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The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
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University of Hawaii Aquaculture Program in association with
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http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

Genetic Perspective on Stress Response and Disease Resistance in Aquaculture

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Key words: stress, immune system, QTL, gene expression

Abstract

Despite continuous progress and improvements in aquaculture technologies and husbandry techniques, fish diseases remain a major limiting factor in the fish culture industry. The aquaculture environment exposes fish to repeated acute stress, which leads to physiological responses that have suppressive effects on growth, reproduction, and immune capacity. The strong link between stress and susceptibility to diseases in farm animals has long been acknowledged, and parameters of high and low stress response are associated with disease resistance in fish. Few studies on genetic aspects of immune response to stress have been conducted. The estimated heritability of several parameters of the innate immune response is mostly moderate. Quantitative trait loci have been found for different responses to stress conditions. Gene expression studies show that hundreds of genes can be involved in fishes' physiological and immunological response to stress. This general response to stress is controlled by a few major genes at the top of the pathway, which activate a cascade of reactions, having a significant effect on the overall health of the fish. Selective breeding for disease resistant fish is an attractive strategy for disease prevention and several studies have reported progress in this field. However, the biological pathways of stress response and disease resistance are not well characterized, and their genetic basis and control are still poorly understood. Extensive research is still needed for a better understanding of these pathways, and this should be a collaborative effort of researchers from different fields: genetics, immunology, pathology, physiology, and endocrinology.

Introduction

It is widely accepted that stress is an important factor to be considered in successful fish husbandry. Indeed, stress has various effects on important traits in cultured fish, including metabolism, growth, social interactions, reproduction, and immunocompetence. Only a limited amount of research has been carried out on the genetic basis of stress response in fish. However, it is clear that stress response has a distinct genetic component and therefore may be modified by selective breeding. The objective of this paper is to review the current

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knowledge about the genetics of stress response and its relationship to disease resistance. The first part of the manuscript is an introduction to the biological basis of stress and stress response, with emphasis on the immune and endocrine systems. The second part is a review of genetic studies on stress response and their relevance to fish health and disease resistance. Finally, I discuss the current status and future directions in applying our knowledge on the genetics of stress response for selective breeding plans in aquaculture.

Stress in Fish and Aquaculture

Stress in fish. The definition of stress has been characterized by many controversies. Stress is usually defined as a condition in which the dynamic equilibrium of the organism, called homeostasis, is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli, commonly defined as stressors (Bonga, 1997). The fact that the concept of stress is widely accepted by cell biologists, physiologists, toxicologists, ethologists, and ecologists demonstrates its vitality. Stress represents different phenomena observed at the organizational levels of cells, organs, organisms, populations, and ecosystems on the basis of a unifying concept.

Physiological processes in fish are often carried out in a harsh aquatic environment, and fish face conditions and challenges that do not exist for terrestrial animals. Concentrations of gases in the water are highly variable and the water serves as both a respiratory medium as well as a sink for toxic metabolic waste products. Living in water requires the investment of a significant amount of energy to overcome drag while swimming, pumping water over the gills, and maintaining osmoregulation in a variety of tissues (blood, kidney, digestion system) that are constantly in osmotic disequilibrium with the aquatic environment (Wedemeyer, 1997).

As in other farm animal species, husbandry conditions are very important in fish production. Aquaculture production systems and stock management practices challenge fish with environmental factors differing from their natural habitats. Under these conditions, physiological challenges caused by water chemistry alterations, culture procedures, and behavioral interactions are added to the normal energy demands imposed by the aquatic environment, resulting in disturbance of the homeostasis of the fish. The maintenance of internal homeostatic equilibrium is essential for the normal function of the animal, and in case of disturbance the fish will try to establish a new equilibrium. However, the harmful effects of many of these stress-induced factors on the physiological condition significantly increase with even a moderate change in stress factors, such as a shift of a few degrees in water temperature or a reduction by a few mg per liter of dissolved oxygen (Pickering, 1993; Wedemeyer, 1997).

