AMER. ZOOL., 39:901–909 (1999)

Heat Shock Proteins and Physiological Stress in Fish[1]

GEORGE K. IWAMA,[2] MATHILAKATH M. VIJAYAN,[3] ROB B. FORSYTH, AND PAIGE A. ACKERMAN

Faculty of Agricultural Sciences and the Canadian Bacterial Diseases Network, 208-2357 Main Mall, Vancouver, British Columbia V6T 1Z4 Canada

SYNOPSIS. **This paper reviews the generalized stress response in fish at the cellular and neuroendocrine levels. The focus of this review is to examine the possible relationships between the stress responses at these two levels in fish. It focuses primarily on the heat shock protein 70 (hsp70). Thus, the descriptions of the endocrine and the cellular stress responses are followed by a discussion of how hsps may be related to the stress hormones adrenaline and cortisol. Preliminary evidence shows that adrenaline causes an increase in hsp70 in primary cultures of rainbow trout hepatocytes. Cortisol does not directly affect hsp70 levels in fish tissues; however, in primary cultures of trout hepatocytes, cortisol decreased the stressor-induced increase in hsp 70. A wide range of abiotic and biological stressors have been shown to induce hsp induction in many types of fish cells, including cell lines, primary cell cultures, and in tissues from whole animals. Heat shock proteins has been implicated in the protection of sulphate transport in the renal epithelium of the flounder against the damaging effects of heat stress. Heat shock proteins likely confer thermotolerance in fish, as well as tolerance to cytotoxic effects of environmental contaminants and other non-thermal stressors.**

INTRODUCTION

Fish are exposed to biological and abiotic stressors in the wild, as well as in captivity. Environmental pollutants, disease, and various aspects of intensive aquaculture are some examples of those stressors. Fish also can become physiologically stressed from psychological stressors such as exposure to predators and crowding. Like other vertebrates, stressed fish exhibit a generalized stress response, that is characterized by an increase in stress hormones and the consequent changes at the physiological, organismal and population levels (see Wendelaar Bonga, 1997; Barton, 1997). Such a generalized stress response also occurs at the cellular level and has been called the cellular stress response (see Hightower, 1991). This paper reviews the generalized stress response in fish from the cellular to the organismal levels. It focuses on the question of whether the cellular stress response is related to the neuroendocrine stress response at the organismal level.

At a very broad level, most investigators subject their biological subjects to specific stressors. This paper, however, discusses the response of cells, tissues and the whole organism that is common to a wide range of stressors. There are many definitions of such a generalized stress response in fish (see Barton, 1997). Although many definitions are restricted to considering only the maladaptive and negative aspects of stress, we must also consider the adaptive aspects of the stress response. The stress response to any exogenous or endogenous perturbation, from the behavioural to the molecular levels, usually works to reestablish homeostasis. The net outcome of that interaction between the stressor and the stress response of the animal determines the success or failure in reestablishing cellular and physiological conditions within the normal range for that organism. The definitions of *stressor* as the causative factor, and *stress* as the response of the animal apply to the whole animal, as well as to each cell in the organism.

The cellular stress response has been described in nearly all cells studied to date.

[1] From the Symposium *Organismal, Ecological and Evolutionary Significance of Heat Shock Proteins and the Heat Shock Response* presented at the Annual Meeting of the Society for Comparative and Integrative Biology, 6–10 January 1999, at Denver, Colorado.

[2] E-mail: giwama@interchange.ubc.ca

[3] Present Address: Department of Biology, University of Waterloo, Waterloo, Ontario, Canada.

One of the most common features of the cellular stress response is the production of heat shock proteins (hsps) in response to stressors that threaten the life of the cell; which is dependent upon the maintenance of protein integrity and function. Although many investigators have described the cellular stress response in different cell types, there is relatively little work on fish cells and especially on whole organisms (see Iwama *et al.,* 1998). Thus, while it may seem intuitive that there should be a simple and direct connection between the organismal and cellular stress responses, there is little direct experimental evidence for this. The emerging evidence supports a rather complex relationship between stress hormones and hsps.

