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Selection programmes for stress tolerance in fish

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SUMMARY - Although breeding programmes in aquaculture are scarce, and most of them seek increase in productivity by selecting traits such as growth rate, there is a new approach which seeks to select additional traits with economic or production implications; such as flesh quality or stress tolerance. Thus, there are new selection programmes being set up to study stress characteristics with the purpose of defining their genetic nature. In this paper, the estimates of genetic parameters are detailed together with the results of some selection experiments, which have been carried out with different stress-associated characters.

Key words: Selection, stress, heritability.

RESUME - *"Programmes de sélection pour la tolérance au stress chez les poissons". Bien que les programmes de sélection soient rares en aquaculture, et que la plupart d'entre eux recherchent une augmentation de la productivité en sélectionnant des caractères tels que la vitesse de croissance, il existe cependant une nouvelle approche qui vise à sélectionner des caractères additionnels ayant des implications économiques ou liées à la production, comme la qualité de la chair ou la tolérance au stress. Il y a donc de nouveaux programmes de sélection mis sur pied pour étudier des caractéristiques liées au stress afin de définir leur nature génétique. Dans cet article sont détaillées des estimations de paramètres génétiques de même que les résultats de quelques expériences de sélection qui ont été menées pour différents caractères associés au stress.*

Mots-clés *: Sélection, stress, héritabilité.*

Introduction

The homeostatic systems for fish in culture conditions are constantly affected by factors associated with fish farming production. These factors may cause stress in the physiological state of the fish, although consensus on the definition of stress has yet to be reached (Pickering, 1993). Factors such as culture density (Pickering and Stewart, 1984; Leatherland and Cho, 1987; Mazur and Iwama, 1993), periodic handling of fish for classification and control of growth (Pickering *et al.,* 1982; Flos *et al.,* 1988; Pages *et al.,* 1995; Waring *et al.,* 1996), transport (Specker and Schreck, 1980; Barton and Peter, 1982; Robertson *et al.,* 1987), water quality (Smart, 1981) or the use of anaesthetics (Thomas and Robertson, 1991) are factors which condition acute stress (ephemeral) and chronic stress (permanent). These, whether acting jointly or separately, may significantly affect factors in fish farming such as survival rate, disease resistance, growth rate and reproduction success (Colombo *et al.,* 1990).

There are various direct and indirect quantitative parameters used as indicators of stress in fish (Barton and Iwama, 1991), some of which we will highlight. Some experimental selection has been carried out, such as the level of cortisol in the plasma, the level of lysozyme in plasma, the immunoglobulin titre, the a_2-antiplasmin titre, the activity of the complement and the activity of the mucus (Cipriano and Heartwell, 1986; Fevolden *et al.,* 1991, 1992; 1993a; Pottinger *et al.,* 1992, 1994; Fevolden and Roed, 1993; Wiegertjes *et al.,* 1994, 1995; Fjalestad *et al.,* 1995). Thus, stress is established as a direct selective agent; improving an organism response to stress would create greater flexibility of the fish to the culture conditions (Pottinger *et al.,* 1992, 1994). Increasing general stress tolerance would also be an indirect means to increase tolerance to other factors such as those mentioned previously (Cipriano and Heartwell, 1986; Fevolden *et al.,* 1991, 1992; 1993a; Fevolden and Roed, 1993; Wiegertjes *et al.*, 1994, 1995; Fjalestad *et al.,* 1995), especially disease resistance since direct exposure to specific pathogens produces risk of infection for broodstocks, commercial fish farms and wild populations (Fjalestad *et al.,* 1993). This review will only deal with selection in fish as regards stress and will not address other traits (see Gjedrem, 1983; Kinghorn, 1983; López-Fanjúl and Toro, 1990; Purdom, 1993; Fjalestad *et al.,* 1993; Afonso, 1996).

Genetic parameters

Breeding programmes in aquaculture are scarce (see Gjedrem, 1997). Thus, there are few experiments which have been carried out to estimate genetic parameters, particularly heritability (the phenotypic variation due to the additive genetic variation, σ_A^2/σ_P^2), of various characters associated with stress, and which are presented in Table 1. The estimates presented here are from sire components. It should be observed that, at times, the standard errors are not as consistent as would be desirable due to the technical difficulties of producing optimum designs in fish farming and thus, the possible conclusions should be accepted with due caution.

