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Genetic variations in survival of rohu carp (*Labeo rohita*, Hamilton) after *Aeromonas hydrophila* infection in challenge tests

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ABSTRACT

A challenge test against *Aeromonas hydrophila* was conducted using 2062 rohu carp (*Labeo rohita*) fingerlings obtained from 52 dams and 87 sires (87 full-sib families) of two year-classes (2003 and 2004). Attempts to establish a cohabitant challenge model were not successful. Therefore, fish were challenged by intraperitoneal injection with *A. hydrophila* in two replicate tanks per year-class, and dead fish were collected hourly. The mortality reached its peak at 16–22 h after challenge and had almost completely stopped after 58 h. The test was terminated after 382 h at which the average survival was 43.9 and 48.9% in the two 2003 year-class tanks, and 77.1 and 35.8% in the two 2004 year-class tanks. Heritability estimates for survival were obtained from sire and dam threshold models (THR) and sire and dam linear repeatability models (LINR). For both types of models the heritability estimates based on data from single tanks were not consistent. For both year-classes, data from one of the two challenged tanks demonstrated significant additive genetic variation in survival during the *A. hydrophila* infection, whereas the heritability estimates were not significantly different from zero for the other tank. Further, genetic correlation between survivals in the two replicate tanks in each year-class was not significantly different from zero. The differential results from the replicate tanks demonstrate that additional challenge test experiments are needed before firm conclusions can be drawn about the magnitude of additive genetic variation for survival to aeromonasis in rohu carp. A cohabitant challenge model that allows the testing of important defence mechanisms in the skin and mucous membranes of the fish might have been more appropriate. To establish a valid cohabitant challenge model for rohu carp should be given high priority.

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1. Introduction

The benefit of selective breeding has been amply demonstrated in several farmed fish species, but still only a small proportion of the aquaculture production is based on genetically improved populations; about 1% in 1997 and 5% in 2004 (Gjedrem, personal communication). Diseases pose a major threat to Indian aquaculture, but neither systematic eradication programmes nor efficient vaccines are yet available to minimize the occurrence of diseases. This leaves the use of antibiotics or chemotherapeutants as the only current option. An extensive and uncontrolled use of antibiotics is not desirable, as it may lead to development of antibiotic resistant bacteria. It also poses a significant risk to consumer's health through the potential transfer of resistance to human pathogens, antibiotic residues and/or chemical contaminants in marketed aquaculture products. Further, widespread use of antibiotics also places the production environment at risk (Sahoo and Mukherjee, 1999).

Fish vaccine design has been significantly improved in recent years, and vaccines are currently important tools to control diseases in many aquaculture productions. Antibiotic treatment and vaccination strategies, however, imply that one is still one step behind the disease problem. An alternative line of action is selection of brood fish from families that show the higher degree of resistance (e.g., measured as survival in challenge tests) to the disease or diseases in question. The approach of selective breeding for increased disease resistance offers possibilities to bring science and fish farmers ahead of the disease problem on a long-term basis. Several research workers have addressed this possibility over the years (Embody and Hayford, 1925; Ehlinger, 1964, 1977; Gjedrem and Aulstad, 1974; Refstie, 1982; Gjedrem, 1983; Bailey, 1986; Cipriano and Heartwell, 1986; Standal and Gjerde, 1987). But it was not until Gjedrem et al. (1991) reported substantial genetic variation in susceptibility to furunculosis (*Aeromonas salmonicida*) in Atlantic salmon from controlled challenge test experiments that it became feasible to

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implement the strategy in aquaculture. The prospect for significant improvements in disease resistance have been confirmed in a series of more recent studies in Atlantic salmon (Gjedrem and Gjøen, 1995; Ødegård et al., 2006), rainbow trout (Henryon et al., 2002, 2005), Atlantic cod (Kettunen and Fjalestad, 2006) and Pacific white shrimp (Argue et al., 2002; Gitterle et al., 2006). Thus, there is substantial evidence that resistance to many infectious diseases in aquaculture has significant genetic components. Experiments in livestock species have likewise demonstrated genetic differences in response to disease challenges (Bishop et al., 2003).

Geneticists and pathologists have shown interest in determining intrinsic resistance factors that protect the fish from various types of diseases. But, despite the efforts directed at studying such factors as candidate marker traits for resistance to a number of diseases (Refstie, 1982; Cipriano and Heartwell, 1986; Grinde et al., 1988; Røed et al., 1990, Fevolden et al., 1991; Røed et al., 1992; Salte et al., 1993), no factor has yet been identified that fulfils the requirements for a marker trait facilitating efficient indirect selection for improved disease resistance. This strongly suggests that whereas there is clearly a genetic component in the resistance of fish to disease the mechanisms by which a particular species and/or population can resist a pathogen are likely to be complex and multifactorial (Secombes and Oliver, 1997).