The paper begins with a description of the physiological stress response in fish. It is then followed by a discussion of the cellular stress response (primarily the hsp70) in fish and how the neuroendocrine and cellular stress responses may be related. We conclude with a brief discussion of the possible physiological roles for hsps in fish.

STRESSORS

The potential stressors to fish discussed here are grouped as being environmental, physical, or biological. They are presented here as a selection of examples in each category and by no means represent a complete or extensive list of published stressors, as reported by Barton and Iwama (1991). Many stressors are unique to certain species or geographic areas. Furthermore, the stress response in fish has a genetic component (see Pottinger and Pickering, 1997). Thus, there are differences in the generalized stress response among different fish species, and different stocks or races of the same species differ in their tolerance to applied stressors. The observed stress response is therefore an expression of both genetic and environmental factors such as season, rearing history, and nutritional state (see Iwama *et al.,* 1992). *Environmental stressors* mainly include adverse physical and chemical conditions of the water. Extreme conditions or changes in water quality such as dissolved oxygen, ammonia, hardness, pH, gas content, partial pressures, and temperature can stress fish. Metals (*e.g.,* copper, cadmium, zinc, and iron) and other contaminants (*e.g.,* arsenic, chlorine, cyanide, various phenols, and polychlorinated biphenyls) in the water can cause severe stress and death in fish. Other potential environmental stressors include insecticides, herbicides, fungicides, and defoliants. Industrial, domestic, and agricultural activities add much of these contaminants to the environment that affect fish at all life stages. Natural changes in water quality, as occurs during low tide in tidepools, may stress the organisms that live in such environments. *Pathophysiological stressors* encompass a wide range of potential stressors including those that disturb the fish physically and psychologically. Handling, crowding, confinement, transport, or other forms of physical disturbance to fish also have psychological components. Many of these are practiced in the intensive culture of fish for both wild stock enhancement, and for the commercial production of food. Chasing fish to exhaustion, or holding them in a net out of water for 30–60 sec have been common protocols utilized to study acute stress responses in fish. Angling also stresses fish in this manner. Other psychological stressors can be manifest in dominance hierarchies, which develop between individuals within confines such as experimental tanks or possibly in natural environments. Pathogens and parasites can also be considered as biological stressors. Fish disease, and outbreaks leading to massive mortalities, occur in nature as well as in cultured stocks. Plankton blooms can stress and kill fish in the wild as well as in aquaculture facilities. The plankton may kill the fish directly by their toxins; irritate or severely damage the gill epithelium with their spines; or kill the fish indirectly by hypoxic conditions by either lowering water oxygen levels directly or by increasing the diffusion distance between blood and water through the stimulation of mucus production on the gill surface.

THE GENERALIZED STRESS RESPONSE

In response to a stressor such as one of those mentioned above, fish undergo a series of biochemical and physiological

changes in an attempt to cope with the challenges imposed upon them. The stress response in fish has been broadly categorized into the primary, secondary and tertiary responses (Barton, 1997; Wendelaar Bonga, 1997).

