 4000 m². All 3 were approximately 0.80 m deep. Each of these ponds contained in the order of several thousand fish, and there was periodic mixing of fish among ponds (R.V. Kirchen, pers. comm.). Thus, it is reasonable to assume the fish originated from a large and stable population in linkage equilibrium.

Breeding protocol and feeding:

Spawning in medaka is primarily controlled by photoperiod (Yamamoto 1975, Kirchen and West 1976, Ueda and Oishi 1982) but also to some extent by temperature (Egami 1954, Sawara and Egami 1977) and food quality. Mature individuals selected as broodstock were gradually conditioned to a 16:8 L:D photoperiod and 26 ± 1 °C water temperature. Breeding

pairs were kept in visual and chemical isolation from conspecifics in 10 l glass containers (25x28x16 cm). They were fed powdered Nutrafin (Hagen) once daily *ad lib.* and live brine shrimp every other day. Oviposition in Medaka usually occurs during the first 2 hours of the light phase (Yamamoto 1975, Kirchen and West 1976). A mature, healthy female may produce broods of between 10 and 30 eggs (max. 40) every other day for the duration of the breeding season. The cluster of eggs remains attached to the female for a few hours after fertilization and can be removed manually. Recently fertilized eggs were carefully removed from the female and treated with methylene blue for prophylactic purposes. Eggs were then raised in petri dishes (ϕ : 5 cm) for approximately 10 days at which time they were transferred to 800 ml cages suspended in large trays with water. Hatching begins approximately 10 days after fertilization. Recently hatched fry and early juveniles were fed powdered Tetraamin babyfish food "E" for egglayers (Tetra), while food for older and larger juveniles consisted of powdered Nutrafin (Hagen Inc.). Food was provided once daily on an approximately 5% total brood biomass basis. This quantity ensured that all fish had an opportunity to become satiated and that food would be available for several hours after feeding time. Under these laboratory conditions individuals matured at around 10 months of age.

Environmental regimes:

A divergent selection program was conducted under two environmental regimes that differed in the way food was distributed. In the high interaction regime (HI) food was provided inside a floating cork ring, positioned in a corner of the tank, which limited access to the food and allowed the possibility of a few fish attempting to monopolize the food supply. Three ring sizes were used depending on individual length and brood size. Initially, rings were 12 mm (internal ϕ); four to six weeks after hatching they were replaced by 15 mm

rings, and four months after hatching they were replaced by 22 mm rings. In the low interaction regime (LI) an equivalent amount of food was spread over the container's surface which allowed greater access to food by all fish.

Selection design:

Thirteen pairs of unselected fish obtained from a large, randomly-breeding, "natural" population (see above) were mated and their offspring were raised and selected for 2 generations under two regimes that differed in the intensity of enforced social interactions (See above and Fig. 2.1a). Fish in each social regime were selected for fast and slow growth rate. Selection was within brood, within spawning date, and it always took place before sexual differentiation, at about day 92 ($SD \approx 10$) since fertilization. There were unequal numbers of broods per family, and also unequal numbers of individuals per brood in all generations. Usually only the single largest and/or smallest fish in the brood were selected. Occasionally, when brood sizes were relatively large, I selected more than 1 individual per direction. Mean (SD) selection intensities (i.e. numbers selected/brood size) in the direction of fast growth were 0.24(0.08) and 0.39(0.14) during G_1 and G_2 respectively. In the direction of slow growth, mean (SD) selection intensities were 0.26(0.10) and 0.53(0.16) during G_1 and G_2 respectively. Four selected lines were established (Fig. 2.1b): fast and slow growth in the high interaction regime (HI), and fast and slow growth in the low interaction regime (LI). In the third generation (after 2 cycles of selection), half the broods within each line were raised in a HI and half in a LI regime, bringing the number of sublines to 8 (Fig. 2.1b). Thus, broods in the different sublines differed in the particular combination of the regime in which the previous two generations had been raised and selected (past regime: PR), the regime in which they are currently raised (current regime: CR), and the direction of selection (DIR).

In the G_1 generation mating was between families within selected lines. Males in the G_2 generation were mated to unselected females to produce the G_3 offspring used to evaluate the results of selection (Fig. 2.1a). This was done to eliminate the possibility of maternal effects confounding the results, e.g. variation in brood size, or covariation between offspring growth and behaviour induced by the rearing environment of the mother.

Growth rate:

All individuals in a brood were measured shortly after behavioural assessment. Lengths were measured to the nearest .05 mm by projecting a photograph of the fish onto a digitizing board. As mentioned above, selection was between siblings originating from eggs fertilized on the same date. Thus final length is the relevant measure of growth. Age was measured in days since fertilization.

Mean brood specific growth rates were calculated as

$$\text{MBSGR} = \log(\text{mean brood length}) \cdot \text{age}^{-1} \cdot 100$$

The realized heritability of growth rate was calculated for generation G_3 using the formula

$$h^2 = R \cdot S^{-1}$$

where R is the response to selection on growth rate and S is the cumulative selection differential after two generations of selection (Falconer 1989). R was measured by taking the difference in mean brood growth rate ($\text{mean}(\log(\text{length}) \cdot \text{age}^{-1} \cdot 100)$) between fast and slow growth lines in G_3 . Both R and S were normalized by the SD of growth rate in the F_1

population after the fraction of the variance due to differences in brood size had been removed by regression. This procedure ensured that remaining differences in growth rate were not due to differences in densities. As stated above, fish in the G_3 generation were the offspring of selected males and unselected females. I assumed genes determining juvenile growth rate are distributed equally in both sexes. Consequently, I doubled the magnitude of the response, when calculating heritability (but not when testing significance levels), on the assumption that, had I used selected females the response would have been twice as large.

Agonistic interactions:

I monitored behavioural interactions among brood members during their juvenile stage in all 3 generations (G_1 , G_2 , G_3). Broods were videotaped from above both in the presence and absence of food. Videotaping took place for 5 min in each instance and the total number of agonistic interactions among brood members (nips, chases, sudden charges, etc.) were counted during minutes 4 and 5.

Statistical analysis

All statistical analyses were performed with the SYSTAT statistical package (Wilkinson 1988).

Growth rate: direct response to selection

Growth rate was analyzed for generation G_3 with an ANOVA design, using brood size (i.e., number of siblings at the time of filming) as a covariate to control for density effects on growth, and current regime, past regime, direction of selection, and their interactions as fixed factors.

Agonistic behaviour: correlated response to selection

In each generation, data on the frequency of agonistic interactions were analyzed by factorial analysis of variance. Current regime (HI vs. LI), and if applicable, past regime (HI vs. LI), direction of selection and their interactions were fixed factors. The frequency of opportunities to engage in social interactions among brood members is related to the number and size of the individuals in the brood. Thus, brood size and mean brood growth rate were included as covariates. Behavioural data obtained in the presence and absence of food were analyzed jointly, with a repeated measures ANOVA design, and also separately. Behavioural data were square-root transformed to meet the normality and homogeneity of variance requirements of ANOVA. Normality and homogeneity of variance were tested with the Kolmogorov-Smirnov (Lilliefors version, Wilkinson 1988), and Bartlett's test respectively.

Other factors that may affect growth or behaviour directly or indirectly, e.g. temperature, light regime, water-borne growth inhibitors, feed ration and feed regime were kept constant. The light regime and room temperature were maintained at 12:12 L:D and $22 \pm 1^\circ\text{C}$ throughout the experiment, except during breeding, as mentioned above.

RESULTS

(a) Growth rate: Direct response to selection

I estimated mean brood growth rates, and also average brood sizes during all three generations (Table 2.1). During generation G_3 most of the lines selected for fast growth showed a higher mean brood growth rate than the lines selected for slow growth (Table 2.1). The only case in which selection seems to have had no effect on subsequent growth rate occurred when past selection had been in a low interaction regime, and the current regime was

also low interaction (Table 2.1). However, the growth rate data in table 2.1 are not corrected for differences in brood size.

By means of ANOVA, I tested for the direct effect of selection on growth, using current and past regime as fixed factors, and brood size as a covariate because of its obvious negative effect on average growth. Broods selected for fast growth grew faster than broods selected for slow growth ($P=0.030$, Table 2.2a). Furthermore, population density depressed growth rate more in the lines selected for slow than for fast growth (brood size*direction $P=0.002$, Table 2.2a; and see also Table 2.1). After adjustment for brood size differences, mean growth rate differed between fast and slow growth lines by 0.453 SD ($P<0.10$) and 0.111 SD ($P>0.50$) in the lines with a past social regime of high and low interaction, respectively (two-tailed t-test for differences between two means performed with residuals before standardization, Sokal and Rohlf 1981, p. 228, see also Table 2.2b). Note that since both lines were mated to the same pool of (unselected) females to produce G_3 , these differences should be multiplied by a factor of 2 to get a true estimate of the magnitude of the divergence under selection. Realized heritabilities in the G_3 generation were calculated for each combination of past and current regime (Table 2.2c). The highest estimate of realized heritability from mass selection (h^2) was obtained in the line with past and current social regime of high interaction ($h^2=0.121$) (with the 2x correction, and estimated using $(h^2=2(1-t) \cdot h^2_w)$ where t is the full sib intraclass correlation and h^2_w is the within family realized heritability (Falconer 1989)(Note: I did not attempt to estimate SD for these heritability estimates because of the dilution effected by mating G_2 males to unselected females). Thus, the direct response to selection on growth rate was highest in the lines with past and current regimes of high social interaction (Table 2.2c). In summary, broods selected for fast growth grew faster than broods selected for slow

growth regardless of past social regime, but this effect was highest in the lines with a past social regime of high social interaction.

(b) Agonistic behaviour: correlated response to selection

I used ANOVA to analyze changes in the intensity of agonistic behaviour occurring as a response to current social regime (for all the three generations), past social regime (for G_3 exclusively), and direction of selection (for G_2 and G_3). A repeated measures design was used because the same broods had been videotaped in the presence and absence of food. Brood size was included as a covariate because of the obvious direct relationship between number of individuals and opportunities to interact. When the terms were significant ($\alpha < 0.05$), I also included mean brood growth rate and its interaction with brood size. Mean brood growth rate was included as a covariate to compare the residuals of behaviour among the selected lines, once the variation in behaviour due to differences in size among broods had been accounted for. For all three generations, results obtained with models containing growth rate as a concomitant variable (covariate) do not differ qualitatively from results obtained with models lacking this variable.

Generation G1

This was the first generation raised in two social regimes: high interaction and low interaction. Thus any difference between regimes in the frequency of agonistic behaviour among brood members is purely phenotypic and no genetic differences can be inferred. The frequency of agonistic behaviour was higher in the high interaction than in the low interaction regime ($P=0.012$, Table 2.3a). This occurred whether food was present or not (Fig. 2.2A). Overall, the number of interactions were higher in the presence than in the absence of food

($P=0.002$ constant term, Table 2.3b). Furthermore, after all food was consumed, agonistic behaviour decreased more in the high interaction than in the low interaction regime ($P=0.045$, Table 2.3b).

Generation G2

After adjusting for differences in brood size, growth rate and their interaction, the repeated measures ANOVA revealed that the frequency of agonistic behaviour depended on the interaction between social regime and direction of selection ($P=0.012$, Table 2.4). In this generation presence or absence of food did not influence the effect of any of the variables considered in the model (Table 2.4: food vs. no food).

I then used a repeated measures ANOVA to analyze data pertaining to each regime separately because of the interaction between direction of selection and regime. When the selection had been in a high interaction regime, broods selected for fast growth were less aggressive than broods selected for slow growth ($N=48$, $P=0.049$). In contrast, when selection had been in a low interaction regime there was no significant difference in the frequency of agonistic behaviour between the lines selected for fast and slow growth rate ($N=133$, $P=0.204$). Next I compared the frequency of behavioural interactions between fast and slow growth lines within each regime and test environment (food vs. no food) separately. In the high interaction regime there was an indication that agonistic behaviour was lower in the line selected for fast growth than in the line selected for slow growth ($P=0.110$ food present, and $P=0.084$ food absent, Fig. 2.2B). In the low interaction regime the lines selected in opposite directions did not differ in the frequency of agonistic behaviour ($P=0.165$ food present, and $P=0.500$ food absent, Fig. 2.2B).

Generation G3

As in the previous generation, the direction of the changes in frequency of agonistic behaviour due to selection depended on the regime in which selection took place in the past (PAST REGIME * DIRECTION $P=0.017$, Table 2.5). When the selection had been in a high interaction regime, broods from lines selected for fast growth were less aggressive than broods from lines selected for slow growth, whereas when selection had been in a low interaction regime, broods selected in opposite directions did not differ significantly (Fig. 2.2C, and see also Table 2.2b).

Overall, the frequency of agonistic behaviour decreased when all food was consumed ($P=0.041$, Table 2.5). This decrease was larger in the high interaction than in the low interaction current regime ($P=0.003$, Table 2.5), and in the broods selected for slow than for fast growth ($P=0.005$, Table 2.5) (See also Table 2.1). The direction of selection strongly affected behaviour in the presence of food ($P=0.000$), whereas in the absence of food, the difference between directions of selection was significant only to $P=0.072$. It is important to note this difference in significance values, because the frequency of agonistic behaviour during feeding is the variable most likely to be relevant to the domestication process.

When selection took place in a high interaction regime the frequency of agonistic behaviour in the presence of food was 0.88 standard deviations lower in the broods selected for fast than for slow growth ($P<0.005$, two-tailed t-test for differences between means, Table 2.2b); whereas when selection took place in a low interaction regime behaviour was 0.39 standard deviations lower in the fast than in the slow growth line ($P\approx 0.05$). When food was absent behaviour was relatively low ($P<0.10$) in the line selected for fast growth only when selection had taken place in a high interaction regime (PR: HI, Table 2.2b). I then analyzed data corresponding to each combination of past and current regime separately.

Past regime: High interaction (HI)

Among the offspring of 2 generations of selection under a high interaction regime, and also raised in a high interaction current regime, frequency of agonistic behaviour was lower in the broods selected for fast than for slow growth ($P=0.003$) when both test environments (food and no food) were analyzed jointly with a repeated measures ANOVA. This difference remained significant when each test environment was analyzed separately ($P=0.016$ food present, $P=0.019$ food absent, Fig. 2.2C and Table 2.6).

There were no behavioural differences between the lines selected in opposite directions when the current regime was low interaction and both test environments were analyzed jointly with a repeated measures ANOVA ($P=0.314$), or separately ($P=0.121$ and $P=0.913$, Fig. 2.2C and Table 2.6).

Past regime: Low interaction (LI)

Among the offspring of 2 generations of selection under a low interaction but raised in a high interaction regime, the frequency of agonistic behaviour did not differ between the lines selected in opposite directions ($P=0.810$, repeated measures ANOVA). I then looked at the data taken in the presence and absence of food separately. There was a difference in behaviour between fast and slow growth lines when food was present ($P=0.008$, Table 2.7). However, direction of selection also interacted with brood size ($P=0.029$) and with growth rate ($P=0.009$, Table 2.7), thus compromising interpretation of its main effects. When, for comparative purposes, I arbitrarily removed the interaction terms involving direction of selection, frequency of agonistic behaviour did not differ between broods selected in opposite directions ($P=0.096$, Fig. 2.2C). Behaviour did not differ between selected lines when food was absent ($P=0.336$, Fig. 2.2C and Table 2.7).

When the current regime was low interaction the frequency of agonistic behaviour did not differ between lines selected in opposite directions ($P=0.255$, repeated measures ANOVA). I then looked at the data taken in the presence and absence of food separately. There was no difference when food was present ($P=0.176$, Fig. 2.2C and Table 2.7). When food was absent the ANOVA showed significant effects due direction of to selection ($P=0.006$, Fig. 2.2C) but it also revealed two and three way interaction terms between direction of selection and brood size and growth rate (Table 2.7). Again, selection had no effect on behaviour when I removed these interaction terms ($P=0.643$, Fig. 2.2C).

DISCUSSION

My results show that under conditions of high social interaction, social behaviour is a major component of growth rate variation in fish (Fig. 2.2, Tables 2.4, 2.5, 2.6, and 2.7). Under these conditions, and given *ad lib.* feeding, it is the animals that are indifferent to other fish (not involved in aggressive behaviour) that grow fastest. In the low interaction regime, behaviour is not a major component of growth variation (Fig 2.2). Therefore, the response to selection under these conditions is low and unpredictable.

After two cycles of selection, broods selected for fast growth grew faster than broods selected for slow growth (Table 2.1 and 2.2a). The realized heritability of growth rate was $h^2 \leq 0.10$ (Table 2.2c), and the response to selection on growth was high under conditions of high social interaction (Table 2.2b). The intensity of agonistic behaviour among brood members also changed as a result of divergent selection on growth rate, but the magnitude, and even the direction of this change depended on the regime in which selection took place (Tables 2.2b, 2.4 and 2.5). When selection had been in an environment where access to food was

spatially restricted (i.e. a high interaction regime) broods selected for fast growth were less aggressive than broods selected for slow growth (Fig 2.2). When divergent selection was carried out in a low interaction regime the changes in the intensity of agonistic behaviour were not significant. When food was evenly distributed, access to the resource apparently did not depend on social interactions (Fig. 2.2).