Cortisol, which is the main corticosteroid in fish (Idler and Truscott, 1972), fluctuates in concentration in relation to confinement stress. In Atlantic salmon, there is a non-significant low additive genetic variation whilst in rainbow trout, this is apparently medium and different from zero. In none of the species there were non-additive genetic variation or common environmental components (Fevolden *et al.*, 1993b, 1994). Furthermore, the absolute levels post-stress cortisol are higher in the Atlantic salmon than in rainbow trout (Fevolden *et al.,* 1991; 1993b; 1994; Fevolden and Roed, 1993), possibly due to the level of domestication which was 4-5 generations for the Atlantic salmon and 20-30 generations for the rainbow trout (Fevolden *et al.,* 1991).

The lysozyme reveals consistent medium values both before and after immunisation (with inactivated or killed *V. anguillarum, V. salmonicida* and *A. salmonicida*) and confinement stress except in the estimates of Roed *et al.* (1993b) for the Atlantic salmon, where there were no non-additive genetic nor common environmental effects (Roed *et al.,* 1993a; 1993b; Fevolden *et al.,* 1994; Lund *et al.,* 1995). Thus, the genetic correlation between the level of lysozyme in the fish before (Lund *et al.,* 1995) and after stressful events was 0.95±0.23 ($P<0.001$)(Fevolden *et al.,* 1994), although the phenotypic correlations between successive samples before and after immunisations were low and non-significant 0.23-0.24 (Roed *et al.,* 1993b). Therefore, the activity of lysozyme is susceptible to selection both in the Atlantic salmon and in the rainbow trout.

The genetic correlation with cortisol does not give conclusive results, -0.58±0.59 (Fevolden *et al.,* 1994), although Fevolden *et al.* (1991, 1992) and Fevolden and Roed (1993) after making divergent selection for stress via cortisol, found that the high cortisol line carried the highest lysozyme activity, both in the Atlantic salmon and the rainbow trout, although only significant in the latter.

The haemolytic activity reveals a medium additive genetic variation (Roed *et al.,* 1992), although it did not differ from zero under stress via confinement (Fevolden *et al.,* 1994). There are no significant non-additive genetic effect nor common environmental component. In spite of being susceptible to selection like lysozyme, the correlations between successive samples before and after immunisation are high and statistically significant, 0.72-0.85 (Roed *et al.,* 1993b).

Finally, both glucose level (under stress via confinement) and the total immunoglobulin level (without stress) reveal non-significant heritability. Only the total immunoglobuline (total IgM) present non-additive genetic and common environmental components although it is highly possible that they are essentially non-additive genetic given the homogeneity of the culture (Lund *et al.,* 1995). The genetic correlation between the levels of post-stress glucose and cortisol are very low and inconsistent, -0.02±0.15 and 0.02±0.02 in Atlantic salmon and the rainbow trout, respectively.

Strategies of selection for stress tolerance

At present, two methods of selection have been used: individual selection and family selection, despite the fact that combined selection is the optimum strategy (Falconer and Mackay, 1996). However, the genotype of a fish can influence the phenotypes of other members of its family reared together (indirect genetic effects) during the pre-tagging period and for this reason family selection is the most efficient method to detect direct genetic effects when heritabilities are low (Nakanishi and Onozato, 1987; López-Fanjúl and Toro, 1990).

In view of the aforementioned effects, the genetic nature of stress-related traits, the ease to produce full sibs and half sibs thanks to the high fecundity of fish, the mortality of broodstocks before selection and the fact that combined selection is costly, family selection is shown to be the best strategy.

Selection experiments

In spite of the ample literature dealing with stress in different fish species, only a few selection programmes are being carried out.

Gilthead seabream

In the Mediterranean area, *Sparus aurata* is becoming increasingly more importance in aquaculture production (Fig.1). For this reason, an improvement programme for stress tolerance was launched in June 1996 via divergent selection for the levels of post-stress cortisol in plasma (high responders and low responders), with unselected fish, in order to establish a protocol for the selective breeding of seabream for increased tolerance to stress. This programme also aimed to confirm that stress-related traits identified in the parents are inherited by the progeny and quantify the heritability of the selected traits, and assess whether the progeny of stress-tolerant parents are at an advantage under aquacultured conditions, in comparison to the progeny of unselected parents, in terms of growth, resistance disease and reproductive performance.