The *primary response* represents the perception of an altered state and initiates a neuroendocrine/endocrine response that forms part of the generalized stress response in fish. This response includes the rapid release of stress hormones, catecholamines and cortisol, into the circulation. Adrenaline is released from the chromaffin tissue in the head kidney of teleosts, and also from the endings of adrenergic nerves (see Randall and Perry, 1992). Cortisol is released from the interrenal tissue, located in the head kidney, in response to several pituitary hormones, but most potently to adrenocorticotropic hormone (ACTH) (see Balm *et al.,* 1994). The resting and stressed levels of adrenaline and cortisol concentrations in the plasma of salmonids are: adrenaline, <3 and 20–70 (nmoles/L) and cortisol, <10 and 40–200 (ng/mL), respectively. A recent study showed that ACTH may also stimulate adrenaline release, and that chronic cortisol treatment may affect catecholamine storage and release in trout (Reid *et al.,* 1996). As both the chromaffin tissue and the interrenal tissue lie in close proximity in fish, there is a possibility that a paracrine control for stress hormone regulation exists in fish (Reid *et al.,* 1996). Environmental contaminants can modulate the primary stress response. Wilson *et al.* (1998) recently showed that beta-naphthoflavone (a potent inducer of cytochrome P450 1A) completely abolished the sensitivity of the interrenal cells to ACTH stimulation.

The *seconday response* comprises the various biochemical and physiological adjustments associated with stress, and is mediated to some extent by the stress hormones. Adrenaline and cortisol activate a number of metabolic pathways that result in alterations in blood chemistry and haematology (see Barton and Iwama, 1991). Stress is an energy demanding process and the animal mobilizes energy substrates to cope with stress metabolically (Barton and Schreck, 1987; Vijayan *et al.,* 1997). The production of glucose with stress assists the animal by providing energy substrates to tissues such as the brain, gills, and muscles, in order to cope with the increased energy demand. The stress hormones adrenaline and cortisol have been shown to increase glucose production in fish, by both gluconeogenesis and glycogenolysis, and likely play an important role in the stress-associated increase in plasma glucose concentration. The rearing history of the fish, including nutritional state, can affect the stress response and glucose clearance rates (Vijayan and Moon, 1992, 1994). Thus, plasma glucose levels may or may not remain elevated despite the continued presence of the stressor. The metabolic aspects of the stress response are discussed in more detail by Iwama *et al.* (1999) and Barton (1997).

The *tertiary response* represents whole animal and population level changes associated with stress. If the fish is unable to acclimate or adapt to the stress, whole animal changes may occur as a result of energy-repartitioning by diverting energy substrates to cope with the enhanced energy demand and away from anabolic activity such as growth and reproduction. Thus, long-term exposure to a stressor, depending on the intensity and duration, can lead to decreased growth, disease resistance, reproductive success, smolting, and swimming performance. At a population level, decreased recruitment and productivity may alter community species abundance and diversity (Barton, 1997).

HEAT SHOCK PROTEINS

A general introduction to hsps and the cellular stress response are given in Chapter 1 of this volume. This cellular stress response, as well as the amino acid sequence identity for any particular hsp group (*e.g.,* hsp70), are both highly conserved across diverse phyla (see Welch, 1993; Hightower, 1991). There is a constitutive (hsc) production of these proteins in the unstressed state. Iwama *et al.* (1998) recently reviewed the subject of hsp expression in fish, and the following summarizes a part of that more extensive discussion.

Fish cells are no exception to the cellular stress response and the expression of various stress proteins has been reported in cell lines, primary cultures of cells, as well as in various tissues from whole animals. The vast majority of studies have focused on various effects of heat shock, but recent studies have shown that hsps levels increase in fish tissues in response to a variety of environmental and biological stressors. Research in this area is very much at a descriptive phase. Emerging evidence, such as that of Deane *et al.* (1999), supports the possibility that the cellular and neuroendocrine stress responses are related in the intact organism, but an unequivocal causal relationship has not been established.