I expected to find genotype-environment interactions in the relation between growth and agonistic behaviour in different selection regimes because of the predicted differences between environments in the optimal level of phenotypic expression of aggression. My predictions were borne out. I obtained a negative association between agonistic behaviour and growth under conditions of spatially localized (but not quantitatively limited) food supply, and no association when food was evenly distributed (i.e. when enforced social interaction was relaxed).

Similar results were obtained in selection experiments with *Drosophila* populations in which the expression of genes for life span varied as a result of gene-environment interaction, and was strongly affected by the environment during development (Luckinbill and Clare 1985, Clare and Luckinbill 1985). Individuals were selected for reproduction at an early, or at a late age in life, and the selection was carried out on populations held in two experimental treatments. Populations held in an environment with high and uncontrolled density of individuals responded to selection for reproduction late in life increasing their life span by up to 50 % (Luckinbill and Clare 1985). In contrast, no response to selection was detected in the populations in which density of larvae was kept constant at a low level (Luckinbill and Clare 1985).

In the present study, selection on growth was carried out for two generations (G_1 and G_2), and the intensity of agonistic behaviour among brood members was analyzed during three

generations (G_1 , G_2 , and G_3). For G_1 , the difference between lines is purely environmental. G_2 individuals are the offspring of 1 cycle of selection on growth. For these fish the difference between lines is due to environmental and genetic causes, and these cannot be distinguished. For generation G_3 I was able to make the distinction between environmental and genetic causes by raising half the number of broods in each line in a high interaction and half in a low interaction regime. The rapid changes in behaviour during selection on growth reported here and in chapter four suggest a substantial proportion of the phenotypic variation of these traits might in fact be based on relatively few genes of relatively major effects, perhaps acting in conjunction with many genes of small effect. Similar genetic mechanisms have been proposed in a variety of other studies (e.g. Parsons 1987, Kohane and Parsons 1988).

The level of escalation in aggressive encounters among fish is usually inversely related to the relative differences in size (Symons 1968, Frey and Miller 1972, Wankowski and Thorpe 1979, Torricelli et al. 1988). In the present study the decreased "fierceness" (Huntingford et al. 1990) or enhanced "uninvolvement" (Doyle and Talbot 1986) of the broods selected for fast growth under a high social interaction regime did not result from relatively high coefficients of variation in length. Coefficients of variation in size did not vary significantly among the selected lines (F-test $p > 0.76$), or between current regimes ($p > 0.22$) or past regimes ($p > 0.41$).

Growth advantages of the socially dominant individuals are usually higher when food is localized than when it is evenly distributed (Magnuson 1962, Rubenstein 1981, Metcalfe 1986, see also chapter 3). Nonetheless, social hierarchies, and consequently growth depensation, do also occur in the presence of excess resources (Purdom 1974, Yamagishi et al. 1974, Jobling and Wandsvik 1983, Abbott et al. 1985, Jobling 1985, Koebele 1985,

Knights 1987), since food defensibility, and not food quantity, appears to be the variable most critically related to growth (Koebele 1985). In my study, even though food was relatively more defensible in the high than in the low interaction regime, after two cycles of selection in the high interaction regime, the level of agonistic interactions was lower in the broods selected for fast growth than in the broods selected for slow growth. This result might be explained by considering the energetic demands on the dominant, more aggressive individuals in a brood. Engagement in agonistic interactions is energetically costly, and diverts the dominant fish's attention from the resource, allowing less aggressive, indifferent fish to gain a growth advantage. Occasionally I observed subdominant individuals gaining access to the food while the dominant, more aggressive fish was engaged in agonistic interactions with another brood member. Alternatively, the pattern I saw could have resulted from a relatively more efficient basal metabolism in the "indifferent" fish.

Behavioural traits are thought to be among the most readily modifiable during the early stages of domestication (Mayr 1963, Hale 1969, Price 1984, Kohare and Parsons 1988). Changes in the frequency with which behaviours occur can result from shifts in physiological thresholds during selective breeding (Hale 1969, Boice 1972, Price 1984). Several behavioural studies with Norway rats (*Rattus norvegicus*) have shown that the frequency of agonistic behaviour among colony members is lower in domestic than in wild colonies (Galef 1970, Harkins et al. 1974, Barnett et al. 1979). Male rats six to nine generations removed from the wild also showed a marked decrease in threat and attack behaviour toward strangers when compared to wild male rats (Barnett and Stoddart 1969). Price (1978), however, found essentially similar results when comparing aggressive behaviour in wild caught Norway rats with laboratory reared descendants of wild parents, where no genotype differences could be inferred. Thus, it is possible that the decrease in aggressive behaviour in domesticated rats

may arise from changes in early experience rather than just from genetic changes accompanying the domestication process (Price 1978). Similarly, prolonged exposure to overcrowded conditions appears to inhibit aggressive behaviour in Atlantic salmon parr (*Salmo salar*) (Kallerberg 1958, Keenleyside and Yamamoto 1962, Fenderson and Carpenter 1971). In the present experiment the possibility of confounding due to this effect was eliminated by using brood size as a covariate.

Domesticated populations of Atlantic salmon (*S. salar*), rainbow trout (*O. mykiss*), and brook trout (*S. fontinalis*) are "tamer" and not so easily "frightened" when disturbed compared to wild populations of the same species (Vincent 1960, Gjedrem et al. 1987; cf. Fenderson and Carpenter 1971). Although the expression of aggressiveness and of timidity or tameness may be two different phenomena (e.g. Swain and Riddell 1990), it appears that for the rat, at least, the degree of aggressiveness toward conspecifics, and of "timidity or shyness" in the presence of an unfamiliar individual are two manifestations of an individual's response to novelty (Galef 1970). It is possible therefore, that a similar common physiological mechanism between aggression and tameness exists in fish as well.

My results show how agonistic behaviour changes during the domestication process, and confirm the predictions of Doyle and Talbot's (1986) game theoretic model. In particular, domestication selection favours fish that are indifferent to the presence of conspecifics, and not those that are most aggressive.

Table 2.1. Number of broods (N), average of mean brood specific growth rate (GROWTH: $\log \text{cm} \cdot \text{day}^{-1} \cdot 100$), average of brood sizes (BROOD SIZE), and intensity of behaviour (BEHAVIOUR) in the presence and absence of food during generations G_1 , G_2 , and G_3 . Data are presented by past regime (PR), current regime (CR), and direction of selection (DIR). Figures in brackets are standard deviations.

G ₁							
PR	CR	DIR	N	GROWTH	BROOD SIZE	BEHAVIOUR	
						FOOD	NO FOOD
-	HI	-	27	0.078(0.082)	6.93(2.63)	5.45(1.32)	3.00(1.23)
-	LI	-	68	0.138(0.073)	7.88(4.64)	4.37(2.08)	2.58(1.48)
G ₂							
PR	CR	DIR	N	GROWTH	BROOD SIZE	BEHAVIOUR	
						FOOD	NO FOOD
HI	HI	FAST	14	0.138(0.076)	3.21(1.58)	2.26(1.63)	2.06(1.61)
		SLOW	34	0.148(0.071)	3.74(1.80)	4.04(3.11)	3.35(2.17)
LI	LI	FAST	110	0.206(0.075)	4.77(2.59)	3.96(2.63)	2.83(2.10)
		SLOW	24	0.226(0.085)	3.79(1.91)	2.86(2.66)	2.21(1.44)
G ₃							
PR	CR	DIR	N	GROWTH	BROOD SIZE	BEHAVIOUR	
						FOOD	NO FOOD
HI	HI	FAST	10	0.268(0.065)	7.70(2.16)	5.93(2.83)	2.89(0.96)
		SLOW	24	0.257(0.156)	6.96(2.68)	7.60(2.55)	4.33(2.27)
HI	LI	FAST	12	0.391(0.112)	7.42(3.37)	5.10(2.12)	4.03(1.54)
		SLOW	28	0.387(0.181)	7.21(4.82)	6.17(2.75)	4.11(2.90)
LI	HI	FAST	42	0.282(0.138)	6.52(2.56)	5.70(2.16)	3.78(1.61)
		SLOW	20	0.252(0.173)	6.60(2.39)	6.29(2.01)	3.42(1.51)
LI	LI	FAST	55	0.334(0.150)	7.11(3.81)	4.96(2.73)	3.33(2.07)
		SLOW	28	0.363(0.128)	6.11(2.42)	5.28(2.62)	3.19(1.71)

Table 2.2. Generation G_3 . (a) Direct response to selection on growth rate. ANOVA table with mean brood growth rate (GROWTH) as the dependent variable, and brood size as covariate. (b) Differences (in standard deviations) in mean growth rate and behaviour (fast growth line minus slow growth line) within each past regime, after adjustment for differences in brood size. T-test for differences between means (P-values refer to 2-tailed tests performed with residual means before standardization, Sokal and Rohlf 1981, p. 228). (c) Realized heritabilities (h^2) estimated from the response to within family selection during two generations of selection for fast and slow growth rate.

(a)

GROWTH = CONSTANT + BROOD SIZE + CURRENT REGIME + PAST REGIME + DIRECTION + BROOD SIZE * CURRENT REGIME + BROOD SIZE * DIRECTION + ERROR (N=219)

SOURCE	S.S.	d.f.	M.S.	F-ratio	p	R ²
DIRECTION	0.037	1	0.037	4.775	0.030	0.681
BROOD SIZE	2.732	1	2.732	352.323	0.000	
CURRENT REGIME	0.001	1	0.001	0.097	0.756	
PAST REGIME	0.101	1	0.101	13.015	0.000	
BROOD SIZE * CR	0.106	1	0.106	13.665	0.000	
BROOD SIZE * DIRECTION	0.077	1	0.077	9.880	0.002	
ERROR	1.644	212	0.008			

(b)

PAST REGIME

	HI (N=74)	LI (N=145)
GROWTH	0.453	0.111
t-test	1.777	0.626
P	<0.10	>0.50
BEHAVIOUR _{food}	-0.882	-0.396
t-test	3.039	-2.152
P	<0.005	<0.05
BEHAVIOUR _{no food}	-0.611	0.067
t-test	-1.730	0.315
P	<0.10	>0.50

(c)

PAST REGIME

		HI	LI
CURRENT	HI	0.092	0.042
REGIME	LI	0.020	0.056

Table 2.3. Generation G₁. REPEATED MEASURES ANOVA table with behaviour (square root transformed) both in presence and absence of food as the dependent variable, brood size, mean brood growth rate, their interaction, and current regime as independent variables.

BEHAVIOUR_{food} BEHAVIOUR_{no food} = CONSTANT + BROOD SIZE + GROWTH + BROOD SIZE * GROWTH + REGIME + ERROR (N=95)

(a)

SOURCE	S.S.	d.f.	M.S.	F-ratio	p
BROOD SIZE	18.678	1	18.678	7.301	0.008
GROWTH	16.582	1	16.582	6.481	0.013
BROOD SIZE * GROWTH	13.256	1	13.256	5.181	0.025
REGIME	16.784	1	16.784	6.560	0.012
ERROR	227.688	89	2.558		

(b)

FOOD vs. NO FOOD (Within subjects effect)

SOURCE	S.S.	d.f.	M.S.	F-ratio	p
CONSTANT	9.493	1	9.493	10.640	0.002
BROOD SIZE	0.018	1	0.018	0.020	0.889
GROWTH	1.454	1	1.454	1.629	0.205
BROOD SIZE * GROWTH	2.726	1	2.726	3.055	0.084
REGIME	3.685	1	3.685	4.130	0.045
ERROR	79.409	89	0.892		

Table 2.4. Generation G₂. REPEATED MEASURES ANOVA table with behaviour (square root transformed) both in presence and absence of food as the dependent variable; brood size, mean brood growth rate, their interaction, regime, direction of selection, and their interaction as the independent variables.

BEHAVIOUR_{food} BEHAVIOUR_{no food} = CONSTANT + BROOD SIZE + GROWTH + BROOD SIZE * GROWTH + REGIME + DIRECTION + REGIME*DIRECTION + ERROR (N=180)

SOURCE	S.S.	d.f.	M.S.	F-ratio	p
BROOD SIZE	0.791	1	0.791	0.149	0.700
GROWTH	14.825	1	14.825	2.798	0.096
BROOD SIZE * GROWTH	94.538	1	94.538	17.846	0.000
REGIME	32.757	1	32.757	6.183	0.014
DIRECTION	7.896	1	7.896	1.490	0.224
REGIME * DIRECTION	34.473	1	34.473	6.507	0.012
ERROR	916.467	173	5.297		

FOOD vs. NO FOOD (Within subjects effect)

SOURCE	S.S.	d.f.	M.S.	F-ratio	p
CONSTANT	0.665	1	0.665	0.318	0.574
BROOD SIZE	0.278	1	0.278	0.133	0.716
GROWTH	0.541	1	0.541	0.258	0.612
BROOD SIZE * GROWTH	1.391	1	1.391	0.664	0.416
REGIME	0.086	1	0.086	0.041	0.840
DIRECTION	0.009	1	0.009	0.004	0.948
REGIME*DIRECTION	1.771	1	1.771	0.846	0.359
ERROR	362.186	173	2.094		

Table 2.5. Generation G₃. REPEATED MEASURES ANOVA table with behaviour (square root transformed) in the presence and absence of food as the dependent variable, brood size, mean brood growth rate, their interaction, current regime, past regime, direction of selection, and significant interaction terms as independent variables.

BEHAVIOUR_{food} BEHAVIOUR_{no food} = CONSTANT + BROOD SIZE + GROWTH + BROOD SIZE * GROWTH + CURRENT REGIME + PAST REGIME + DIRECTION + PAST REGIME * DIRECTION + ERROR (N=214)

SOURCE	S.S.	d.f.	M.S.	F-ratio	p
BROOD SIZE	79.670	1	79.670	22.119	0.000
GROWTH	31.540	1	31.540	8.756	0.003
BROOD SIZE * GROWTH	67.759	1	67.759	18.812	0.000
CURRENT REGIME	45.855	1	45.855	12.731	0.000
PAST REGIME	0.841	1	0.841	0.234	0.629
DIRECTION	4.057	1	4.057	11.508	0.000
PAST REGIME * DIRECTION	21.002	1	21.002	5.831	0.017

FOOD vs. NO FOOD (Within subjects effect)

SOURCE	S.S.	d.f.	M.S.	F-ratio	p
CONSTANT	7.954	1	7.954	4.210	0.041
BROOD SIZE	0.427	1	0.427	0.226	0.635
GROWTH	7.595	1	7.595	4.020	0.046
BROOD SIZE * GROWTH	22.195	1	22.195	11.747	0.001
CURRENT REGIME	17.191	1	17.191	9.098	0.003
PAST REGIME	0.001	1	0.001	0.000	0.984
DIRECTION	15.095	1	15.095	7.989	0.005
PAST REGIME * DIRECTION	0.377	1	0.377	0.200	0.655
ERROR	389.226	206	1.889		

TABLE 2.6. Generation G₃. Past Regime: High Interaction (HI). ANOVA table for the model containing behaviour as the dependent variable, brood size and direction of selection as independent variables. Growth rate and interaction terms had no effect.

BEHAVIOUR = CONSTANT + BROOD SIZE + DIRECTION + ERROR

Data pertaining to each current regime (CR), as well as data obtained in the presence and in the absence of food were analyzed separately.

(A) Current regime: High interaction (HI) (N=33)

(i) Food

SOURCE	S.S.	d.f.	M.S.	F-ratio	p	R ²
BROOD SIZE	86.271	1	86.271	20.084	0.000	0.451
DIRECTION	28.006	1	28.006	6.520	0.016	
ERROR	128.864	30	4.295			

(ii) No Food

SOURCE	S.S.	d.f.	M.S.	F-ratio	p	R ²
BROOD SIZE	50.440	1	30.440	9.978	0.004	0.329
DIRECTION	18.655	1	18.655	6.115	0.019	
ERROR	91.523	30	3.051			

(B) Current regime: Low interaction (LI) (N=38)

(i) Food

SOURCE	S.S.	d.f.	M.S.	F-ratio	p	R ²
BROOD SIZE	135.804	1	135.804	46.178	0.000	0.585
DIRECTION	7.411	1	7.411	2.520	0.121	
ERROR	102.931	35	2.941			

(ii) No Food

SOURCE	S.S.	d.f.	M.S.	F-ratio	p	R ²
BROOD SIZE	156.415	1	156.415	68.831	0.000	0.663
DIRECTION	0.027	1	0.027	0.012	0.913	
ERROR	79.534	35	2.272			

TABLE 2.7. Generation G₃, Past Regime: Low Interaction (LI). ANOVA table for the model containing behaviour (square-root transformed) as dependent variable, brood size, mean brood growth rate, their interaction, direction of selection, and significant interaction terms as the independent variables:

BEHAVIOUR = CONSTANT + BROOD SIZE + GROWTH + BROOD SIZE*GROWTH + SELECTION + BROOD SIZE*SELECTION + GROWTH*SELECTION + ERROR

Data pertaining to each current regime, as well as data obtained in the presence and in the absence of food were analyzed separately.