Rainbow trout

Pottinger *et al.* (1992) began a selection programme of breeding fish using the level of post-stress plasma cortisol, with multiple measurements, in order to establish an improvement in the tolerance of stressful factors in fish farming. To this purpose, they carried out the same experiment with two different strains (Annandale and Stirling), using 240 tagged fish, all of which were 22 months old. The fish were submitted to confinement stress (concentration of fish in one part of the tank for 1 hour) once a month for over five months. The fish with highest values (HR = high responders) over the fives months were selected; 34 in Annandale and 43 fish in Stirling. The fish with lowest value (LR = low responders) were also selected; 38 fish in Annandale and 38 in Stirling. There were significant statistical differences between HR and LR in Annandale six months after selection (161.9±10.2 ng ml^{-1}, 83.4±4.9 ng ml^{-1}, P<0.001), and 28 months after selection (404.1±28.2 ng ml^{-1}, 172.8±20.0 ng ml^{-1}, P<0.0001), whilst there were no differences between them at basal level, without stress, (12.2±3.3 ng ml^{-1}, 8.4±2.2 ng ml^{-1}). Similarly there were significant differences between HR y LR in Stirling 5 months after selection (258.0±7.9 ng ml^{-1}, 182.2±10.9 ng ml^{-1}, P<0.001). In each line (HR, LR), Pottinger *et al.* (1994) carried out crosses of approximately 15 males with 15 females and found significant differences in the levels of post-stress cortisol in the offspring of the lines (113.7±2.8 ng ml^{-1} in HR and 96.7±2.2 ng ml^{-1}, P<0.05).

In the rainbow trout and the Atlantic salmon, Refstie (1986) and Fevolden *et al.* (1991) began a programme of divergent selection via family selection for the levels of post-stress cortisol in plasma. Later, they analysed the offspring (F$_1$) of the high responder (HR) and low responder lines (LR) for the levels of post-stress cortisol in plasma, post-stress glucose in plasma, post-stress lysozyme in plasma, post-stress haemolytic activity (only in Atlantic salmon) and for post-stress antibodies against *Vibrio anguillarum* (this only in rainbow trout) in fish which had been previously immunised. The rainbow trout only revealed significant statistical differences in the lysozyme, which was higher in HR than in the LR. Although there were no significant differences in the cortisol and the glucose, the HR line had levels of cortisol and glucose which were almost always higher than the LR with both parameters correlated in two successive samples (0.101, P<0.001, for the first sample and 0.294, P<0.001, for the second sample). In the second generation of selection of the rainbow trout, Fevolden *et al.* (1992) exposed the F$_2$ of both lines to *Aeromonas salmonicida* (at 15 months old) and *Vibrio anguillarum* (at 16 months old) and found significant statistical differences in accumulated mortality between the lines, 29% in the HR line as opposed to 19% in the LR line for *Aeromonas salmonicida* (P£0.01) and 63% in the LR line as opposed to 42% in the HR line for *Vibrio anguillarum* (P<0.001). In the lysozyme analysis, the significant statistical difference detected in the previous generation is lost although the HR line shows greater lysozymic activity than the LR line. The serum haemolytic activity was statistically higher in the LR line than in the HR line (complete, spontaneous and spontaneous-absorbed haemolytic activity). Due to the inverse mortality rate between lines according to the pathogenic agent, Fevolden and Roed (1993) carried out a second analysis of the F_2 of the HR and LR lines with subsamples (S1 in June and S2 in October) where the post-stress lysozyme, the post-stress cortisol and the post-stress haemolytic activity were evaluated. Besides, when fish were 2 years old, the fish of both the HR an LR lines were immunised against *Aeromonas salmonicida (As)*, *Vibrio anguillarum (Va)* and *Vibrio salmonicida (Vs)* and the titre of antibodies were determined on the following day (the SRO sample) and six weeks (the SR1 sample) after immunisation, together with the lysozyme level and the activity of the complement. The post-stress cortisol presented higher values in the HR than in the LR in S1 (non-significant statistically) since the situation was the opposite in S2 and the difference was statistically significant ($P<0.001$). In spite of this there was a significant correlation between the measurements of cortisol in both subsamples ($P<0.001$). The lysozyme was always higher in the HR than in the HL although it is only statistically significant in S1 and SR1 and showed significant correlations between subsamples, between samples and between samples with subsamples (0.110-0.476), with the exception of S1 with SR1. The spontaneous haemolytic activity (SH) and complete haemolytic activity (CH) were statistically lower in the HR line than in the LR line in S1, S2, SR1 and SR2 except for the SH in S1 although all are significantly correlated (0.144-0.430). Furthermore, in SR1, there were significant correlations between the activity of complement and the titre of antibodies (*As*, *Va* and *Vs*) and between titres of antibodies against *As*, *Va* and *Vs* (0.108-0.513). The antibody titres for *Aeromonas salmonicida* were significantly higher in the HR line than in the LR line for SR1 since the accumulated mortality for furunculosis was greater in the HR than in the LR (Fevolden *et al.,* 1992). Moreover, there were no differences between lines for the antibody titres against *Vibrio anguillarum* whilst there were clear differences in accumulated mortality between lines (Fevolden *et al.,* 1992). These apparent inconsistencies demonstrate the difficulties associated with investigating the mechanisms of complex, immune and cortisol responses, even though experiments in the latter were carried out under standardized conditions.