Lethal stress is rare in well-managed aquaculture systems. Chronic stress, eventually affecting health and physiological condition is the more common phenomenon. The fish's behavioral and physiological reactions to stressors are commonly called 'stress response'. Cultured fish, especially under intensive conditions, are in a prolonged stress situation and their physiological response under these circumstances affects energy-dependent activities such as growth, maturation, and disease resistance (Pickering, 1993).

Responses to stress have been classified as primary, secondary, and tertiary, according to the level of organization of the response (Barton and Iwama, 1991). Many of these responses are used as quantitative indicators of stress in fish. The primary response to stress is indicated by a rapid elevation of the concentrations of plasma catecholamines and corticosteroids. The variety of secondary physiological responses may be considered as metabolic (e.g., changes in concentrations of plasma glucose, lactic acid, liver and muscle glycogen, and in adenylate energy charge), hematological (e.g., changes in hematocrit, leukocrit, hemoglobin, blood clotting time, and numbers of erythrocytes, leukocytes, and thrombocytes), immunological (e.g., phagocytic activity, ratios of circulating B- and T-lymphocytes, antibody response to an antigen), hydromineralic (e.g., changes in plasma chloride, sodium, potassium concentrations, and osmolality), or structural (e.g., changes in renal cell size and nuclear diameter, gastric tissue morphology). Tertiary response is detected at the whole-animal level and is measured as changes in growth rate, metabolic rate, disease resistance, thermal and hypoxia tolerance, and reproductive capacity (Barton and Iwama, 1991). Most investigations with fish have focused on measuring the primary and secondary stress responses to a specific stress factor (Barton, 2002). It is important to realize that many environmental, non-stress factors act as modifiers affecting the magnitude of these physiological responses. These influences include acclimation temperature, salinity, water quality, and nutritional state (beyond a certain threshold these factors are also stressors).

The fish innate immune system. In fish, like other vertebrates, the immune response is a combination of two systems, the innate (non-specific) immune system and the acquired (specific) immune system. Both systems may be divided into two major pathways: cellular and humoral responses. The two systems are not separated; they interact and activate each other for an overall immune response of the organism (Ellis, 1999; Magnadottir, 2006).

The first defense line of fish against microorganisms is represented by the epithelia covering gills, skin, and gut. These cells secrete a layer of mucus that contains a variety of substances, mainly proteins and glycoproteins that nonspecifically inhibit the growth of infectious microorganisms. They are specific in that they react with just one chemical group or configuration, but the targeted substances are very common and influence the growth of a variety of microorganisms. When microorganisms penetrate the body, tissue macrophages, circulating monocytes, and granulocytes enter the infected area to attack the pathogens through phagocytosis, release of oxidative and lytic enzymes, and reactive oxygen species (respiratory burst) as nonspecific defense activities. Phagocytosis by macrophages of antigenic material can start the specific humoral and cellular immune response (Bayne and Gerwick, 2001; Magnadottir, 2006).

In spite of their importance in the innate defense, the biological properties of these immune-related substances are not fully known. Some of the better-characterized humoral defense factors are the complement system, interferon molecules, lysozyme, C-reactive protein, transferrin, and lectin (Yano, 1996; Ellis, 2001). A variety of leukocyte types is involved in non-specific cellular defenses of fish and includes macrophages, granulocytes, and non-specific cytotoxic cells. They can engulf bacteria and kill them by production of reactive oxygen species (ROS), which have bacteriocidal activity. These cells are found in the blood and lymphoid tissues and are particularly important in inflammation, which is the cellular response to microbial invasion. The lack of specificity is an important feature as it allows a large number of cells to be mobilized quickly. However, unlike specific defenses, there is no memory component, and thus there is no higher or faster response due to subsequent exposure to the same pathogen. Components of the non-specific immunity are able to interact with and be stimulated by the specific immune system (Secombes, 1996; Ellis, 1999).

Endocrinology of stress response. The immune and the neuroendocrine systems are tightly linked, with bi-directional communication, which is essential to maintain homeostasis. In bony fish, two major neuroendocrine pathways control the stress response. The first major pathway is the hypothalamus-sympathetic nerves-chromaffin cell (HSC) axis. It results in the release of catecholamines (adrenaline and noradernaline), primarily from cromaffin cells scattered throughout the kidney and the posterior cardinal vein to the general circulation, under conditions requiring enhanced oxygen transport and mobilization of energy substrates. The release of the catecholamines initiates a series of integrated responses, aiming to overpower the disruptive effects of acute stress on normal physiology. In particular, the catecholamines serve to optimize cardiovascular and respiratory functions while also mobilizing energy stores for the increased metabolic requirements associated with stress (Sumpter, 1997; Harris and Bird, 2000).