(A) Current regime: High interaction (HI) (N=62)

(i) Food

SOURCE	S.S.	d.f.	M.S.	F-ratio	p	R ²
BROOD SIZE	0.285	1	0.285	0.122	0.728	0.527
GROWTH	24.041	1	24.041	10.305	0.002	
BROOD SIZE * GROWTH	30.345	1	30.345	13.007	0.001	
DIRECTION	17.483	1	17.483	7.494	0.008	
BROOD SIZE * DIRECTION	11.781	1	11.781	5.050	0.029	
GROWTH * DIRECTION	17.143	1	17.143	7.348	0.009	
ERROR	128.313	55	2.333			

(ii) No Food

SOURCE	S.S.	d.f.	M.S.	F-ratio	p	R ²
BROOD SIZE	2.129	1	2.129	1.028	0.315	0.208
GROWTH	5.103	1	5.103	2.465	0.122	
DIRECTION	2.869	1	2.869	1.386	0.244	
ERROR	120.05	158	2.070			

(B) Current regime: Low interaction (LI) (N=81)

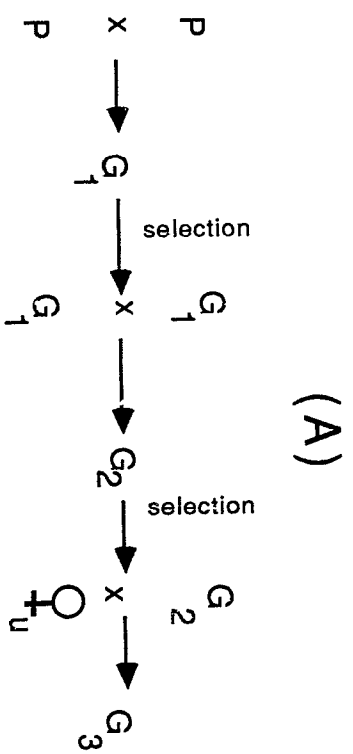
(i) Food

SOURCE	S.S.	d.f.	M.S.	F-ratio	p	R ²
BROOD SIZE	11.660	1	11.660	3.483	0.066	0.557
GROWTH	18.295	1	18.295	5.464	0.022	
BROOD SIZE * GROWTH	58.147	1	58.147	17.367	0.000	
DIRECTION	6.253	1	6.253	1.868	0.176	
ERROR	254.455	76	3.348			

(ii) No Food

SOURCE	S.S.	d.f.	M.S.	F-ratio	p	R ²
BROOD SIZE	1.113	1	1.113	0.612	0.437	0.562
GROWTH	11.810	1	11.810	6.495	0.013	
BROOD SIZE * GROWTH	27.201	1	27.201	14.959	0.000	
DIRECTION	14.599	1	14.599	8.029	0.006	
BROOD SIZE * DIRECTION	18.172	1	18.172	9.994	0.002	
GROWTH * DIRECTION	13.952	1	13.952	7.673	0.007	
BROOD SIZE * GROWTH * DIR	12.427	1	12.427	6.834	0.011	
ERROR	132.739	73	1.818			

Fig. 2.1. (A) Experimental design: **PxP** (parental generation): 13 pairs of unselected mature individuals were mated to produce the G_1 generation. They produced a total of 95 broods (mean and SD: 7.4 ± 2.4 broods per family). First selection episode: within brood selection for fast and for slow growth conducted on G_1 broods before sexual differentiation. $G_1 \times G_1$: 54 pairs of selected males and females of the G_1 generation were mated within lines between families. (There was no brother-sister mating.) They produced a total of 182 broods (G_2) (mean and SD: 9.54 ± 5.90). Second selection episode: Within brood selection for fast or for slow growth conducted on G_2 broods before sexual differentiation. $G_2 \times \text{♀}_u$: 31 selected males of G_2 were mated to unselected females. Of these males, 1 was mated to 3 females, 18 were mated to 2 females each, and 12 were mated to 1 female, producing a total of 219 G_3 broods. Thus, each male produced on average 6.71 ± 2.56 G_3 broods, some of which were fullsibs and some were halfsibs. In all generations there was an unequal number of broods per family, and also an unequal number of individuals per brood. (B) Environmental regimes and selection design: Fish in each of the 2 environmental regimes (high interaction: HI, and low interaction: LI) were selected for fast and slow growth rate in generations G_1 and G_2 . In generation G_3 half the broods within each line were raised in a HI and half in a LI regime, bringing the number of sublines to eight. I recorded agonistic interactions among brood members during each generation's juvenile stage. All brood members were measured to the nearest 0.05 mm.



(B)

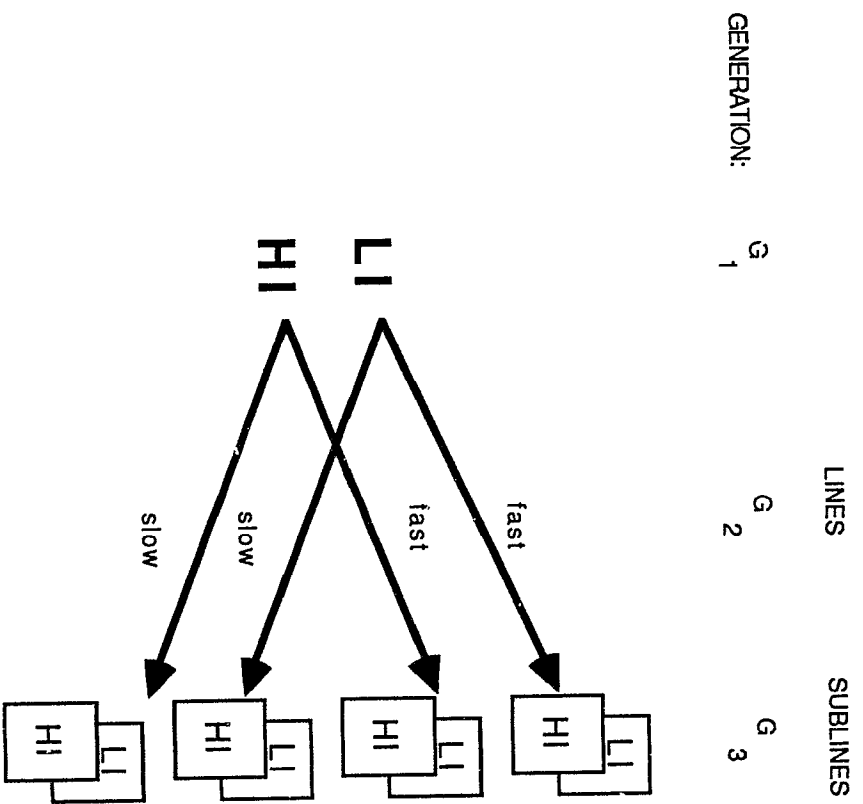


Fig. 2.1

Fig. 2.2. Number of agonistic interactions among brood members (square-root transformed) in each environment and selected line, controlled by covariates. Y-axis: residuals after adjusting for brood size and mean brood growth rate, and their interaction (if significant). X-axis: Current environment. Hatched bars: strictly environmental effect (G_1). Filled (black) bars: selected for fast growth rate. Open bars: selected for slow growth rate. A: Generation G_1 ; differences between lines (HI vs. LI) are strictly phenotypic (p-values indicate differences between environments). B: Generation G_2 . C: Generation G_3 . In generation G_3 a distinction is made between current regime (X-axis) and past selection regime. For generations G_2 and G_3 p-values indicate differences between directions of selection. Error bars indicate standard error of the means.

MEAN NUMBER AGONISTIC INTERACTIONS

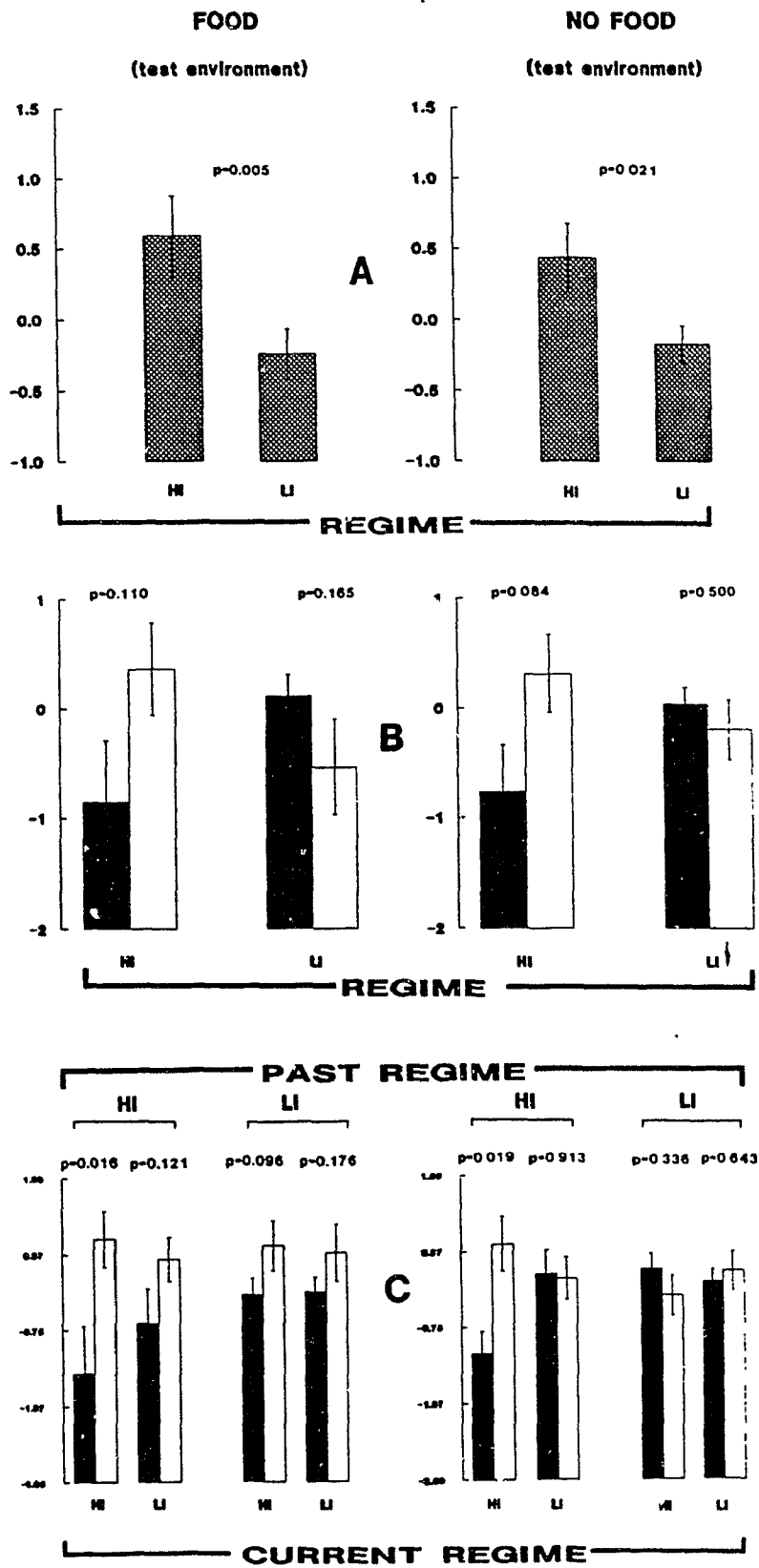


Fig. 2.2

CHAPTER 3

**BEHAVIOURAL AND GROWTH RESPONSES TO THE INTENSITY OF SOCIAL
INTERACTION AMONG MEDAKA FROM THE BASE POPULATION**

ABSTRACT

When access to food is restricted, faster growing fish may be those whose behaviour is relatively unaffected by the presence of nearby conspecifics. Behavioural experiments were carried out to determine the relation between growth and motor activity levels in crowded and uncrowded conditions, and measures of aversion/attraction to groups of conspecifics. Two experimental groups of fish (*Oryzias latipes*) were grown for several weeks in two environments manipulated so as to maximize differences in social interactions. In the high interaction environment (HI), food was provided inside a floating cork ring. In the low interaction environment (LI), food was spread over the container's surface. Fish were measured at the end of the growth period and tested for their activity levels in the presence of conspecifics and for their preference for, or tolerance of crowded conditions. The correlation between motor activity and growth was significantly more positive in the HI environment than in the LI environment. The relationship between preference for crowded conditions and growth was negative for both groups of fish, although less so for HI than for LI. I conclude that artificial selection for faster growth may produce more aggressive fish only under very high levels of forced social interaction (competition), if at all. Under conditions of reduced social interaction, the social-averse or socially indifferent fish grow faster.

INTRODUCTION

Growth depensation, a term coined by Magnuson (1962), refers to the increase in the size variance due to differential growth rates among individuals in a population. The causes of differential growth rate may be both genetic and environmental but these differences are amplified by social interactions including competition (Jobling and Reinsnes 1986). This phenomenon has long been recognized in fish populations (Magnuson 1962, Moav and Wohlfarth 1974, Yamagishi et al. 1974, Doyle and Talbot 1986, Davis and Olla 1987). Nonetheless, there have been relatively few experimental studies in which the relationship between growth rate and social behaviour has been analyzed in environments with different intensities of social interaction.

Intraspecific competition for food is potentially a major problem for aquaculture geneticists who seek to change the characteristics of a population by artificial selection. There is a widespread belief that under competition faster growing fish may simply be the more aggressive ones. If this is true, selection for higher growth rate may lead to fish that are more aggressive but not necessarily to fish with higher productivity if aggressive behaviour is a heritable trait (Purdom 1974). Doyle and Talbot (1986) demonstrated that in theory this positive correlation between aggressive behaviour and growth is likely to occur only in the situation where aggressive behaviour is independent of relative size, and where the intensity of competition is very high.

The more limited and defensible the food supply, the more intense is the competition, resulting in an increase in variation in growth over time (Rubenstein 1981b, Davis and Olla 1987). The converse is not necessarily always true. Food need not be limiting for growth depensation to take place. Smaller fish can be inhibited by larger fish from seeking access to

the food, even when food is abundant (Allee et al. 1948, Abbott et al. 1985, Jobling 1985, Koebele 1985). Thus, an increase in growth depensation with time may indicate the presence of social interactions, but it would not necessarily indicate that resources are limiting (Doyle and Talbot 1986, Davis and Olla 1987). In experiments with European eels Knights (1987) found that inhibition of feeding in established communities occurs after larger fish are satiated even when food is available.

The work described here was designed to determine: (1) the correlation between growth and measures of aversion/attraction to conspecifics, (2) the correlation between growth and activity levels in crowded and uncrowded conditions, and (3) the way these correlations differ in environments of varying food accessibility.

MATERIALS AND METHODS

Growth period

Two sequential experiments were carried out to determine the relationship between social behaviour (specifically: tolerance for the presence of conspecifics) and growth in a laboratory population of Medaka (*Oryzias latipes* Temminck and Schlegel). In the first experiment, two groups of 12 fish were grown for 17 weeks. In the second experiment, two groups of 13 fish were grown for 12 weeks. The two experiments were performed 8 months apart. In all cases fish were grown in 4 l rectangular containers under constant environmental conditions. In each experiment, one group was raised in a high interaction environment (HI) and another in a low interaction environment (LI). In the HI environment food was provided inside a floating cork ring positioned in a corner, which limited access to the food and allowed a few fish to monopolize the food supply. In the LI environment food was spread over the container's surface. Food consisted of powdered Nutrafin (Hagen Inc.). Both groups were fed

between 20 mg and 30 mg of food once daily. Preliminary trials had shown that with this quantity no food remained uneaten by the end of the day. The light regime and room temperature were maintained at 12:12 L:D and $22 \pm 1^\circ\text{C}$ throughout the experiment.

Test protocol

At the end of the growth period the fish were individually marked and total length was measured to the nearest 0.05 mm. The fish were subsequently tested for their preference for (or aversion to) crowded or empty conditions. Individual test fish were placed inside a moveable glass tube that could be slid across a three-compartmented plastic box (Fig. 3.1). At any given time half the tube was outside the central compartment and half the tube was inside it. Five boxes containing 15 tubes in total were set in parallel in a tray filled with water. By adding approximately 250 fish to the central compartment, the test fish could be exposed to visual contact with conspecifics if they were in the appropriate half of the glass tube. During the experiment the tubes were slid back and forth every 2 min, changing the environment of the test fish with respect to the visual presence of other fish, and thus forcing the test fish to make a decision whether to move or not. The position of the fish relative to the central compartment was recorded at the end of each 2 min period, just before sliding the tubes. Fish were sequentially tested in the presence and absence of conspecifics over a 2 h period as follows:

- (a) 30 min with conspecifics ABSENT from the central compartment (Fig 3.2a).
- (b) 60 min in the PRESENCE of a crowd of conspecifics in the central compartment (Fig. 3.2b).
- (c) 30 min in the absence of conspecifics (repeating condition (a)).

Fish were tested once per day. In the first experiment the test was repeated 7 times on each fish over a period of 9 days. In the second experiment the test was repeated 5 times on each fish over a period of 5 days.