Atlantic salmon

In the selection programme above mentioned (Refstie, 1986; Fevolden *et al.,* 1991), as far as the Atlantic salmon is concerned, in the F_1 significant differences were found between the lines in the cortisol and the glucose levels, which were significantly correlated (0.3, $P<0.001$). The haemolytic activity also showed significant differences between lines, higher in the HR line than in the LR line, for the spontaneous haemolytic and spontaneous-absorbed activities (P£0.01) (Fevolden *et al.,*1993a). For the lysozyme, there were no significant statistical differences although HR revealed greater lysozymic activity than the LR. In the second generation of selection of Atlantic salmon, Fevolden *et al.* (1993a) challenged the offspring of both lines to *Aeromonas salmonicida* (furunculosis), *Vibrio salmonicida* (coldwater vibriosis) and *Renibacterium salmoninarum* (bacterial kidney disease), and obtained significant statistical differences in the accumulated mortality only in furunculosis and coldwater vibriosis, where the HR line showed higher cumulative mortality than the LR line for both diseases, ($P<0.001$).

Fjalestad *et al.* (1995) carried out a programme of divergent selection for disease resistance via indirect selection of various traits such as plasma lysozyme activity, serum haemolytic activity, the total immunoglobin (total IgM) and a_2-antiplasmin. They used two control groups for each trait submitted for selection. Once the selection had been made, significant differences in the total level of IgM were found between the lines for survival to furunculosis and coldwater vibriosis whilst there were only significant differences in the a_2- antiplasmin for survival in the case of coldwater vibriosis between the two lines. There were no significant differences between the lines in the case of infectious salmon anaemia.

Common carp

Wiegertjes *et al.* (1994) carried out a divergent selection for the antibody titre against DNP-KLH in females at thirteen months old. Via individual selection, they chose three female fish showing high and consistent response at 12 and 21 days after immunisation (named 17, 61, 69) together with another three low responders (named 51, 60, 85). There were significant differences between lines (1.67±0.03 versus 0.38±0.06 at 12 days and 2.28±0.04 versus 1.13±0.11 at 21 days, P£0.0001). To value the selection response, the females were gynogenetically reproduced and their offspring evaluated at six months of age, revealing that there were significant differences between high and low responders at 28 days after immunisation (P£0.05) since at 12 and 21 days there were inbreeding depression. In order to prove that age was not responsible for this, Wiegertjes *et al.* (1995) kept the offspring of the females 69 and 85 until they were 12 months old and confirmed the significant differences between the lines at 21 and 28 days after immunisation (P£0.001) and the persistence of the depression.

Brown trout

Cipriano and Heartwell (1986) carried out individual selection for mucus precipitin activity against *Aeromonas salmonicida* over a population of 243 fish, measuring via simple-radial immunodiffusion (diameter > 12 mm). They selected eight fish (six males and two females) with which they formed two couples due to lack of females and kept a control group via random mating with the rest of the population. 2000 descendants of the control group and 600 of the selected were infected with *Aeromonas salmonicida* and registered, at six months, a cumulative mortality of 48% in the control group as opposed to 2% in the F_1 of the selected fish. Moreover, the inspecificity of the mucus to other pathogens was made manifest and the diameter of simple-radial immunodiffusion of a sample of 20 rainbow trout disease free was defined for *Aeromonas salmonicida* (17.0±1.7 mm), *Vibrio anguillarum* (16.6±1.4 mm) and *Aeromonas hydrophila* (10.3±1.4 mm).

Table 1. Estimates of heritabilities (h^2±se) in Atlantic salmon, rainbow trout and common carp to various stress and immunological traits