The second major pathway controlling the stress response in fish is the hypothalamus-pituitary-interrenal (HPI) axis. This pathway, which is a cascade of hormones that results in the release of the physiologically important hormone cortisol, is responsible for the physiological stress response. The first hormone in the cascade is corticotrophin-releasing hormone (CRH). The main role of CRH, at least in the HPI axis, appears to be regulation of the release of the adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. ACTH, like CRH, is synthesized as part of a larger precursor, termed proopiomelanocortin (POMC). It is the common precursor for ACTH, β-endorphin, α-melanophore-stimulating hormone (α-MSH), and other peptides. These peptides have a significant role in modulating immune responses in fish. ACTH is secreted rapidly in response to stress and acts on the interrenal tissue to stimulate the release of corticosteroids, particularly cortisol (Sumpter, 1997; Harris and Bird, 2000).

Immune response to stress. The effects of stress on the immune system are difficult to interpret simply in terms of catecholamine immunosuppression, as a number of different hormones are involved via the HPI axis, including CRH and ACTH, which in turn, affect the release of other hormones. Thus, it is likely that immunological responses to stress are dependent on the actions of various hormones and their interactions with each other and with immunocompetent cells as well as with other endogenous factors such as cytokines (Harris and Bird, 2000).

A wide array of influences cause stress situations. Natural defense against these insults is provided by the immune system, which is thus considered as part of the adaptive repertoire. Pathogens are prevented from penetrating the organism by a barrier or destructive effect of mucus, gastric, or intestinal secretions. When they do penetrate, pathogens may be inactivated by spontaneous or induced mechanisms (Chevassus and Dorson, 1990; Bayne and Gerwick, 2001; Magnadottir, 2006). The immune system responds to stress mainly through the nonspecific pathway, as only recognized specific pathogens can alter the acquired immunity. Nevertheless, stress influencing immune capacity and therefore stress response can be measured in both specific and non-specific pathways. In general, stress in fish causes a rapid increase in circulating neutrophils and a reduction in circulating lymphocytes levels. At least part of the reduction in circulating lymphocytes and macrophages may be caused by the extravasation of these cells and their penetration into the epithelia of gills, skin, and intestine of stressed fish. Both B and T lymphocyte functions are affected and this is frequently associated with decreased resistance to opportunistic pathogens, resulting in disease and mortality (Bonga, 1997).

Components of the nonspecific immune system are widely used as indicators for stress response in a variety of fish species, e.g., Atlantic salmon (*Salmo salar*; Fevolden et al., 1994), common carp (*Cyprinus carpio*; Yin et al., 1995), rainbow trout (*Oncorhynchus mykiss*; Fevolden et al., 1999), bass (*Morone* spp.; Noga et al., 1999), sea bream (*Sparus aurata*; Ortuno et al., 2001), tilapia (*Oreochromis* spp.; Cnaani et al., 2004a), and goldfish (*Carassius auratus*; Dror et al., 2006). These components include the complement pathway, lysozyme activity, respiratory burst activity, spontaneous hemolytic activity, ceruloplasmin activity, phagocytosis, number of circulating lymphocytes, and level of IgM (Rotllant et al., 1997; Palti et al., 1999; Sarder et al., 2001; Cnaani et al., 2004a). Along with the major role that innate immunity is thought to have in disease resistance of fish, these parameters are associated with disease resistance in several fish species (Wiegertjes et al., 1996; Balfry et al., 1997ab; Dror et al., 2006).

Genetics of Stress Response

Selective breeding for stress response. Considering the correlation that was found between stress, innate immunity, and disease resistance, and based on experience from other farmed animals, selective breeding for stress response as a method of improving disease resistance seems attractive. Several breeding plans used cortisol levels in response to stress as selection parameters. Two lines were selected in rainbow trout and Atlantic salmon for high stress and for low stress response (Fevolden et al., 1991). These lines were challenged with bacterial pathogens to evaluate their disease resistance.