The use of *cell lines* to study hsp expression has the convenient features of simple maintenance, uniformity, and abundance of the cells. This allows for detailed studies involving many different experimental treatments to be conducted quickly, in contrast to studies with whole animals. Such studies have enabled the separation of various isoforms within families or groups of induced hsps; the description of the time course of novel protein induction; and various studies of the function of hsps in the cell. Comprehensive studies such as those on the chinook salmon embryonic cell line (CHSE-214; Heikkila *et al.,* 1982; Gedamu *et al.,* 1983; Misra *et al.,* 1989), as well as those on the rainbow trout gonadal cell line (RTG-2; Mosser and Bols, 1988; Kothary and Candido, 1982; Kothary *et al.,* 1984*a,b*; Mosser *et al.,* 1986; Burgess, 1984), and on the rainbow trout hepatoma cell line (RTH-149; Misra *et al.,* 1989; Heikkila *et al.,* 1982) have all shown increased expression of various hsps (*e.g.,* 28, 41, 46, 51, 65, 68, 70, and 84 kDa) in response to heat shock and metal exposure. Cho *et al.* (1997) reported a novel 90 kDa protein, that was different from hsp90, to increase in the cell line CHSE-214 in response to the infectious haematopoietic necrosis virus. Slight differences in the molecular masses of individual hsps may well be attributable to differences in techniques, such as in the experimental set-up, isolation of tissues, and the molecular weight markers used in each study. Such factors may also be particularly important for various temporal factors such as time taken to show significant increases in hsps or maximum increase in concentration, or the time taken to return to control levels. Focusing on classes of hsps, rather than on those minor differences in individual proteins, may be more meaningful in gaining an understanding about the effects of specific stressors on hsps in a particular cell line.

Most of the above studies report that the increase in hsp70 is the most prominent response to heat shock. In contrast to such a generalized response, Misra *et al.* (1989) found the metallothionein protein to increase only in response to metal stress and only in CHSE. Studies with transcription blockers (*e.g.,* actinomycin D) have suggested that the transcription step of protein synthesis plays a major role in the regulation of which hsps are produced in response to a given stressor (Heikkila *et al.,* 1982; Currie and Tufts, 1997). Airaksinnen *et al.* (1998) have recently provided further evidence from primary cultures of rainbow trout hepatocytes and gill epithelial cells, as well as the RTG-2 cell line that the transcriptional step is involved in the regulation of hsp70 levels in those fish cells. There is one report of cold shock inducing a 70 kDa protein in the RTG-2 cell line (Yamashita *et al.,* 1996). That protein was different from hsp70, but more closely related in some residues to proteins associated with cell division. Yamashita *et al.* (1996), therefore, suggested that the response was a metabolic compensation to the cold-induced reduction in cell division. There is a pattern for larger hsps (*e.g.,* 60, 70, 90 kDa) to be more highly conserved between organisms of different taxa. Smaller hsps (*e.g.,* 16–30 kDa) may be more species-specific, and as such may offer reasonable potential for diagnostic purposes among species, whereas the larger hsps may serve as indicators of non-specific stressors in a wide range of organisms.

A number of studies with fish cells in *primary culture* have been valuable in providing information that is perhaps closer to the in-vivo condition because such cells maintain their differentiated characteristics. The work of Brown *et al.* (1992), Renfro *et*

al. (1993), and Sussman-Turner and Renfro (1995) using the primary culture of renal proximal tubule cells of the winter flounder, *Pleuronectes americanus,* have shown both mild heat stress (+5°C) and zinc to abolish the negative effects of severe heat shock (+10°C) or 0.5 mM 2,4-dichlorophenoxyacetic acid on sulphate transport across that epithelium. Since several hsps (28, 68–70, and 90 kDa) were induced with the heat shock, and blockage of protein synthesis by cyclohexamide abolished the protective effect, those hsps may be vital to the transport functions of that renal epithelium. The use of primary cultures of hepatocytes from catfish, *Ictalurus punctatus,* (Koban *et al.,* 1991), rainbow trout, *Oncorhynchus mykiss* (M. M. Vijayan, C. Pereira and G. K. Iwama, in preparation), and desert topminnow, Poeciliopsis spp., (White *et al.,* 1994; Norris *et al.,* 1995) have shown the induction of a number of hsps (*e.g.,* 30kDa, 60kDa, 70kDa class, 90kDa class, and 100kDa) in response to heat shock and various environmental contaminants. The studies on the topminnows have shown that the constitutive hsc70 was identical among various species of this genus, while the heat-inducible hsp70 had significant polymorphism among species. They concluded that a high hsc70 content and the ability to quickly increase the synthesis of hsp70 were vital to thermotolerance in that genus. While primary cell cultures may be closer to the in-vivo condition, relative to cell lines, there are some discrepancies between results from primary culture studies and in-vivo experiments. Koban *et al.* (1991), for example, found that the acclimation temperature of catfish hepatocytes did not affect the hsp induction temperature. However, Dietz and Somero (1992) showed in whole goby fish (genus *Gillichthys*), that the acclimation temperature did affect the induction temperature of a 90 kDa hsp; this showed that the hsp induction temperature was not strictly determined by genetic code, but could be modified by environmental factors.