Species (Traits)	h^2±se	Authors
Atlantic salmon		
Total IgM level	0.00±0.14	Lund *et al.* 1995
Lysozyme activity 12 weeks after first immunisation	0.16±0.10	Lund *et al.*, 1995
Lysozyme activity 2,5 years old	0.08±0.05	Roed *et al.*, 1993b
Lysozyme post-stress 2 years old	0.19±0.11	Fevolden *et al.*, 1994
Cortisol post-stress 2 years old	0.07±0.09	Fevolden *et al.*, 1994
Cortisol post-stress 18-19 months old	0.05±0.03	Fevolden *et al.*, 1993b
Glucose post-stress 18-19 months old	0.03±0.11	Fevolden *et al.*, 1993b
Complete haemolytic activity post-stress 2,5 years old	0.04±0.05	Fevolden *et al.*, 1994
Complete haemolytic activity 2,5 years old	0.35±0.16	Roed *et al.*, 1992
Spontaneous haemolytic activity post-stress 2 years old	0.02±0.04	Fevolden *et al.*, 1994
Spontaneous haemolytic activity 2,5 years old	0.32±0.13	Roed *et al.*, 1992
Spontaneous haemolytic activity first sampling at 2,5 years old	0.23±0.15	Roed *et al.*, 1993b
Spontaneous haemolytic activity second sampling at 2,5 years old	0.19±0.13	Roed *et al.*, 1993b
Spontaneous haemolytic activity third sampling at 2,5 years old	0.04±0.09	Roed *et al.*, 1993b
Spontaneous haemolytic activity –absorbed 2,5 years old	0.32±0.14	Roed *et al.*, 1992
Naturally occurring haemolysins 2,5 years old	0.20±0.09	Roed *et al.*, 1992
Rainbow trout		
Lysozyme activity 18 months old	0.22±0.06	Roed *et al.*, 1993a
Cortisol post-stress 18-19 months old	0.27±0.10	Fevolden *et al.*, 1993b
Glucose post-stress 18-19 months old	0.07±0.04	Fevolden *et al.*, 1993b
Haemolytic activity first sampling at 1,5 years old	0.13±0.32	Roed *et al.*, 1990
Haemolytic activity second sampling at 1,5 years old	0.33±0.27	Roed *et al.*, 1990
Haemolytic activity third sampling at 1,5 years old	0.08±0.33	Roed *et al.*, 1990

Table 1. Estimates of heritabilities (h^2±se) in Atlantic salmon, rainbow trout and common carp to various stress and immunological traits (cont.)

Species (Traits)	h^2±se	Authors
<u>Common carp</u>		
Antibody concentration after immunisation against DNP-KLH. 6 months old	0.37±0.36*	Wiegertjes *et al.*, 1994
Antibody concentration after immunisation against DNP-KLH. 6 months old	0.29*	Wiegertjes *et al.*, 1995

* = Realized

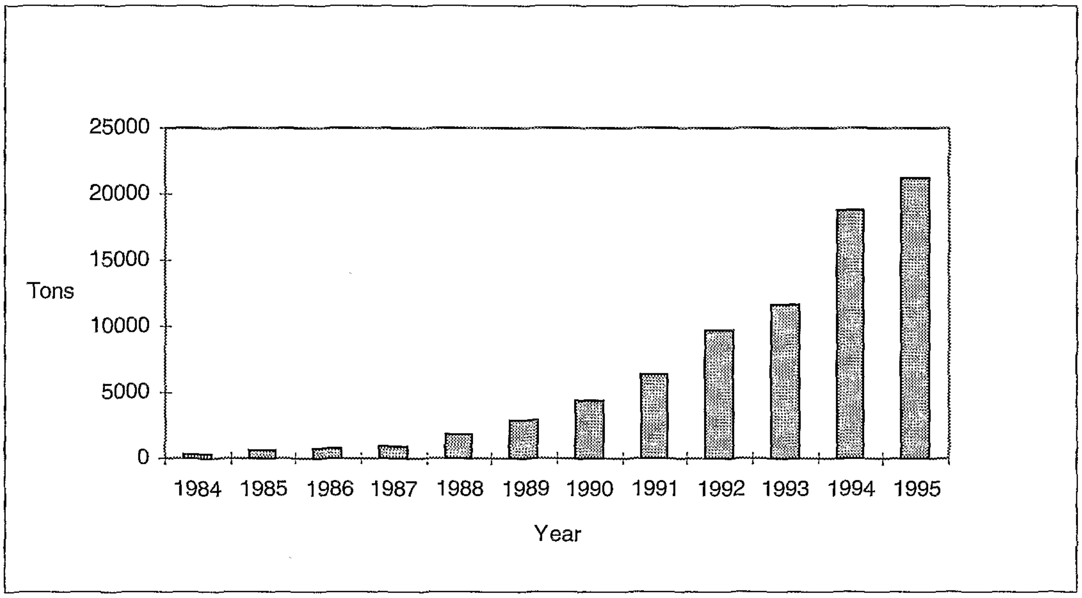

Fig.1. Aquaculture production in tons of *Sparus aurata*, in Mediterranean countries. Data from FAO.

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