The selected rainbow trout lines were challenged by *Aeromonas salmonicida* causing furunculosis and *Vibrio anguillarum* causing vibriosis. In the furunculosis challenge, the death rate in the high stress response line was higher than in the low stress response line, but the opposite was true for the vibriosis challenge (Fevolden et al., 1992). The selected Atlantic salmon lines were challenged by *A. salmonicida*, *V. anguillarum*, and *Renibacterium salmoninarum* causing bacterial kidney disease (BKD). While the low stress response line preformed better against furunculosis and vibriosis, there was no difference between the lines in the BKD challenge (Fevolden et al., 1993).

These lines were also tested for lysozyme levels in response to stress. Estimates of heritability of lysozyme and cortisol were moderate and both traits displayed positive genetic correlations in pair-wise comparisons of stress exposures. It was suggested that lysozyme is not directly correlated with resistance to the challenged pathogens but rather is another indication of stress response, though not superior to cortisol in consistency of stress response (Fevolden et al., 1994; Fevolden et al., 1999). To evaluate the possibility of using post-stress lysozyme activity as a selection criterion, rainbow trout progenies were selected for high and low post stress lysozyme activity (Fevolden et al., 2002). The low response line had higher resistance to furunculosis but there were no differences in a vibriosis challenge. After vaccination, the levels of antibodies were higher in the high response line though it seems that the association between lysozyme and disease resistance is complicated and influenced by the fish immune status (Roed et al., 2002).

Additional studies were conducted in different fish species that demonstrated the feasibility of selective breeding for high and low stress response. Rainbow trout were selected for confinement stress response as indicated in post stress cortisol and glucose levels (Pottinger and Carrick, 1999). Isogeneic lines of common carp were selected for high and low cortisol levels in response to cold stress. These lines were used to analyze the genetic basis of stress response in common carp and the genetic variation was estimated as mostly additive (Tanck et al., 2002). Progenies of sea bream from parents selected for high or low cortisol response to handling and confinement stress had divergent cortisol responses after stress similar to their parents (Rotllant et al., 2003).

Gene expression in response to stress. Microarray and real-time PCR technologies are rapidly becoming available in some fish species. However, they are still limited in use, mainly because relatively few genes have been adequately characterized in most of the commonly studied species.

The utility of microarray for studying the physiological response to stress in fish was demonstrated when analysis of hypoxia induced gene expression in gobies (*Gillichthys mirabilis*) revealed 126 distinct hypoxia-regulated cDNAs (Gracey et al., 2001). An extensive study of gene expression in response to cold stress in common carp revealed a large set of more than 3000 unique cDNAs affected by cold temperatures, about 2000 of them with homology to previously described genes (Gracey et al., 2004). However, in poikilothermic organisms such as fish, a large number of genes are affected by temperature changes as part of physiological thermal adaptation and are probably not stress related. In channel catfish (*Ictalurus punctatus*), 61 genes were differentially expressed in the brain in response to cold stress. These were mainly chaperones, transcription factors, and genes involved in signal transduction, lipid metabolism, and translational machinery (Ju et al., 2002).

In a study on gene expression in the brain and kidney of rainbow trout in response to handling stress, differences were found between organs in both function and time course. A total of 264 genes were differentially expressed in response to stress, 48 of which were identified as being regulated by stress in both tissues and in three time intervals (Krasnov et al., 2005).

Expression differences of the prolactin 1 (prl1) gene in tilapia were associated with a polymorphism of a microsatellite within its promoter and correlated with differences in growth rate in a high salinity challenge; higher expression of prolactin was correlated with slower growth in high salinity (Streelman and Kocher, 2002).

QTL for stress response. So far, only a few QTL studies were conducted in cultured fish species but the traits of interests were stress related in several of them. QTL studies aimed to detect loci associated with traits related to common stressors in commercial culture such as temperature tolerance and response to air exposure.