The observational data consisted of three types of behaviour in response to a change in the visual environment. Transition in (TRIN): The fish is initially in the half of the tube that is inside the box, which may (treatment b) or may not (treatment a or c) contain conspecifics. Thus when the tube is slid the fish finds itself outside the box. If at this time it makes an active choice to swim back to the opposite end of the tube, which is now inside the box, then TRIN is incremented by 1 (Fig 3.1a). Transition out (TROUT): The fish is initially outside the box. If when the tube is slid it finds itself inside the box but chooses to swim back outside, then TROUT is incremented by 1 (Fig 3.1b). No transitions: The fish remains passively in the same part of the tube regardless of whether it is inside or outside the box (Fig 3.1c), then neither TRIN nor TROUT are incremented. Thus, for any given fish and treatment the variable TRIN indicates the number of active transitions in and the variable TROUT indicates the number of active transitions out of the central compartment where conspecifics may or may not be present.

Four derived variables were used in the analyses:

$ACT = TRIN + TROUT$ (total activity or movement)

$SCORE = TRIN - TROUT$

$DACT = ACT_{(presence)} - ACT_{(absence)}$. (refers to presence or absence of conspecifics)

$DSC = SCORE_{(presence)} - SCORE_{(absence)}$. (preference for, or tolerance of crowded conditions)

DACT: A positive value of DACT indicates a higher activity (number of transitions) in the presence of conspecifics than in their absence. A negative value indicates a decrease in activity. $DACT=0$ indicates no change.

DSC: Positive values of DSC reflect what I will call the response of a "social" fish: when forced to make a decision, prefers to be among a crowd of conspecifics. It is a fish that has had a higher SCORE during treatment (b) than during treatment (a) and (c), ie. it has chosen to swim into the central compartment (TRIN) rather than out of it (TROUT) more often in the presence of conspecifics than in their absence. A negative value of DSC reflects the opposite behaviour, which I will call the response of an "antisocial" fish, ie. a fish that swims away from a crowd of conspecifics.

The growth (DL) of a fish during the weeks prior to the testing was obtained by taking the difference between mean initial length (L_0) and individual final length (L_f). The individual growth was usually considerably larger than the initial S.D.

Preliminary experiments were carried out to detect learning or habituation effects within the 2 h time frame of the test. Two groups of fish were tested continuously for 2 h, and each individual was tested 3 times on 3 consecutive days. One group of fish was tested in the absence of conspecifics, the other in the presence. An ANOVA model revealed significant differences across fish, but no significant effect due to the time elapsed since the start of the experiment. There was evidence for non-systematic changes in individual behaviour between trials. For this reason I considered the means of those trials to calculate the correlations between the behavioural data and growth (7 trials in experiment 1, and 5 trials in experiment 2).

RESULTS

In experiment 1, 7 fish were tested from the HI environment, and 8 fish were tested from the LI environment. These represented all surviving fish at the termination of the growth period (Table 3.1). In experiment 2, 9 fish were alive in each of HI and LI environments at the termination of the growth period (Table 3.1). 8 and 7 fish were tested from the HI and LI environments respectively.

Social behaviour (DSC) in relation to growth (DL)

Experiment 1:

The correlation between "social preference" (DSC) and growth (DL) was negative and not significant for HI fish ($r=-0.446$, $n=7$, $P=0.32$, Fig 3.3a), and negative and highly significant for LI fish ($r=-0.837$, $n=8$, $P=0.01$, Fig 3.3b).

Experiment 2:

For fish raised in HI the correlation between "social preference" (DSC) and growth was consistent with replicate 1 ($r=-0.416$, $n=8$, $P=0.31$, Fig. 3.3a). For fish raised in LI however, the correlation was not significant in the 2nd experiment ($r=-0.039$, $n=7$, $P=0.93$, Fig. 3.3b). This may be due to the fact that 2 of the 7 fish grew hardly at all (Fig. 3.3b). When I exclude these 2 fish from the calculations, the correlation becomes more negative but remains non-significant ($r=-0.430$, $n=5$, $P=0.47$).

Difference in activity (DACT) in relation to growth

Experiment 1:

For fish raised in HI the correlation between difference in activity and growth was positive but non-significant ($r=0.133$, $n=7$, $P=0.78$, Fig 3.4a), while for fish raised in LI it was strongly negative ($r=-0.685$, $n=8$, $P=0.06$, Fig 3.4b).

Experiment 2:

For fish raised in HI the correlation between difference in activity and growth was positive and highly significant ($r=.901$, $P=0.002$) (Fig 3.4a). For fish raised in LI the correlation was still positive but 1/3 the magnitude of the previous correlation ($r=0.362$), and non-significant ($P=0.43$) (Fig 3.4b).

High vs. low interaction: comparing correlation coefficients

The correlation coefficients between "social preference" and growth, and between difference in activity and growth were compared across the high and low interaction environments (Table 3.2). For "difference in activity" differences between environments were significant at $\alpha=0.051$ for both experiments. The correlation coefficient in HI environment was significantly more positive than in LI environment (1-tail test because the correlations are hypothesized *a priori* to be more positive in the HI environment than in the LI environment; see Discussion) (Table 3.2). Differences for "social preference" across environments fail to reach significance in either experiment (Table 3.2).

DISCUSSION

The correlations between growth and social preference, and between growth and activity levels depend on the type of environment fish are raised in. The intensity of social interaction among individuals in a population affects the magnitude and in some cases even the sign of these correlations. In this study, factors that may affect growth directly or indirectly, e.g. temperature, light regime, water-borne growth inhibitors, feed ration and feed regime were either held constant or eliminated altogether. The hypotheses that the behavioural observations were designed to test can be described as follows:

"Social preference" and growth

In an environment where food is limited either in quantity and/or accessibility (i.e. HI environment) fish often have to interact with each other to acquire food (Magnuson 1962). These interactions do not necessarily involve "aggression". Thus, in such an environment, fish that exhibit a preference, or at least a high tolerance, for a crowd of conspecifics are expected to grow faster than fish that display no such preference or tolerance. Crowd seeking or "social-tolerant" individuals are at an advantage over "antisocial" or "asocial" fish.

Fish with a positive "social behaviour" ($DSC > 0$) are fish that display a willingness to follow conspecifics, or that display a high tolerance to be among conspecifics.

Phillips (1974) studied the relation between measures of attraction/repulsion and dominance hierarchies between males of the territorial benthic fish *Chasmodes bosquianus*. In that study, individuals with a history of dominance were attracted to prior residents, whereas subordinate individuals preferred to be in visual isolation from the prior resident (Phillips 1974). These experiments were not designed to estimate the relationship between "social

preference" and dominance. Nevertheless, available evidence (Phillips 1974) seems to indicate that, at least in some species, fish that are attracted to conspecifics are indeed the dominant individuals.

In an environment where food is patchily distributed high rank, or dominance is likely to translate into benefits to the individual (Hodapp and Frey 1982). A number of studies have demonstrated a positive correlation between dominance and growth rate in fish (Yamagishi et al 1974, Barlow et al. 1975, Li and Brocksen 1977, Doyle and Talbot 1986 and references therein, Knights 1987). Nevertheless, whether high ranking or dominant individuals benefit at all depends on the overall abundance and distribution of food (Koebele 1985, Huntingford and Turner 1987). There is ample evidence that this is true in a variety of organisms. For example, in a study on monkeys, it was shown that not only the distribution of food determines the intensity of competition among individuals, but also that dominants do better only when the intensity of competition is high (reviewed by Harcourt 1989).

In contrast, in an environment where food is evenly distributed fish do not necessarily have to interact with each other to acquire food. Engagement in social interactions, agonistic or otherwise, is energetically costly, and in this environment is not rewarded with better access to food (Huntingford and Turner 1987, Harcourt 1989). Similarly, retreat from an agonistic encounter is not punished by reduced access to food. Thus, indifferent or "timid" individuals should grow faster than aggressive or "social" fish.

For these reasons I expected the correlation between "social tolerance" and growth to differ across environments: It should be negative where interaction is low, and positive, or at least less negative, where interaction is high (Doyle and Talbot 1986). (This *a priori* hypothesis is the rationale for the use of the one-tail test in the results section).

The results of my experiments are consistent with the predictions: the correlation between social behaviour (DSC) and growth (DL) in the high interaction environment was negative but non-significant and was half the absolute magnitude of the homologous correlations in the low interaction environment.

Many aquaculturists believe that selection for faster growing fish will produce fish that are more competitive, not more productive (Purdom 1974). In the experiments described here, even the fish raised under high interaction intensities showed a negative (although non-significant) relationship between preference for crowded conditions and growth. Similarly, for pygmy sunfish (Rubenstein 1981b), even at high competition intensities the advantage acquired by dominant individuals did not translate into faster growth. Thus, these results seem to indicate that a trade-off between competitive success and maximum production might occur only at very high intensities of competition, if at all.

Activity and growth

I also expected the correlation between DACT (difference in locomotor activity) and growth to differ across environments. In a HI environment, an increase in activity during the presence of conspecifics may lead to an increase in food acquisition, e.g. as a manifestation of aggressive or territorial behaviour in the presence of defendable resources, or simply as a manifestation of "crowd following behaviour" to an unpredictable resource. It could also be argued that low locomotor activity is associated with low growth when access to food is limited since available evidence indicates that dominance inhibits the general activity of social subordinates (Baenninger 1970, Frey and Miller 1972, Li and Brocksen 1977, Hodapp and Frey 1982, Abbott and Dill 1988).

Competition for food in a LI environment is not necessary to gain better access to the resource. Thus, any increase in the level of activity will lead to an increase in metabolic cost that does not necessarily translate into an increase in food acquisition. The energy channelled into increased locomotor activity is unavailable for physiological growth. Again my expectations were borne out. In the first experiment the correlation between difference in activity and growth was positive for fish raised in the high interaction environment and negative for fish raised in the low interaction environment. In the second experiment this correlation was positive in both environments, but it was much lower (and non-significant) in the low than in the high interaction environment. These results are consistent with the game-theoretic analysis of Doyle and Talbot (1986), who predicted that in well managed environments the process of "domestication selection" should favour fish that are more or less completely indifferent to the presence of conspecifics.

Table 3.1. Mean initial and final length (cm) and standard deviation (SD), and mean growth for LI and for HI fish.

GROUP	n	INITIAL LENGTH(cm)		FINAL LENGTH(cm)		MEAN GROWTH
		MEAN	S.D.	MEAN	S.D.	
LI, exp.1	8	1.52	0.08	2.49	0.12	0.97
LI, exp.2	9	1.58	0.10	1.87	0.13	0.29
HI, exp.1	7	1.56	0.07	2.47	0.14	0.90
HI, exp.2	9	1.54	0.15	2.01	0.17	0.47

Table 3.2. Test of significance for differences between correlation coefficients across environments. (From Sokal & Rohlf 1981, p 589, corrected for small sample size). 1-tail test.

$$H_0: r_{HI} = r_{LI}$$

$$H_1: r_{HI} > r_{LI}$$

		HI vs. LI	P-value
		t	
$r_{DSC,DL}$	EXPERIMENT 1	1.159	0.123
	EXPERIMENT 2	0.633	0.264
$r_{DACT,DL}$	EXPERIMENT 1	1.643	0.051
	EXPERIMENT 2	1.749	0.040

FIG. 3.1. (A) TRANSITION IN: The fish is initially in the half of the tube that is inside the central compartment. Next, the tube is moved, so that now the fish finds itself outside the central compartment but it actively chooses to swim back inside. (B) TRANSITION OUT: The fish is initially in the half of the tube that is outside the central compartment. It is then moved inside, but actively chooses to swim back outside. (C) NO TRANSITION: The fish remains passively in the same half of the moveable tube regardless of whether it is inside or outside the central compartment.

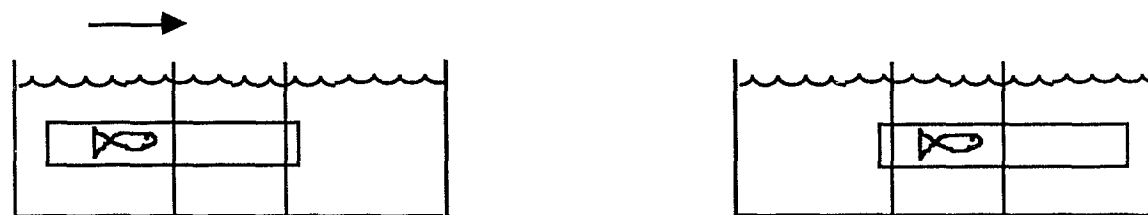
A. TRANSITION IN**B. TRANSITION OUT****C. NO TRANSITION**

Fig. 3.1

FIG. 3.2. (A) Absence of conspecifics. One test fish in each of 15 moveable tubes. (B) Presence of conspecifics in the central compartment. Fish were tested for 30 min in the absence of conspecifics, then for 60 min in their presence, and finally for another 30 min in their absence.

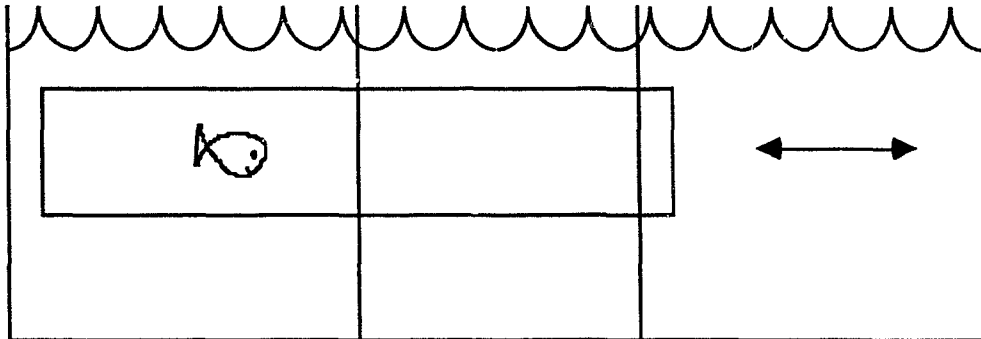
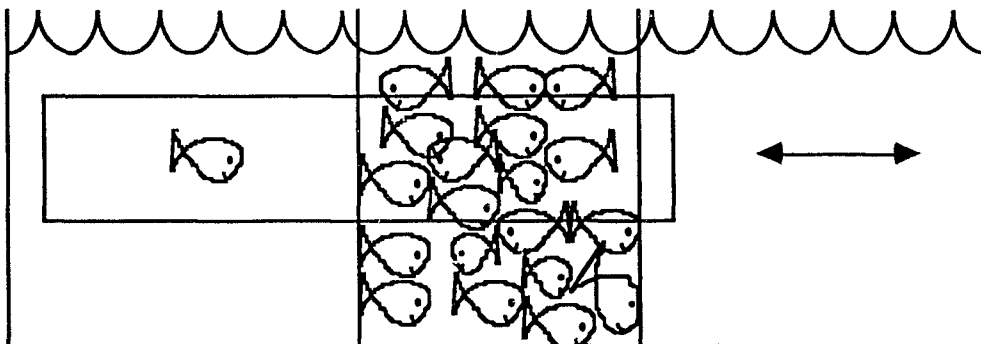
A.**B.**

Fig. 3.2

FIG 3.3. (A) High interaction environment (HI). (B) Low interaction environment (LI). The 2 plots on the left correspond to experiment 1, and the 2 plots on the right correspond to experiment 2. For experiment 1 both the regression of DSC on growth and the correlation between the 2 variables are more negative (although not significantly so) in LI than in HI (See also Table 3.2). For experiment 2, the correlations between DSC and growth are not significant in either environment. Points represent results for individual trials. Correlations were calculated using the means of 7 trials in experiment 1 and of 5 trials in experiment 2. Error bars indicate standard error of the means.