There are several studies of hsp expression in *intact fish.* The extensive range of species, tissues, and stressors, as well as the hsp responses precludes a detailed description here. Studies in a number of fish species (Koban *et al.,* 1991; Dyer *et al.,* 1991; Dietz and Somero, 1992, 1993; Mazur, 1996; Forsyth *et al.,* 1996; Koban *et al.,* 1991; Kikuchi *et al.,* 1993; and Vijayan *et al.,* 1997*b*; 1998) have shown hsp levels of various classes (*e.g.,* small hsps, 60 kDa, 70kDa, 90kDa) increased in a wide range of tissues in response to stressors such as heat shock, environmental contaminants, and bacterial disease (see Iwama *et al.,* 1998). Liver, kidney and gill tissues seem to be sensitive tissues to the hsp response. It is also noteworthy, from the perspective of the potential use of this response as a biomarker of environmental quality, that handling stress does not elicit hsp70 expression (Vijayan *et al.,* 1997*b*). The potential artifact that handling and sampling procedures can induce in stress studies is a constant and significant problem. Vijayan *et al.* (1998) showed that the induction of hsp70 in the liver of rainbow trout exposed to two toxicants occurred at toxicant concentrations several-fold lower than the LC_{50} value (lethal concentration to 50% of the population in 96h; a standard index of toxicity). They concluded that changes in hsp may be a sensitive indicator of stressed states in fish. The study of Forsyth et al. (1996) was the first to document an hsp response to a fish disease (*Renibacterium salmoninarum* in coho salmon, *Oncorhynchus kisutch*).

THE GENERALIZED STRESS RESPONSE AND HSPS

The relationship between the cellular and neuroendocrine stress responses in tissues of intact animals is important in several regards. Whether it is the study of the physiological and ecological significance of hsp expression in fish in natural environments, or the development of hsp-based probes for determining stressed states in fish, elucidation of the relationship between the stress hormones and hsps is an important area of research. Most of the evidence about how the generalized stress response, as discussed, and hsp expression may be related comes from studies on mammals, but some evidence is available for fish.

The interaction of hsp70 and hsp90, with

the steroid receptor has been characterized using cell line models (see Bohen and Yamamoto, 1994). A number of studies on heat stressed and restraint-stressed rats have shown that various components of a state of physiological stress can act as stressors and do elicit hsps. Blake *et al.* (1990) demonstrated hsp gene expression in rats exposed to heat shock. Blake *et al.* (1991) and Udelsman *et al.* (1993) have shown the expression of hsp70 after 3h to 6h of restraint stress in adrenal cortical tissue and thoracic aorta tissue of rats. The observation that hypophysectomized rats did not show the hsp-gene expression in response to restraint stress, and that addition of ACTH to those rats induced hsp70 expression in the adrenals, (Blake *et al.,* 1991) indeed supports the possibility that a functional relationship between hsp expression and the hypothalamus-pituitary-adrenal axis exists. Udelsman *et al.* (1994*b*) have shown an α_1-mediated adrenaline effect on hsp70 expression in aortic tissue of rats. Udelsman *et al.* (1994*a*) showed that the glucocorticoid dexamethasone attenuated the induction of hsp70 mRNA expression in the adrenal, but not in the aortic tissue of rats undergoing restraint stress. Thus, such responses can be tissue specific.