Studies on temperature tolerance were conducted on both cold and warm water species. In rainbow trout and Arctic charr (*Salvelinus alpinus*), QTL for upper thermal tolerance were detected (Jackson et al., 1998; Danzmann et al., 1999; Perry et al., 2001; Somorjai et al., 2003). In tilapia, QTL for cold tolerance were found (Cnaani et al., 2003; Moen et al., 2004). In all these studies, temperature tolerance was measured as survival time under a regime of temperature change, thus, the various physiological responses for thermal stress were measured as a single trait, namely the tertiary response to stress. Two QTL were found for upper temperature tolerance in the two salmonids and comparative analysis demonstrated that they are on homologous chromosomes in rainbow trout and Arctic charr (Somorjai et al., 2003). One QTL was found for cold tolerance in tilapia. The location of this QTL was consistent in two hybrid crosses, a two-species F_2 hybrid (Cnaani et al., 2003) and a four-species F_3 hybrid (Moen et al., 2004).

A genome scan was conducted in tilapia to search for QTL for innate immunity response to stress and blood biochemical parameters, both of which are indicators of fish health (Cnaani et al., 2004b). Unlike the thermal stress studies, the measured traits were the secondary responses to stress. Several QTL were detected, including one in the same chromosomal location as the tilapia cold tolerance QTL. Most of the traits in this study did not correlate with each other, however, all the QTL were associated with several traits. This may indicate that these are QTL for some regulatory genes in the upper level of the stress response biological pathway.

Discussion and Conclusion

The few studies in which selective breeding for stress response was implanted in fish demonstrate the feasibility of using the genetic variance of post-stress levels of cortisol as a selection criterion. These breeding plans were based on the hypothesis that reducing stress responsiveness will result in improved disease resistance. However, it has not yet been proven that there is genetic gain in any economically valued trait, or demonstrated that high stress response is a disadvantage and low stress response an advantage in respect to disease resistance (Pottinger and Carrick, 1999; Fevolden et al., 2002).

Stress response measurements were based on levels of cortisol in these breeding programs, but it might be better to characterize the stress response with an index of immunological parameters, thus obtaining a better estimation of the immunocompetence state in response to stress, a trait likely to be associated with disease resistance. The relationship between stress, the innate immunity response, and disease resistance has been little studied. We should explore the genetic variance of stress response in specific pathways of the innate immunity system, as we should be interested in differences in immunological activity, not only alteration of the immune system. Further research is needed to develop a method to evaluate fish immunocompetence in regard to innate immunity and its effect on susceptibility to pathogens. It seems likely that the immune state of the fish affects overall susceptibility. Therefore, a different pathogen challenge should be developed. Such a challenge should simulate the natural conditions of pathogen invasion and disease outbreaks rather than directly injecting bacteria and bypassing some of the defensive mechanisms of the fish.

Research on biological pathways that are altered in response to stress has increased our knowledge and understanding of the complex interactions and feedback systems involved in the endocrinological control of stress response. The initial secretion of a few stress related hormones causes activation of hundreds of genes that are involved in the fish response to stress, as demonstrated in gene expression studies and expected from the variety of responses observed after stress in several tissues and organs. The QTL study in which loci were found to be associated with several unrelated traits also supports the idea that a few genes at the top of the cascade control a variety of responses (Cnaani et al., 2004b). It is generally accepted that all these responses are mediated by cortisol. Thus, selecting for stress response as reflected by cortisol levels can affect a variety of traits that are unrelated to the selective breeding goal and have unpredictable influences on other economically important traits. The genetic variance of stress related traits should be studied under different conditions of fish culturing, as alleles that can be advantageous under acute stress conditions might be disadvantageous under normal or chronic stress conditions.

Studies aimed at decreasing disease susceptibility in response to the stressful conditions of aquaculture practices will benefit from collaborative efforts of different fields of research such endocrinology (to characterize the hormonal control of stress response), immunology (to study the immune response), pathology and veterinary medicine (to analyze disease infections), physiology (to characterize stressors and their effect on the homeostasis of fish), and genetics (to explore genetic variance and the potential for applying selective breeding for these traits).

Acknowledgements

I would like to thank Prof. T.D. Kocher for his support, advice, and useful comments. Avner Cnaani was supported by a Vaadia-BARD post-doctoral fellowship award from the US-Israel Binational Agricultural Research and Development Fund (no. FI-338-2003).

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