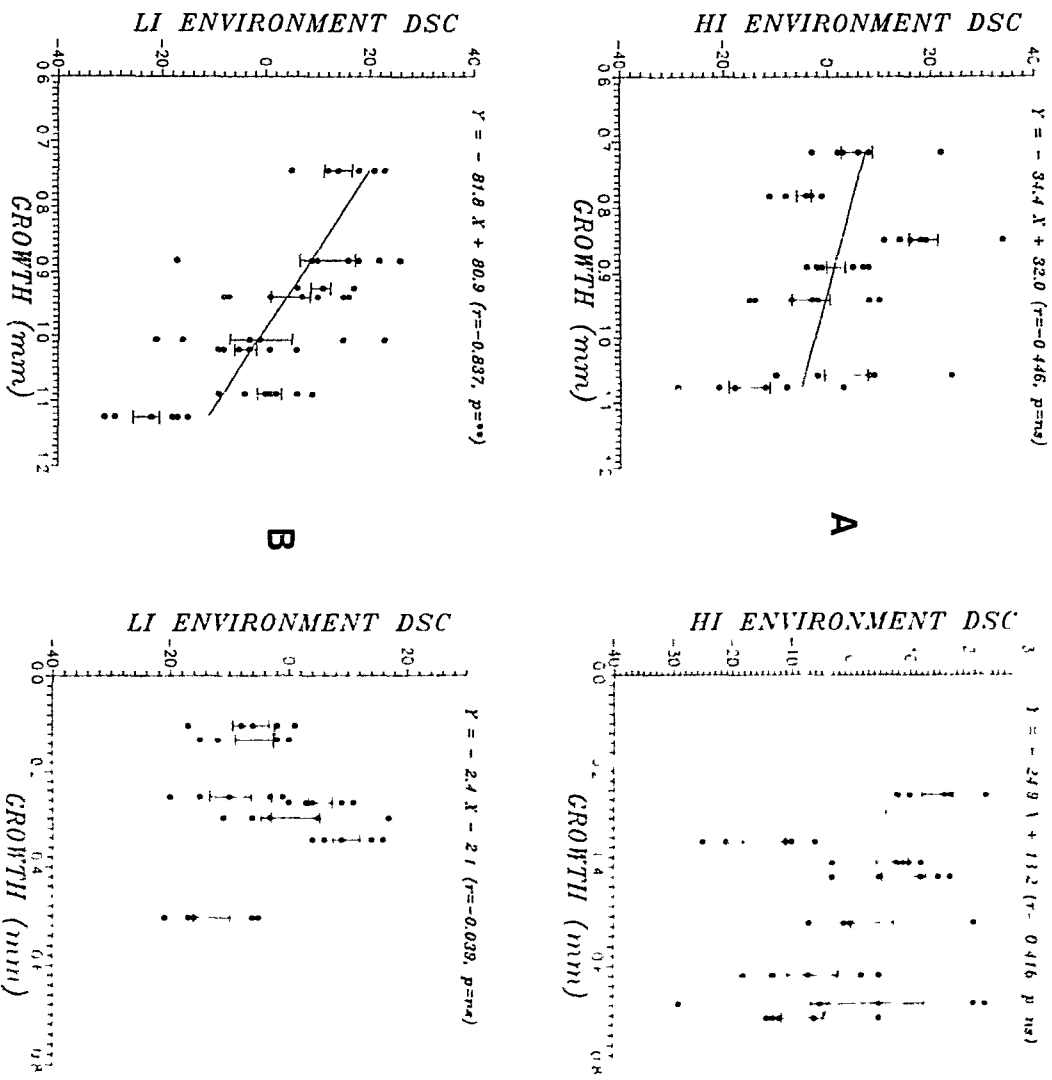


Fig. 3.3

Fig 3.4. (A) High interaction environment (HI). (B) Low interaction environment (LI). The 2 plots on the left correspond to experiment 1, and the 2 plots on the right correspond to experiment 2. In both experiments the correlations between DACT and growth were significantly ($\alpha=0.05$) more positive in HI than in LI (See also Table 3.2). Points represent results for individual trials. Correlations were calculated using the means of 7 trials in experiment 1 and of 5 trials in experiment 2. Error bars indicate standard error of the means.

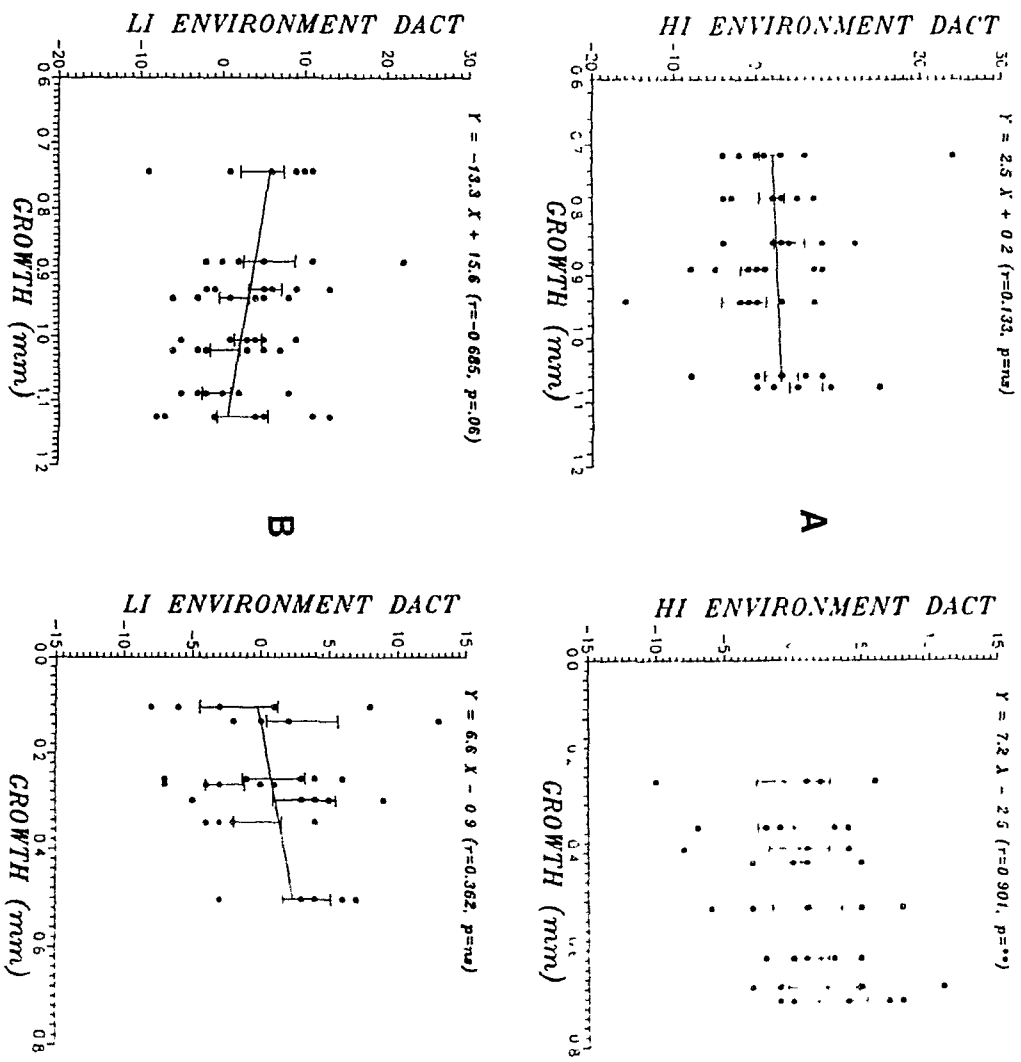


Fig. 3.4

CHAPTER 4
RAPID CHANGE IN THE GENETIC CORRELATION OF SOCIAL BEHAVIOUR
AND GROWTH DURING SELECTION

ABSTRACT

Genetic correlations are thought to be a basic constraint on responses to selection. Here I demonstrate that the correlation of social behaviour and growth in fish can be modified by two generations of selection on growth. The results also suggest that at least some of the variability of the behavioural traits is controlled by a few major genes.

INTRODUCTION

Genetic variances and covariances are constraints on the evolutionary response to selection (Wright 1977, Lande 1982). When phenotypic characters are genetically correlated an evolutionary change in one of them will in general be accompanied by changes in the other (Lande and Arnold 1983). Genetic correlations among traits are usually assumed to remain relatively constant or to evolve much more slowly than the traits themselves (Lande 1979, 1982, Arnold 1981, Via and Lande 1985). This assumption has been said to be unwarranted as a basis for general theory (Bohren et al. 1966, Turelli 1988). I demonstrate experimentally that in the medaka (*Oryzias latipes*) the genetic correlation between growth and behaviour can indeed be modified by selecting on growth. By selecting fish for fast growth for just 2 generations under a highly interactive regime (a situation resembling domestication (Price

1984, Kohane and Parsons 1988)) I modified the correlation between growth and behaviour. The results are consistent with the assumption that some of the variability in these traits is dependent on a few major genes (Parsons 1987, Kohane and Parsons 1988).

MATERIALS AND METHODS

Thirteen pairs of unselected fish obtained from a large, randomly-breeding population were mated and their offspring raised and selected in 2 environments that differed in the intensity of agonistic interactions (Fig. 4.1). In the "high interaction regime" (HI) food was provided inside a floating cork ring, positioned in a corner of the tank, which limited access to the food and allowed a few fish to monopolize the food supply. In the "low interaction regime" (LI) an equivalent amount of food was spread over the container's surface (see chapter 2). Broods were raised in individual 800 ml cages suspended in large trays. Individual trays contained a maximum of 15 cages (broods) each. Fish were selected for fast and slow growth. Selection was within brood, within spawning date, before sexual differentiation. I usually selected only the largest and/or smallest individual in the brood, but occasionally more than one fish were selected in each direction to account for mortality and sex. Selection intensities were usually between 10% and 50% depending on brood size. Four selected lines were established: fast and slow growth in a HI regime, and fast and slow growth in a LI regime. In the third generation (after 2 episodes of selection), half the broods within each line were raised in a HI and half in a LI regime, bringing the number of lines to 8 (Fig. 4.1). Here I report the response to selection of 3rd generation fish from the four lines raised in a high interaction regime. I estimated phenotypic correlations in each line. Since the environmental component of the phenotypic variance was the same in all 4 lines and possible maternal covariances were

eliminated by the breeding plan (Fig. 4.1), the observed differences in the phenotypic correlations are presumably due to changes in genetic variances and covariances. I selected fish on the basis of growth and measured the direct (growth) and correlated (behavioural) response to selection.

Fish were tested for activity level in the presence and absence of conspecifics, and for their preference for, or tolerance of crowded conditions (Fig. 4.2). Two behaviours were tested: (1) the change in locomotor activity when exposed to a crowd of conspecifics. (2) the degree of "tolerance" of crowds of conspecifics.

RESULTS

Within the line selected for faster growth in a HI regime (Fig. 4.3a) the slope of the regression of activity on growth rate is positive ($r=0.43$, $P=0.06$). Within this selected line the faster growers show a higher increase in locomotor activity in the presence of conspecifics than the slower growers do. There is no relationship between activity and growth in the line selected for slower growth in the same regime (Fig. 4.3b) or in the line selected in either direction in the LI regime (Fig. 4.3c and 4.3d). The slopes of these activity-growth regressions differ between fast and slow growth lines only when these descend from 2 generations of selection under a HI regime ($P=0.036$, Fig. 4.3a vs. 4.3b) but not when selection took place under a LI regime ($P=0.684$, Fig. 4.3c vs. 4.3d). Similarly for social tolerance score (Fig. 4.4), the slopes differ between the fast and slow growth lines only when selection was performed under a HI regime ($P=0.05$, Fig. 4.4a vs. 4.4b), and not when it was performed under a LI regime ($P=0.75$, Fig. 4.4c vs. 4.4d).

Among offspring of fish selected for fast growth rate in a HI regime where access to food was restricted, growth was positively correlated with activity and social tolerance. In contrast, the correlations of these behaviours and growth rate were not significant among offspring of fish selected for slow growth rate in the same regime. Selection on growth in a low interaction regime did not cause differences in the correlation of behaviour and growth. I infer from these results that in a regime where access to food is spatially unrestricted, higher levels of activity and social tolerance were not components of fitness (growth) (Doyle and Talbot 1986, Huntingford and Turner 1987, this dissertation). The changes in the correlations observed under a HI regime are in fact conservative, because genetic differences were diluted by using unselected females (Fig. 4.1).

DISCUSSION

I induced changes in the phenotypic (and presumably genetic) correlations between social behaviour and growth after only 2 generations of selection on growth, when the selection was practised under conditions where access to the resource depended mainly on social behaviour. (The mean value of growth and a related behavioural trait also changed as a result of selection (see chapter 2).) Changes in the genetic variance-covariance structure following moderate to strong selection are expected on theoretical grounds (Bohren et al. 1966, Turelli 1988). Here, I emphasize the rapid change in the correlation structure following just two generations of selection on growth. These rapid behavioural changes are inconsistent with the assumption that these traits are determined solely by many genes of small effect. It is more likely that a few major genes are responsible for a significant proportion of the variability (Parsons 1987, Kohane and Parsons 1988).

Modifications in behaviour are a major feature of the early stages of the domestication process (Kohane and Parsons 1988). They often involve changes in the frequency or intensity with which behavioural patterns are manifested (Hale 1969, Price 1984). Territorial patterns of behaviour tend to break down under conditions of intense competition, and selection pressures seem to favour animals with more flexible social behaviour (Hale 1969). Selection for dominance or aggression (Farr 1983, Francis 1984, Bakker 1986), back-crosses (Parzefall 1979), and hybridization experiments (Moav and Wohlfarth 1970, Ferguson and Noakes 1982, 1983, Csányi and Gervai 1986) have all demonstrated that behaviour in fish has a strong genetic component. Domesticated stocks of brook trout (*Salvelinus fontinalis*) and of Atlantic Salmon (*Salmo salar*) are considerably tamer and less easily frightened than wild stocks (Vincent 1960, Holm and Fernö 1986, Gjedrem et al. 1987). These results of domestication in fish have been formally predicted using a game-theoretic argument (Doyle and Talbot 1986) and have also been demonstrated experimentally (see chapter 2).

Despite the conflicting evidence derived from different experiments and theoretical models, data documenting the rate of genetic changes in behaviour during domestication are limited (Parsons 1987), and in the case of fish, somewhat speculative (Moav and Wohlfarth 1970). The application of quantitative genetics theory to the study of the evolution of natural populations has usually relied on the simplifying assumption of relatively constant genetic correlations under weak selection (Lande 1979, 1982, Arnold 1981). This assumption may frequently be unrealistic (Turelli 1988) since genetic covariances are inherently less stable than genetic variances (Bohren et al. 1966). Strong environmental shifts (e.g. long periods of intense drought followed by heavy rainfall) can cause selection producing changes in genetic correlations over a few generations in the wild (Grant and Grant 1989). Doyle and Hunte (1981) described significant evolutionary changes in the mean values and correlations of

demographic traits of an estuarine amphipod (*Gammarus lawrencianus*) kept under constant laboratory conditions, which occurred within a time scale short enough to be of "importance to ecologists". Comparative studies with migratory and non-migratory populations of the milkweed bug (*Oncopeltus fasciatus*) revealed significant differences in genetic variance-covariance structure across populations (Palmer and Dingle 1986, Dingle et al. 1988).

These findings provide experimental support for the expressed doubts concerning the robustness of evolutionary inferences derived under the constant covariance assumption (Turelli 1988). They also provide evidence that "tameness" or intensity of "involvement" may be under considerable selective pressure in fish populations (Moav and Wohlfarth 1970, Doyle and Talbot 1986). The rapid changes in genetic correlations following 2 generations of selection observed in the present experiment raises the following evolutionary questions: Does the probably small number of major genes affecting behavioural variability facilitate domestication? Or from a different perspective, do species that differ in the ease with which they can be domesticated also differ in the number of genes affecting variability in social behaviour? Overall, widespread species tend to show a higher degree of adaptability than geographically limited species (Holt 1990, Pease et al. 1990). Do these differences in geographic distributions (or in tolerance to environmental degradation) correlate with differences in the number of major genes affecting behavioural variability? Parsons (1987) argued that genetic variability may often be unmasked under conditions of stress, and that a stressful environment has the potential to maximize the rate and direction of evolutionary change. Service and Rose (1985) showed that negative additive genetic correlations among life history traits in *Drosophila* became significantly less negative when measured under novel environmental conditions. In the present experimental environments, variation in the genes (possibly major genes) affecting behaviour may be selectively neutral under some

environmental conditions (e.g. low interaction regime), but strongly correlated with fitness under other, more stressful conditions (e.g. high interaction regime).

FIG. 4.1. Experimental design: Fish in each of the 2 environments (High Interaction: HI and Low Interaction: LI) were selected in fast (F) and slow (S) growth lines for 2 generations. Selected males from generation G_2 were all mated to unselected females to eliminate the possibility of maternal effects affecting covariances. In the third generation half the broods within each line were raised in a HI and half in a LI regime (see chapter 2). For the present experiment I tested 3rd generation fish from the four sublines currently raised in HI. Fish were tested from 48 broods. Total length was measured to the nearest .05 mm for all brood members, and 2 fish per brood were tested for behaviour (96 fish in total). These were the largest and smallest fish in the brood. This procedure did not induce a bias because growth was normally distributed. Each fish was tested once a day on 5 consecutive days (i.e. 5 trials per fish). Test individuals were marked at the start of the experiment and reintroduced in their respective broods after each trial.