The above findings suggest that direct selection of the most resistant families based on challenge test survival data where all fish are subjected to infection with a specific pathogen in a common environment, is the best way to improve the innate disease resistance of farmed fish; large full- and half-sib groups of fish can be easily obtained allowing accurate ranking of the families even for traits with low heritability.

The present experiment was conducted to investigate the possibility of improving the innate resistance to *Aeromonas hydrophila* in rohu carp by selection based on challenge test survival data.

2. Materials and methods

2.1. Genetic material and production of families

The fingerlings used in this study were from year-classes 2003 and 2004 of the selective breeding program for rohu carp initiated at the Central Institute of Freshwater Aquaculture (CIFA), India in 1992 under an Indo-Norwegian collaboration between CIFA and Institute of Aquaculture Research (AKVAFORSK), Norway (Anonymous, 2003). The fish represented the fifth generation of selection for increased growth. The families of the two year-classes were produced in the month of July–August 2003 (year-class 2003) and 2004 (year-class 2004) using a nested mating design with two males nested within each female (producing full-sibs and maternal half-sibs). Dry stripping methodology was followed to obtain milt and eggs from the Ovaprim-injected breeders (Reddy et al., 2002). First, milt was obtained from all males and stored at 4 °C in different containers. Subsequently the ready females were stripped. The eggs from each female were divided into two portions, and each portion was fertilized with the milt from a different male. Mating of full-and/or half-sibs was avoided. After fertilization individual full-sib families were incubated in separate incubation jars. The eggs hatched between 18 and 24 h after fertilization and the resulting hatchlings of individual full-sib families were reared separately in $1\times1\times1.5$ m^3 hatching hapas. The number of dams and sires used to produce the experimental fish in each year-class are shown in Table 1.

2.2. Rearing and tagging

After approximately 72 h in the incubation jars and hatching hapas, spawn from individual full-sib families were collected and stocked in separate earthen nursery ponds (100 m^2), at a stocking density of 5000 spawn per pond. When the fish had attained a mean weight of about 44 g (2003 year-class after ~200 days of rearing), ~25 g (2004 year-class after 160 days of rearing) (Table 1), approximately 30 fingerlings from each full-sib family were collected randomly from the nursery ponds. After being kept overnight without any artificial feed, tagging was performed by injecting a Passive Integrated Transponder (PIT-tag) (11×2.1 mm) into the abdominal cavity of each MS-222 anaesthetized fingerling following the procedure described by Das Mahapatra et al. (2001). The tagged fingerlings were then monitored over night to observe any mortality associated with the tagging procedure and subsequently transferred to two outdoor $10\times5\times1$ m^3 cement cisterns where they were reared for about 30 days until the challenge test was initiated. The number of fish challenged in each year-class and tank are given in Table 1.

Table 1
Number of dams and sires, number of challenged offspring within tank, and mean and standard deviation of body weight at tagging

Year-class (Tank)	Number of			Body weight (g)	
	Dams	Sires	Offspring	Mean	SD
2003 (1)	22	40	426	44.7	38.1
2003 (2)	22	40	446	42.5	34.7
2004 (1)	30	47	607	25.1	26.4
2004 (2)	30	47	583	23.8	27.7

2.3. Challenge test

It was planned to use a cohabitant challenge model, i.e., challenge the experimental fish by adding cohabitants of the same size injected with *A. hydrophila* to the test tanks. However, attempts to establish a valid cohabitation challenge model were not successful; the cohabitants died within 48 h while no mortality occurred among the experimental fish. Consequently, it was decided to challenge the fish using i.p. injection.

The fingerlings in the two replicate tanks of each year-class were challenged with *A. hydrophila*; the 2003 year-class in month of May 2004 and the 2004 year-class in month of April 2005. For practical reasons the fish in the two replicate tanks of each year-class were challenged on two different, but subsequent days. Individual fish were injected intraperitoneally with a predefined LD_{50} dose of live cells of *A. hydrophila* (5×10^6 CFU/gram body weight) of a virulent strain of *A. hydrophila* obtained from the College of Fisheries, Mangalore, India. Bacteria were grown in tryptone soya broth at 30 °C for 20 h. The LD_{50} bacterial dose required was determined in separate experiments with a random sample of untagged fingerlings from the same families and of the same size as the experimental fish used in the challenge test experiments. Dead fish were collected hourly over a period of 382 h. Bacteria could be reisolated from the kidney of 10% of the dead fish as described by Kumari et al. (2003).

2.4. Statistical analysis

Survival during the challenge test was defined using two different trait definitions.

Binary test-period survival (TPS), which was scored 0 if the fish died during the challenge test period, and 1 if the fish survived. Generally, variance components of binary traits on the observable scale (as in a linear model) are highly frequency-dependent (Dempster and Lerner, 1950). However, using threshold models, variance components on the underlying liability scale are independent of the binary frequencies. As the survival rates differed between tanks (Fig. 1), a cross-sectional threshold model (THR, see Section 2.4.1) was chosen for the analysis of TPS.

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