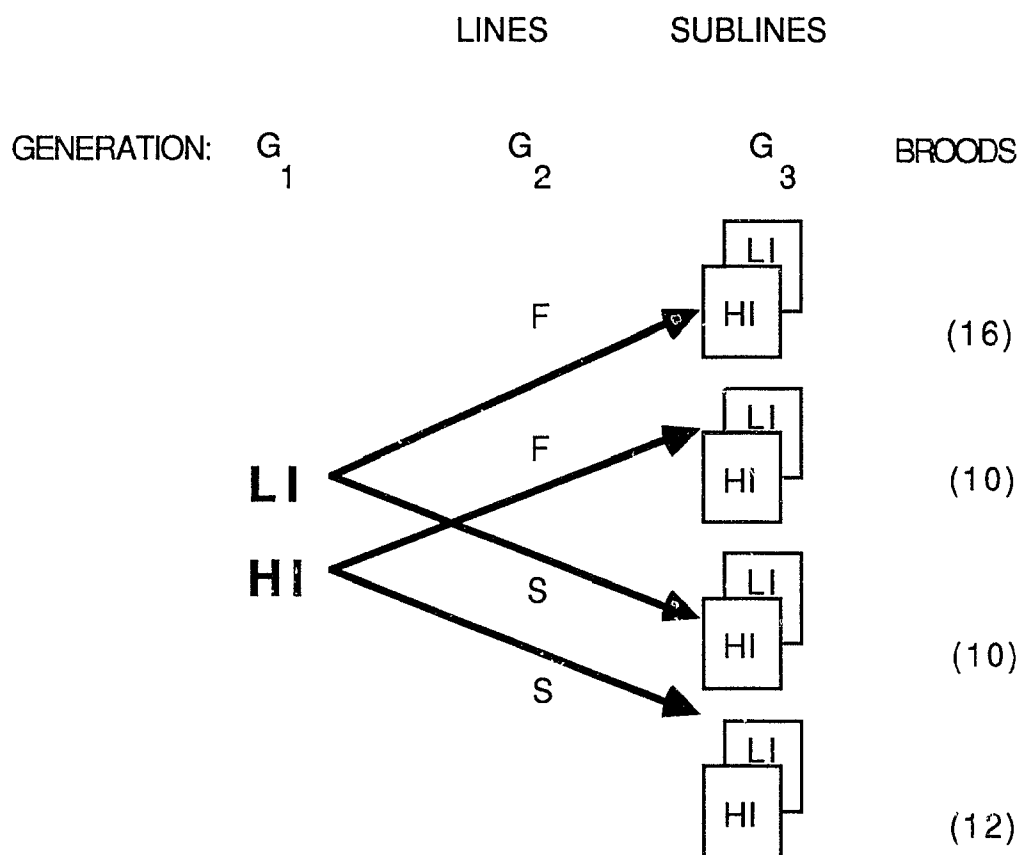


Fig. 4.1

FIG. 4.2. (A) Individual test fish were placed inside a moveable glass tube that could be slid inside a box with three compartments. At any given time half the tube was inside the central compartment and half the tube was outside it. Five boxes containing 16 tubes were set in parallel in a tray filled with water. (B) By adding approximately 250 fish to the central compartment, the test fish could be exposed to visual contact with conspecifics if they were in the appropriate half of the glass tube. During the experiment the tubes were slid back and forth every 2 min, changing the environment of the test fish with respect to the visual presence of other fish, and thus forcing the test fish to make a decision whether to move or not. Details of the experimental protocol are described elsewhere (see chapter 3).

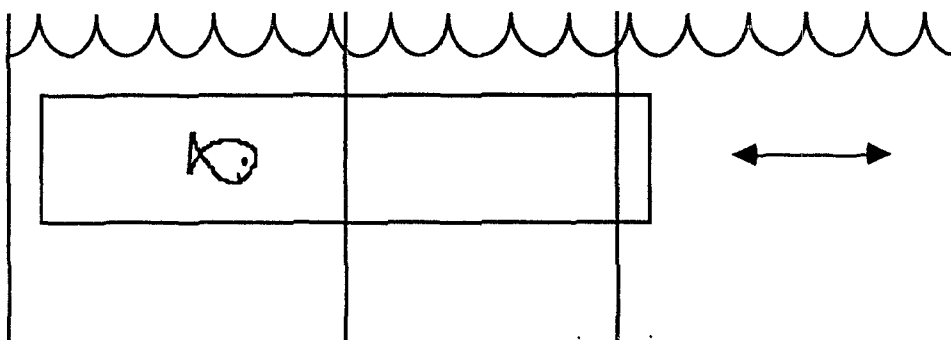
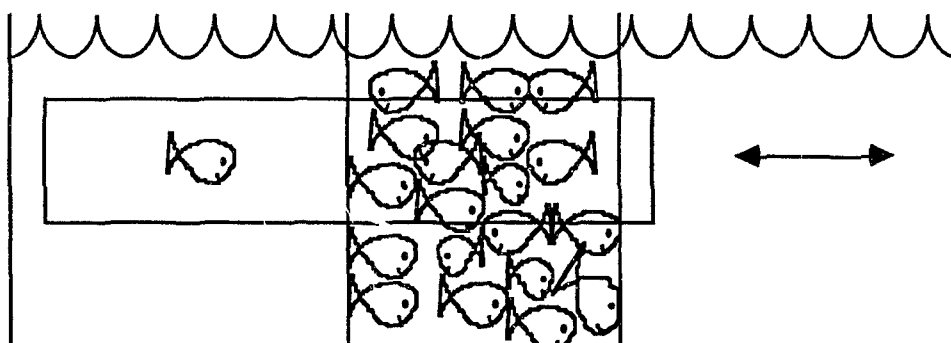
A.**B.**

Fig. 4.2

FIG. 4.3. Change in locomotor activity vs. growth rate (mm.day^{-1}). The change refers to the activity during the presence minus the activity during the absence (P-A) of conspecifics in the central compartment. The slopes of the regressions of activity on growth rate differ between the fast and slow growth selected lines only when the divergent selection on growth rate was carried out under a HI environment ($P=0.04$, a vs. b), and not when it was carried out under a LI environment ($P=0.68$, c vs. d).

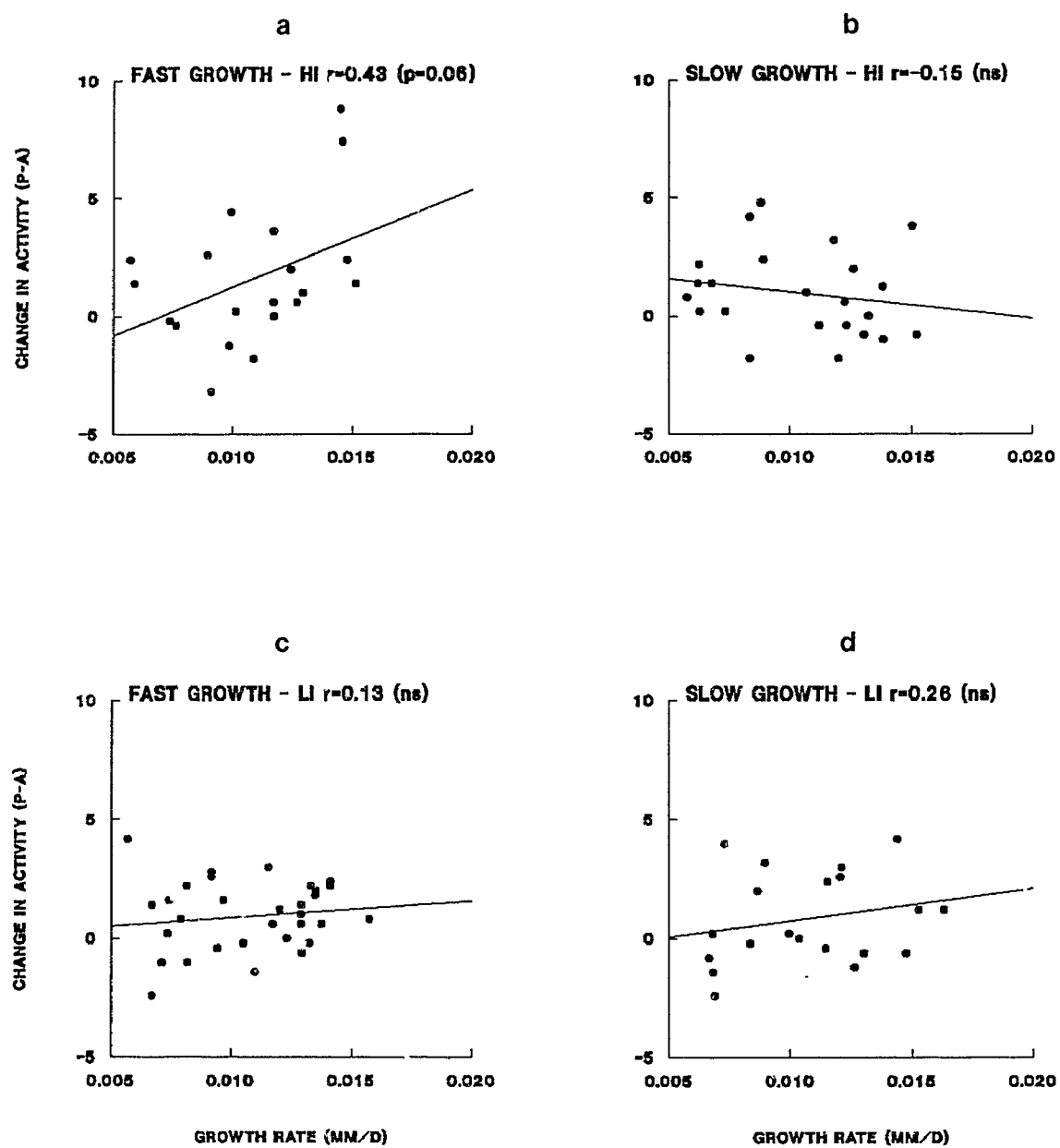


Fig. 4.3

FIG 4.4. Change in score vs. growth rate (mm.day^{-1}). The change refers to the score during the presence minus the score during the absence (P-A) of conspecifics in the central compartment. Even though both sets of variables, the change in score and the change in locomotor activity (Fig.4.3) are based on different combinations of the same set of observations (see chapter 3) they are uncorrelated with each other.

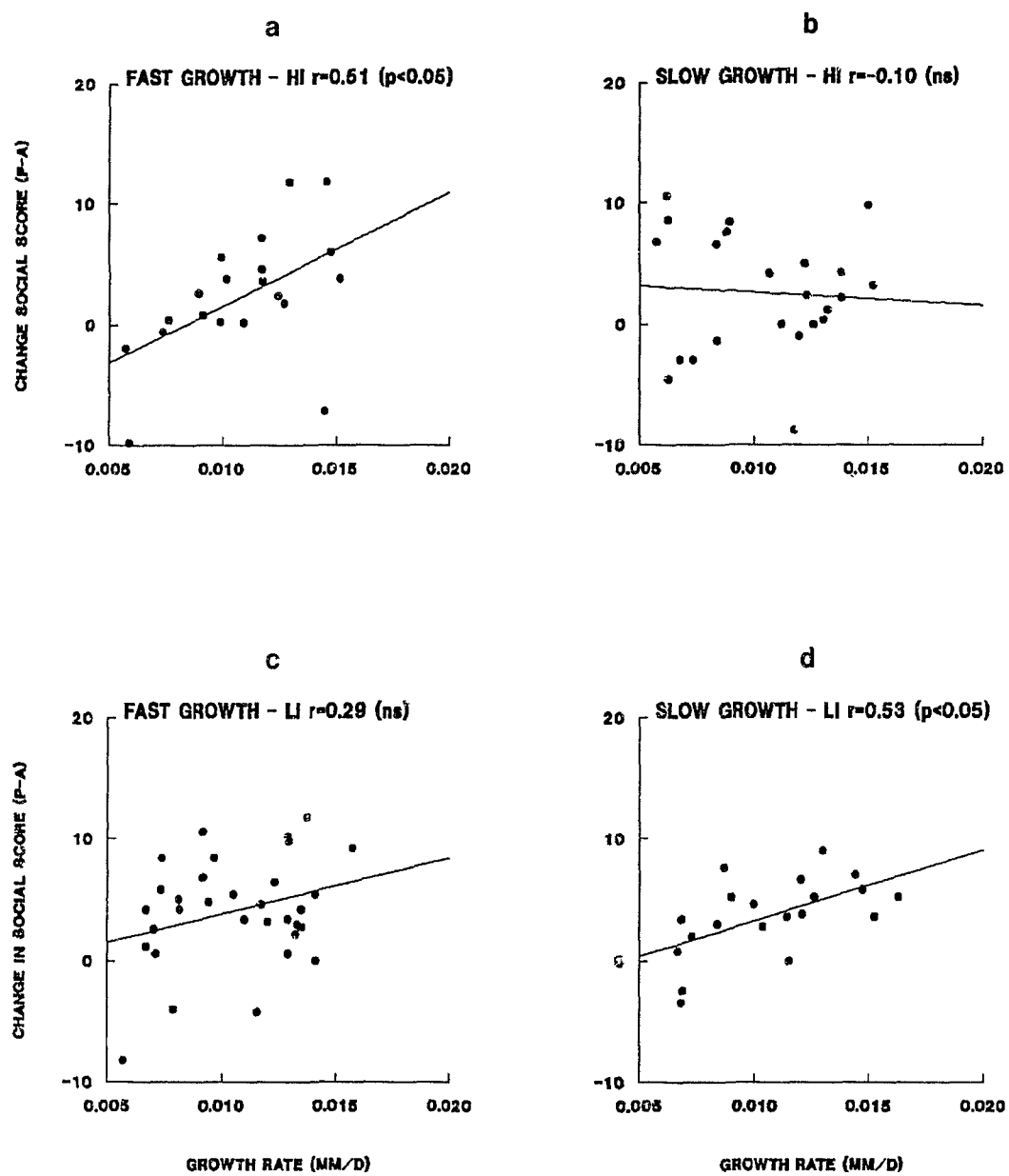


Fig. 4.4

CHAPTER 5

RAPID CHANGE IN SCHOOLING BEHAVIOUR FOLLOWING SELECTION FOR COMPETITIVE AND NON-COMPETITIVE GROWTH.

ABSTRACT

Whether in any particular instance fish behave "cooperatively" by schooling, or competitively by engaging in agonistic interactions, is often determined by an array of environmental circumstances (e.g risk of predation vs. intraspecific competition) and by the individuals' physiological state. In this chapter I present evidence that the tendency to form cohesive schools among medaka can be modified by two generations of divergent selection on growth, indicating that schooling behaviour in this species is influenced by genetic components that are related to growth. Medaka derived from two generations of selection for fast growth in a high interaction environment, and also raised in a high interaction environment, responded to the sight of a predator by swimming more closely together than medaka derived from the other selected lines. Conversely, medaka derived from the line selected for slow growth in a low interaction environment, and also raised in a low interaction environment, responded by swimming further apart than medaka derived from the other selected lines. The broods that responded to the sight of a predator by increasing school cohesiveness are also those that exhibited a relatively low level of agonistic behaviour (Chapter 2), suggesting that one consequence of selection for fast growth may have been an increase in the threshold level of crowding that elicits agonistic responses.

INTRODUCTION

Whether an animal lives in a social group or is solitary can be described in terms of the fitness costs and benefits of alternative strategies (Pulliam and Caraco 1984). Some of the benefits of group living were initially mentioned in a pioneering 1922 study by R.C. Miller, who described the protective advantages of the gregarious habit in a passerine bird exposed to constant high predation risk in the wild (Miller 1922). Schooling or shoaling behaviour in aquatic vertebrates is also thought to have evolved mainly as an antipredator strategy (see Partridge 1982, and Pitcher 1986 for reviews). Once a school is detected by a predator, the probability that any one individual might actually be eaten is lower than if the individual was found on its own, a phenomenon described as the "dilution effect" (Neill and Cullen 1974, Major 1978, Foster and Treherne 1981, Morgan and Godin 1985). The probability of a predator catching a prey may also be inversely related to shoal size due to what is referred to as the "confusion effect" (Milinski and Heller 1978, Milinski 1979, Ohguchi 1981). In addition, fish in larger shoals may be able to detect a predator earlier than in smaller groups (Pitcher and Magurran 1983, Magurran et al. 1985, Godin et al. 1988, cf. Godin and Morgan 1985).

Living in groups can also provide benefits associated with foraging activities. For instance, experiments with foraging minnows (*Phoxinus phoxinus*) and goldfish (*Carassius auratus*) demonstrated that the time spent foraging for patchily distributed resources decreased with increasing shoal size providing evidence that fish acquire information from the behaviour of other shoal members (Pitcher et al. 1982).

Living in groups, however, can have associated costs (Pulliam and Caraco 1984). In particular, competition for available resources increases with increasing shoal size (Eggers

1976, Street et al. 1984), as hunger levels increase (Morgan 1988), or the availability of spawning sites decrease (Magurran and Bendelow 1990). Eggers (1976) suggested that schooling by predators occurs at the expense of prey consumption, and that this cost was reduced if distance between schooling members was high, school size was small or prey density was very high. In addition, prey handling time appears to be inversely related to shoal size in goldfish (*C. auratus*) (Street et al. 1984) and medaka (*Oryzias latipes*) (Uematsu and Takamori 1976, Suehiro and Uematsu 1976), apparently in response to an increase in interference competition (Pitcher 1986).

Despite the well documented knowledge of the functional aspects of schooling behaviour in fish (e.g. Pitcher 1936, Magurran and Pitcher 1987), relatively little is known about its genetic basis. Seghers (1974) demonstrated that the tendency to form cohesive schools among predator naive, laboratory reared guppies (*Poecilia reticulata*) originating from different natural populations was correlated with predation pressures characteristic of their respective populations of origin. Similarly, European minnow (*Phoxinus phoxinus*) from populations subjected to intense predation in nature perform more "effectively integrated evasive tactics" (Magurran and Pitcher 1987), and form more cohesive schools (Magurran 1990) than those from populations characteristic of streams where predation is low. The ability to increase the degree of school cohesiveness as a response to early experience with a predator appears also to be genetically determined (Magurran 1990). Finally, predator-naive, laboratory reared guppies (*Poecilia reticulata*) from different populations in Trinidad differ in their ability to recognize different types of predators (Magurran and Seghers 1990). This variation in predator recognition ability correlates well with the predators' relative abundance in the populations of origin. This suggests that, given a genetic basis to the behaviour

differences, predation is a selective factor promoting genetic divergence (Magurran and Segners 1990).

My objectives in this study were to determine whether selection on growth in environments that differed in the intensity of social interaction induced correlated changes in the tendency to form cohesive shoals among medaka (*Oryzias latipes*), and if so, whether the direction of these changes was affected by the environment and the direction in which selection took place. I tested fish after 2 generations of divergent selection on growth. The selection had been conducted with fish raised in a high interaction environment and in a low interaction environment. In chapter two I have shown that agonistic behavior in medaka was rapidly reduced as a consequence of selection for fast growth, when selection took place under conditions of high social interaction. Whether or not a particular fish joins a school of conspecifics depends, to a large extent, on a tradeoff between its cooperative and competitive tendencies (Magurran 1990b), which, in turn, may be influenced by environmental cues (Reimers 1968, Magurran and Bendelow 1990, Christiansen and Jobling 1990), and by the individual's physiological state (Morgan 1988). For the present study I predicted that under a standard predation threat, fish in the selected line that showed a relatively low level of agonistic interactions would also show a tendency to form more cohesive schools than fish in the selected lines that did not exhibit changes in agonistic behaviour.

MATERIAL AND METHODS

Selection design

Thirteen pairs of unselected fish from a large, randomly-breeding population (purchased from Carolina Biological Supplies) were mated and their offspring were raised and

selected under two regimes that differed in the intensity of agonistic interactions (see below). Selection took place during two generations (G_1 and G_2) (Fig. 2.1a), and here I present results of schooling tests performed with individuals of the third generation (G_3) (Fig. 2.1a). Fish in each social regime were selected for fast and slow growth rate (Fig. 2.1b). Selection was within brood, within spawning date, and it always took place before sexual differentiation. There were unequal numbers of broods per family, and also unequal numbers of individuals per brood in all generations. Usually only the single largest and/or smallest fish in the brood were selected. Occasionally, when brood sizes were relatively large, I selected more than 1 individual per direction. Mean (\pm SD) selection intensities in the direction of fast growth were $0.24(\pm 0.08)$ and $0.39(\pm 0.14)$ during G_1 and G_2 respectively. In the direction of slow growth, mean (\pm SD) selection intensities were $0.26(\pm 0.10)$ and $0.53(\pm 0.16)$ during G_1 and G_2 respectively. Four selected lines were established during the first episode of selection (Fig 1b): fast and slow growth in a high interaction (HI), and fast and slow growth in a low interaction (LI) regime (Fig. 2.1b). In the third generation (after 2 episodes of selection), half the broods within each line were raised in a high and half in a low interaction regime, bringing the number of sublines to 8 (Fig. 2.1b). Thus, each subline differed from the rest in the combination of past regime, current regime, and direction of selection. Further details of the experimental design are described in chapter 2.

In the G_1 generation mating was between families within selected lines. Males in the G_2 generation were mated to unselected females to produce the G_3 offspring used to evaluate the results of selection (Fig. 2.1a). This was done to eliminate the possibility of maternal inheritance confounding the results, e.g. variation in brood size or covariation between offspring growth and behavior induced by the rearing environment of the mother.

Environmental regimes

The divergent selection was conducted under two environmental regimes that differed in the way food was distributed. In the high interaction regime (HI) food was provided inside a floating cork ring, positioned in a corner of the tank, which limited access to the food and allowed the possibility of a few fish monopolizing the food supply. Three ring sizes were used depending on individual length and brood size. Initially, rings were 12 mm (internal ϕ); four to six weeks after hatching they were replaced by 15 mm rings, and four months after hatching they were replaced by 22 mm rings. In the low interaction regime (LI) an equivalent amount of food was spread over the container's surface. Food was provided once daily on an approximately 5% total brood biomass basis. This quantity ensured that all fish had an opportunity to become satiated and that food would be available for several hours after feeding time.

Test arena and protocol

Nearest neighbour distances were measured in groups of 3 fish in the absence and in the presence of a predator. The tests were performed in circular pools with 2 concentric walls (diameter outer wall $\phi_o=45$ cm, diameter inner wall $\phi_i=30$ cm, water depth=10 cm). The outer wall was opaque. The inner wall was transparent, and it had small holes that allowed water to flow between the 2 compartments. The test fish were placed in the inner compartment and the predators in the outer compartment. Thus, predator and test fish were in visual and chemical but not physical contact. Fish were tested in the absence and in the presence of a predator. Convict cichlids (*Cichlasoma sp.*) were used as predators, each measuring approximately 7 to 8 cm in total length. All tests were conducted under constant room temperature ($22\pm1^\circ\text{C}$) and 12:12 L:D light regime.

Preliminary experiment

Preliminary tests indicated that medaka swim closer to each other in the presence of a predator than when no predator is present. A group of 3 fish was observed during 6 consecutive days, on 3 of which a predator was added to the outer pool compartment (days 2,4, and 6). Fish were transferred back to their rearing tanks and kept in isolation from the rest of the population between trials.

Main experiment

During the main experiment broods were first videotaped from above for 5 min in the absence, and then for 5 min in the presence of a predator, and both tests were conducted on a single day. Tests in the presence of a predator were usually started within 5 to 10 min after the predator had been introduced to the outer pool compartment, thus allowing enough time for the test fish to see the predator. I tested a total of 97 broods of the third selected generation (G_3). For each brood I calculated the mean nearest neighbour distance in the presence and in the absence of a predator, and also the difference between the two. Nearest neighbour distances between fish were measured from the tapes by stopping the image every 20 to 30 s. In each frame, and for each fish I measured the distance to its nearest neighbour. I then calculated a mean value for the frame. I measured 7 or 8 frames per brood, and then averaged over all frames to obtain a single value for the brood in the absence and presence of predator.

Data Analysis

For each combination of past regime, current regime, and direction of selection I calculated the mean nearest neighbour distance in the absence of a predator (D_A), in its presence (D_P), the difference between the two (REACTION), and the average of the two

distances ($MD_{(A,P)}$) with its associated standard error. The reaction to the sight of a predator was estimated by subtracting the mean nearest neighbour distance between medaka in the presence of a predator from that when the predator was absent. Thus, a negative REACTION value indicates that the fish swam closer to each other when a predator was present, whereas a positive value indicates the fish reacted by swimming further apart.

Each test group consisted of 3 individuals of the same brood (i.e. they were full-sibs of the same age) that had also been raised together. An effort was made to select the 3 siblings in the brood that were most closely matched in size. Individual sizes (total length) were usually uniform within broods and also between broods. Tests were conducted during the juvenile stage when no sexual differentiation was apparent.

Statistical Analysis

All statistical analyses were performed with the SYSTAT statistical package (Wilkinson 1988). Mean nearest neighbour distances were normally distributed (Kolmogorov-Smirnov test, as modified by Lilliefors), and variances were homogeneous across past regimes, current regimes, and direction of selection (Bartlett test). Thus, no transformations were required.

Data on the REACTION to the presence of a predator were analyzed by factorial ANOVA with past regime, current regime, and direction of selection as fixed factors.

RESULTS

The broods derived from 2 generations of selection for fast growth under a high interaction regime (past regime:HI) and also raised in a high interaction regime (current

regime:HI) (See Fig. 2.1b) showed the closest association in the presence of a predator ($D_p=6.140$ cm), and also the highest increase in group cohesiveness (REACTION) due to the addition of a predator (Table 5.1). Fish in this group swam, on average, 1.8 cm closer to each other when a predator was present than when it was absent. No other subline showed higher increase in cohesiveness when a predator was added to the test pool (Fig. 5.1, Table 5.1).

An ANOVA conducted with REACTION data for all the broods tested ($N=97$) with past and current regimes, direction of selection, and their three way interaction as covariates revealed a significant effect due to the three way interaction among past regime, current regime, and direction of selection ($P=0.026$) (Table 5.2). This interaction indicates that the effect on the reaction to a predator of each of these 3 variables depended on the combination of the other two. I then conducted an ANOVA for each direction of selection combining past and current regime under a single variable with 4 categories. Among the broods selected for fast growth ($N=41$), the overall reaction to a predator did not vary significantly among the 4 lines ($F_{3,37}=1.878$, $P=0.150$). Contrast analysis showed, however, that the reaction in the line selected and raised in a high interaction regime (past and current regimes of high interaction) differed from the other 3 lines ($F_{1,37}=4.489$, $P=0.041$). Among the broods selected for slow growth ($N=56$), the overall reaction to a predator varied among the 4 selected lines ($F_{3,52}=3.731$, $P=0.017$). Contrast analysis showed, however, that this difference was not caused by the line selected and raised in a high interaction regime ($F_{1,52}=1.318$, $P=0.256$), but by the line selected and raised in a low interaction regime ($F_{1,52}=8.977$, $P=0.004$, see also Fig. 5.1).

In summary, among the lines selected for fast growth, the nearest neighbour distance decreased as a response to a predator more in the line selected and raised in a high interaction regime than in any other line. Conversely, among the lines selected for slow growth, the

nearest neighbour distance increased in response to a predator more in the line selected and raised in a low interaction regime than in any other line.

I then conducted a two-way ANOVA for each past regime to analyze the effect of current regime and direction of selection on the REACTION to a predator. In both past regimes the interaction effects between current regime and direction of selection were apparent but non-significant (past regime high interaction: $F_{1,35}=0.283$, $P=0.140$, and past regime low interaction: $F_{1,54}=8.164$, $P=0.102$). These results suggest the effect of direction of selection on the reaction to a predator depended on the environmental regime in which the broods had been raised. Observation of Fig. 5.1 reveals, however, that the direction of this interaction between current regime and selection depended on past regime.

I also tested whether direction of selection had affected the nearest neighbour distance averaged over the absence and presence of predator. This analysis was conducted separately for each combination of past and current regime (Table 5.3). When selection in the past 2 generations took place under a high interaction regime, and the current regime was also high interaction differences in school cohesiveness between lines selected in opposite directions were apparent and significant at $P=0.097$ level (Table 5.3). No such differences were observed in the other comparisons ($P>0.66$ or higher, Table 5.3).

DISCUSSION

The results described in this chapter indicate that the degree of school cohesiveness shown in response to a predation threat is, at least in part, a genetically based trait that can be modified by two generations of divergent selection on growth (Fig. 5.1). Among the broods derived from two generations of selection for fast growth, those raised in a high interaction

environment, and also derived from broods selected in high interaction responded to the sight of a predator by swimming more closely together than fish originated from the other selected lines (Table 5.1, Fig. 5.1). Among the broods derived from selection for slow growth, those raised in a low interaction environment, and also derived from broods selected in low interaction responded to the sight of a predator by swimming further apart (Fig. 5.1). Evidence that the difference between the lines is partly genetic and not solely environmental is provided by the fact that school cohesiveness did not increase or decrease when past and current environments differed from each other (Fig. 5.1).

In chapter two, I showed that the level of agonistic behaviour among brood members was rapidly reduced after two generations of selection for fast growth, when the selection took place under conditions of high social interaction. Here I demonstrated that individuals in that same selected line also exhibited higher school cohesiveness in the presence of a predator than individuals in any of the selected lines, for which agonistic behaviour had not changed during selection (Table 5.1, Fig. 5.1). The findings reported in the present chapter suggest that selection for fast growth in a high interaction environment, and selection for slow growth in a low interaction environment may have caused shifts in the threshold level of crowding that elicit agonistic responses.

Whether in any particular instance fish behave "cooperatively" by schooling, or competitively by interacting agonistically, will depend on an array of environmental circumstances, and also on the individual's physiological state. For instance, in bluntnose minnows (*Pimephales notatus*) school cohesiveness decreased with increasing hunger level (Morgan 1988). Similarly, agonistic behaviour among schooling White Cloud Mountain minnow (*Tanichthys albonubes*) increased with decreasing availability of spawning sites (Magurran and Bendelow 1990). In the present study I have shown that, at least to some

extent, the genetic composition of the fish also affects their tolerance to conspecifics.

Variability among the selected lines in this tolerance was reflected as variation in the degree of school cohesiveness shown when faced with a standard predation threat.

So far, experiments conducted to demonstrate a genetic basis to schooling behaviour in fish have been restricted to interpopulation comparisons of laboratory raised fish. For instance, laboratory-reared, naive guppies (*Poecilia reticulata*) and minnows (*Phoxinus phoxinus*) showed differences in school cohesiveness that correlated with predation intensities in their respective populations of origin, i.e. fish from "high predation" environments exhibited more cohesive schooling tendencies than fish from "low predation environments (Seghers 1974, Breden et al. 1987, for guppies; Magurran 1990, Magurran and Seghers 1990, for minnows). Recently, additional evidence has accumulated suggesting a genetic basis for certain components of anti-predator behaviour in spiders as well (Riechert and Hedrick 1990).

The growth rate and food conversion efficiency of medaka raised at constant densities increases with increasing group sizes of up to six fish (Kanda and Itazawa 1978), suggesting one of the benefits of shoaling in this species may be increased growth rate. The present study demonstrates the existence of genetic factors related to growth that also affect schooling behaviour in this species. In this study, differences in the shoaling tendencies are demonstrated at the intra-population level, following two generations of divergent selection on growth in high and low interaction environments. In conclusion, I have demonstrated that in medaka the tendency to swim in a school is modified as a result of selection on growth. I predicted that the line selected for fast growth under conditions of high social interaction, and also raised in a high interaction environment, would exhibit a relatively high level of school cohesiveness under predation. The fact that my predictions were borne out suggests schooling behaviour in medaka is influenced by genetic factors that are also related to growth.

TABLE 5.1. Mean nearest neighbour distance (cm) in the absence of a predator (D_A), and in its presence (D_P); reaction due to the sight of a predator ($REACTION = D_P - D_A$); number of broods tested (N); and nearest neighbour distance averaged over absence and presence ($MD_{(A,P)}$) with corresponding standard error (SE). Data are presented by past regime, current regime, and direction of selection.

PAST REG	CURRENT REG	DIR	D_A	D_P	REACTION	N	$MD_{(A,P)}(SE)$
HI	HI	FAST	7.934	6.140	-1.795	6	7.037(0.412)
		SLOW	8.335	7.772	-0.563	15	8.054(0.327)
HI	LI	FAST	7.104	7.620	0.516	6	7.362(0.519)
		SLOW	7.571	6.315	-1.256	12	6.943(0.602)
LI	HI	FAST	7.126	8.349	1.223	15	7.738(0.499)
		SLOW	7.254	7.769	0.514	12	7.512(0.784)
LI	LI	FAST	7.927	8.306	0.379	14	8.117(0.701)
		SLOW	7.217	9.404	2.187	17	8.311(0.462)

Table 5.2. Three-way ANOVA with reaction to the sight of a predator as the dependent variable, past regime, current regime, direction of selection, and three-way interaction term as the independent variables.

$$\text{REACTION} = \text{CONSTANT} + \text{PAST REGIME} + \text{CURRENT REGIME} + \text{DIRECTION} + \text{PAST REGIME} * \text{CURRENT REGIME} * \text{DIRECTION} + \text{ERROR}$$

(N=97)

SOURCE	S.S.	d.f.	M.S.	F-ratio	P	R ²
PAST REGIME	84.011	1	84.011	10.570	0.002	0.157
CURRENT REGIME	7.761	1	7.761	0.976	0.326	
DIRECTION	1.262	1	1.262	0.159	0.691	
PR * CR * DIR	40.917	1	40.917	5.184	0.026	
ERROR	731.238	92	7.948			

TABLE 5.3. One-way ANOVA table for the mean nearest neighbour distance averaged over absence and presence of a predator ($MD_{(A,P)}$) as the dependent variable, and direction of selection as the independent variable. Data were analyzed for each combination of past and current regime.

$$MD_{(A,P)} = \text{CONSTANT} + \text{SEL} + \text{ERROR}$$

PR	CR	N	SOURCE	S.S.	d.f.	M.S.	F-ratio	P	R ²
HI	HI	21	SEL	4.429	1	4.429	3.057	0.097	0.139
			ERROR	27.531	19	1.449			
HI	LI	18	SEL	0.701	1	0.701	0.201	0.660	0.012
			ERROR	55.921	16	3.495			
LI	HI	27	SEL	0.341	1	0.341	0.064	0.802	0.003
			ERROR	133.372	25	5.335			
LI	LI	31	SEL	0.289	1	0.289	0.057	0.813	0.002
			ERROR	147.647	29	5.091			

Fig. 5.1. Shoaling tendencies in medaka during exposure to a predation threat after two generations of selection on growth. Y-axis: The reaction to the presence of a predator was measured by subtracting nearest neighbour distance (cm) between medaka in the presence of a predator from that in its absence ($REACTION = DP - DA$). Data are presented by past and current environmental regimes, and direction of selection. X-axis: Current environmental regime (HI: high interaction, LI: low interaction). Solid bars: fish derived from the lines selected for fast growth. Empty bars: fish derived from the lines selected for slow growth. Error bars are standard error of the mean of several broods. Number of broods tested per line as in table 5.1.

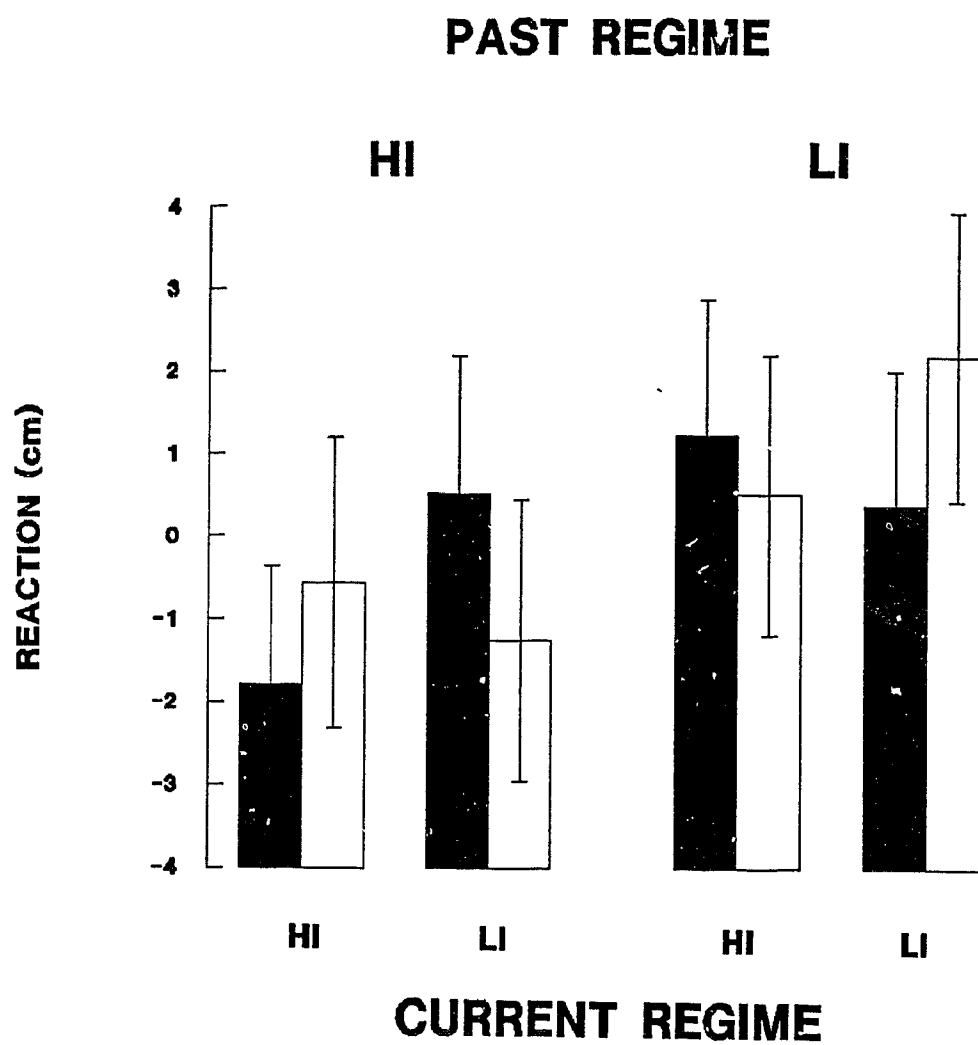


Fig. 5.1

CHAPTER 6

GENERAL DISCUSSION

In this dissertation I have shown that fish derived from 2 generations of selection for fast growth rate grow faster than those derived from selection for slow growth rate (Tables 2.1 and 2.2a). The direct response to selection was highest under conditions of high social interaction (Table 2.2c). In addition, under those same conditions social behaviour is a major component of growth rate variation as demonstrated in chapter 2. Medaka derived from the line selected for fast growth in an environment where food was highly localized (but provided *ad lib.*) were less interactive and more indifferent to each other than those derived from the line selected for slow growth in the same environment (Fig. 2.2). No significant changes in agonistic behaviour were detected when selection was conducted under conditions of low social interaction (i.e. where equivalent amounts of food were evenly distributed). These results contradict the predictions expressed in the literature (Purdom 1974, Kinghorn 1983) that artificial selection for enhanced growth is likely to result in an increase in agonistic behaviour (Chapter 2).

Experiments with unselected medaka demonstrated that the phenotypic correlations between growth and locomotor activity, and between growth and tolerance to crowds of conspecifics (chapter 3) were more positive with fish raised in a high interaction environment than with those raised in a low interaction environment. When resources are clumped (i.e. in a high interaction environment), activity can be interpreted as a manifestation of territorial behaviour in defence of a patchily distributed resource. If this interpretation is correct, high levels of activity in the presence of conspecifics may lead to an increase in food acquisition,

and thus, they may be correlated with fast growth. Low levels of activity may be associated with slow growth in a high interaction regime, since dominants appear to inhibit the activity of social subordinates (Abbott and Dill 1989). For these reasons, individuals that exhibit a high level of activity in a high interaction environment are expected to grow faster than those that exhibit low levels of activity. In an environment where resources are evenly distributed or unpredictably scattered, and thus, where territorial defence is uneconomical (Davies and Houston 1984), a high level of activity is energetically costly and is not rewarded with better access to the resource. Medaka that exhibit high activity levels in this environment may be associated with slow growth. In summary, I expected the phenotypic correlations between growth and activity to be more positive in the high interaction environment than in the low interaction environment.

For similar reasons, I expected the phenotypic correlation between growth and social tolerance to be more positive (or less negative) in the high than in the low interaction environment. In an environment where food is patchily distributed, individuals are expected to interact with each other to acquire food (Magnuson 1962). Fish exhibiting a high tolerance, or preference to be among a crowd of conspecifics are expected to grow faster in such environments than fish that exhibit no such tolerance. When food is evenly distributed, on the other hand, fish do not have to interact with each other to grow fast. High levels of social tolerance are not rewarded with better access to food, and the energy (or time) used in social interactions will not be available for physiological growth. Low levels of social tolerance are not penalized with lower access to food. My predictions were borne out. The phenotypic correlation of social tolerance and growth was less negative in a high than in a low interaction environment (Chapter 3).

I also presented evidence that the genetic correlation between growth and activity, and between growth and social tolerance can be modified by 2 generations of divergent selection on growth when selection takes place in a high interaction environment (Chapter 4). These experiments were conducted with selected fish of the third generation (F₃) that had all been raised in a high interaction environment (current regime). They differed, however, in the environment in which selection had taken place during the previous 2 generations (past regime) and/or the direction of selection. A common current environment for all selected lines insured that the environmental component of the phenotypic variance was the same for all selected lines. The maternal effects were also the same across all lines because of the breeding scheme (see chapter 2). Thus, differences in the phenotypic correlations between lines are presumably due to differences in the genetic component of the correlation.

The application of quantitative genetics theory to the study of the evolution of natural populations has for the most part relied on the simplifying assumption that genetic correlations and covariances remain relatively constant (Lande 1979, 1982, Arnold 1981, Via and Lande 1985). The concern has been expressed that this assumption may not be correct as a basis for general theory (Turelli 1988), since genetic covariances are inherently less stable than genetic variances (Bohren et al. 1966). The results described in chapter 4 provide experimental support for this concern.

In chapter five, I showed that the degree of school cohesiveness exhibited by medaka in response to a predation threat is, at least in part, influenced by genetic factors related to growth (Chapter 5). Fish derived from 2 generations of selection for fast growth in a high interaction environment, and also raised in a high interaction environment, responded to the sight of a predator by swimming more closely together than fish originated from the other selected lines (Table 5.1, Fig. 5.1). This is also the selected line that exhibited a relatively low

level of agonistic behaviour, suggesting that one consequence of selection may have been an increase in the threshold level of crowding that elicits agonistic responses.

That individual differences in competitive abilities can influence the dynamics of populations (e.g. Begon 1982, Łomnicki 1988) has often been a major source of concern among aquaculture geneticists. For instance, Purdom (1974) and Kinghorn (1983) have both discussed the possibility that selection for fast growth might favour fish that are more aggressive. They argued that if this view is correct selection for enhanced growth may lead to higher levels of overall aggression and not to higher levels of assimilation efficiency, thus resulting in an ineffective selection enterprise (Kinghorn 1983). Doyle and Talbot (1986) modelled the consequences of selection for enhanced growth using a game theoretic argument derived from the hawk-dove-assessor model of Maynard Smith (1982). Doyle and Talbot's model assumed the existence of basically four alternative behavioural phenotypes: pure aggressors (i.e. they always attack, regardless of relative size), pure submitters (i.e. they always retreat regardless of relative size), contingent (attack when relatively large, retreat when small), and uninvolved (i.e. a less aggressive variant of the contingent phenotype) (Doyle and Talbot 1986). Their model predicted that selection for enhanced growth in an environment where food is not limited in quantity would result in an increase in the frequency of the uninvolved phenotype, a result that fits quite well with available evidence from domesticated animals (Price 1984). Overall aggressiveness can indeed increase as a result of selection for enhanced growth, but only if agonistic behaviour is independent of relative size (i.e. only if the population consisted of pure aggressors and submitters exclusively), and competition intensity was extremely high (Doyle and Talbot 1986). The evidence I presented in the introduction concerning the adaptive flexibility of fish behaviour to changing environmental circumstances (e.g. Dill 1983b) appears to indicate that a behavioural trait (e.g. agonistic behaviour) that is

solely dependent on the absolute size of the individual, and independent of relative size is unlikely to be of importance in an evolutionary sense. That fish adjust their aggressive behaviour to changing environmental conditions, in particular to changes in relative size, makes sense from an evolutionary standpoint, since this kind of flexibility would minimize the negative effects that aggressive behaviour might otherwise have on the fitness of the individual (Dill 1983b).

There can be little doubt that the intensity with which agonistic behaviour is expressed in fish is, to some extent, a genetically determined trait (e.g. Parzefall 1979; Ferguson and Noakes 1982; Bakker 1986; this dissertation). The adaptive flexibility of agonistic behaviour, i.e. the ability to perceive changes in the environment and to adjust behavioural patterns accordingly (Dill 1983b), could also be influenced by genetic factors. If this is true, then the possibility exists that selection for enhanced growth may result in changes in this flexibility, if flexibility and intensity are correlated at the genetic level. In the present study, selection for enhanced growth in a high interaction environment favoured less aggressive, "uninvolved" (Doyle and Talbot 1986) fish. The question remains, however, whether the apparent increase in "uninvolvement" or indifference is not an incidental consequence of decreased flexibility of behaviour. Current hatchery practices often involve the production of fish for restocking of natural populations. Given the potential serious consequences of a decreased flexibility of behaviour for individual fitness in a natural environment, I believe research efforts should be made to answer this question.

In chapter four, I described the changes in the genetic correlations between growth and two behavioural traits following two generations of divergent selection on growth. These changes occurred only when selection was practised under conditions where access to the resource depended mainly on social behaviour (i.e. high social interaction environment). That

these changes in genetic correlations took place after only two generations of selection suggests a significant proportion of the variability of the behavioural traits in question is likely to be determined by a few genes of major effect. Kohane and Parsons (1988) suggested it is only under such a scenario that we can interpret the rapid behavioural changes that commonly occur when species are forced to occupy new environments, like those provided by man for domestication purposes. Comparison of migratory and non-migratory populations of milkweed bugs (*Oncopeltus fasciatus*) are a possible example of this effect, as strong differences in the variance covariance structure among life-history and behavioural traits have been demonstrated in this species (Palmer and Dingle 1986, Dingle et al. 1988). The migratory population studied is seasonal, and thus, presumably, derived each year from the non-migratory population (Leslie 1990). The differences between the two populations may evolve annually, during the course of 2 to 4 generations, which in turn suggests that "only a few loci with major effects are involved, or that selection pressures are very strong, or both" (Leslie 1990).

The rapid changes in genetic correlations among behavioural and growth traits that followed just 2 generations of selection, and the implication of a relatively low number of genes of major effect being responsible for these rapid changes raise several evolutionary questions. For instance, species can respond to climate change or environmental deterioration by tracking the changes in the environment (Pease et al. 1990), by evolving, or by going extinct (Holt 1990). We may ask whether species that differ in the number of genes affecting variability of behaviour also differ in the way they respond to shifts in the environment. Similarly, do species that differ in the ease with which they can be domesticated also differ in the number of genes affecting behavioural variability?

The experiments described in this dissertation can be considered from another perspective: as an assessment of the genotype-by-environment interaction between growth and

several behavioural traits, including agonistic behaviour, activity levels, tolerance for crowds, and schooling behaviour. Genotype-environment interactions are of paramount importance when considering the extent to which a particular trait or set of traits is influenced by the genetic composition of the individual. Changes in certain components of the environment are likely to affect different genotypes in ways that can alter the ranking of the corresponding phenotypes. At the very least, changes in the environment are likely to alter the magnitude of the environmental component of the phenotypic variance. Such changes are likely to alter the heritability of the character in question and the phenotypic correlations among characters. Based on the game theoretic model of Doyle and Talbot (1986), I expected to detect a genotype-environment interaction in the relationship between growth and agonistic behaviour. These predictions were confirmed by the results described in this dissertation if it is accepted that the behavioural differences produced by selection have a genetic basis. Agonistic behaviour was negatively associated with growth under conditions of spatially localized, but abundant food supply, and it was not associated with growth when the intensity of enforced social interactions was relaxed (i.e. when equivalent amounts of food were evenly distributed). These results showing genotype-environment interaction underscore the need to evaluate the results of selection and crosses in more than just one environment when the objective is to assess the efficacy of selection programs.

Domestication is usually characterized as the adaptation of animals to environmental circumstances which have been defined by humans (Price 1984). Domesticated populations of Atlantic salmon (*Salmo salar*), rainbow trout (*O. mykiss*), and brook trout (*S. fontinalis*) have been reported to be "tamer" and "less frightened" when disturbed by humans than wild conspecific populations (Vincent 1960, Gjedrem et al. 1987; cf. Fenderson and Carpenter 1971). Such evidence may be relevant to changes in the intensity of agonistic behaviour

during domestication if tameness and aggressiveness are behavioural indicators of the same underlying trait, or represent different but highly genetically correlated traits. Swain and Riddell (1990) noted the important distinction between tameness and lack of intraspecific aggression, and argued that they may be two different behavioural traits. I have been unable to find evidence in the literature of any study conducted on fish that discusses whether "tameness" (or lack thereof) and "aggressiveness" are linked through a common causal mechanism. In the rat (*Rattus norvegicus*), however, the relationship between the degree of aggressiveness toward conspecifics, and of "timidity or shyness" in the presence of an unfamiliar individual are both manifestations of an individual's response to novelty, and the two share a common physiological link (Galef 1970). In that study domesticated rats were less aggressive, and also more curious than their wild counterparts, which, in turn were more aggressive and also showed a strong tendency to "avoid any novel object in an otherwise constant environment" (Galef 1970).

Similar questions arise concerning the relationship between social aggression and "timidity" or fear of predators. A literature review of studies on social aggression and predatory or antipredator behaviour across several taxa revealed that in some cases the patterns of inter- and intraspecific aggression exhibit a tendency to covary (Huntingford 1976a). In sticklebacks (*Gasterosteus aculeatus*) for instance, they covary along several time scales between breeding and non-breeding males (Huntingford 1976b), over the course of the breeding season (Huntingford 1976c), and across populations subjected to contrasting predation pressures (Huntingford 1982, Tulley and Huntingford 1988). Fish from a heavily predated location are both less "bold" toward predators and also less aggressive toward conspecifics than sticklebacks from a location where predation is low (Tulley and Huntingford 1988). Although a complete knowledge of the motivational and mechanical aspects of inter- and

intraspecific aggression in fish is by no means available, the possibility exists that at least in some cases both behaviours may be correlated with "fearfulness", and thus partially connected through common physiological factors.

It may be that the sensitivity to experience "fear" can be thought of as the common motivational link (or underlying trait) that is indicated by aggressive behaviour, tameness, and response to predators. Fear has been suggested as one of the underlying internal motivational forces responsible for the expression of aggressive behaviour (Huntingford 1976a, Tulley and Huntingford 1988). It is therefore not unreasonable to think that the intensity of "fear" in a given situation is the trait that is modified by domestication selection. If this assumption is correct, then a reduction in this sensitivity to fear-inducing stimuli will likely result in fish that are both less aggressive and more tame. Obviously, more detailed studies are necessary to elucidate the relationship between tameness and agonistic behaviour in fish.

In conclusion, aquaculture geneticists are right in being concerned about unwanted behavioural changes that may occur during selection for enhanced growth rate. I believe, however, and the evidence presented in this dissertation seems to confirm, that they have been worried for the wrong reason. Agonistic behaviour does indeed change during domestication selection, however, it changes in the direction of decreased intensity, and not toward increased levels. In addition, it may also be that domesticated strains also experience a decrease in the behaviour flexibility they can exhibit in any given circumstance.

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