Until recently, there has been no work relating stress hormone levels and cellular hsp levels in fish. Vijayan *et al.* (1997*b*) showed that physical handling, which caused an increase in circulating cortisol levels, did not affect liver hsp70 levels, or affect the β-naphthoflavone (BNF)-induced hsp70 increase in rainbow trout. Most recently, Deane *et al.* (1999) also found that daily intraperitoneal injections of 4ug/g cortisol did not affect either hsp70 mRNA or hsp70 levels in the liver tissue of the silver sea bream (*Sparus sarba*). Deane *et al.* (1999) also observed that similar injections with 1 ug/g recombinant bream growth hormone, and in another group 6 ug/g ovine prolactin, both caused decreases in both hepatic hsp70 mRNA (42% and 54%, respectively) and hsp70 (76% and 64%, respectively) of the sea bream. However, the potential state of physiological stress in their fish, due to the daily handling, was not assessed (*e.g.,* through the measurement of plasma cortisol levels in control and treatment fish). In contrast to the above findings, cortisol significantly decreased the hsp 70 induction associated with temperature or BNF exposure in primary cultures of trout hepatocytes (M. M. Vijayan, C. Pereira and G. K. Iwama, in preparation). It is theoretically possible that higher levels of circulating cortisol could inhibit hsp70 synthesis by binding with cortisol receptors in the cytosol. Glucocorticoid receptor complexes contain hsp70 which would be released upon binding with cortisol (see Pratt, 1993). Increased levels of free hsp70 as a result may inhibit the likelihood of HSF1 trimerization and subsequent hsp70 expression through: HSF1 binding to the promoter region of the hsp70 genes; stressor-induced activation of HSF1 through phosphorylation; and hsp70 transcription (see Morimoto *et al.,* 1996). The relative quantities of cellular glucocorticoid receptor complexes relative to the total hsp70 pool, would likely vary from tissue to tissue, and among different species. This theoretical speculation, however, has no supporting experimental evidence, and warrants further research.

We are clearly at the very early stages of understanding what seem to be complex interactions among stressors, stress hormones, and hsp expression in fish. Elucidation of the various signal pathways that regulate hsp production and degradation will contribute significantly in this regard.

Physiological Role of Hsps in Fish

Some of the vital roles that hsps play in the cell include the maintenance of protein integrity, preventing premature folding and aggregation of proteins, protein translocation, and mediating steroid and receptor binding (see Chapter 1 of this volume). Although these intracellular roles have been clearly shown for the cell, the body of knowledge concerning the physiological and ecological importance of hsps to the survival of fish and other animals is relatively small. There are data showing the increase in hsps in various tissues of many different fish species subjected to stressors. However, few studies clearly show the physiological significance of changes in hsp concentration in various cells. The imposed

degree of the stressor, in many cases, is beyond that which the animal would encounter in nature. Most of the data from studies where the animals are exposed to stressors within an ecologically normal range, are correlative. Thus, one can only imply from such data that the increase in hsps must be playing some role in enhancing the survival of the stressed fish. While most of these studies have involved temperature as a stressor, many studies show that stressors other than heat shock can increase hsp concentration. Several studies show that hsp induction can increase tolerance to subsequent stressors. A good example is the work showing that hsp28, hsp70, and hsp90 induction in the renal epithelium (in primary culture) of the white flounder protects the cells against the damaging effects of severe heat and 2,4-dichlorophenoxyacetic acid on sulphate transport (Brown *et al.,* 1992; Renfro *et al.,* 1993; Sussman-Turner and Renfro, 1995).

Stress-induced increases in hsps in fish may occur in a threshold manner, rather than in a graded way dependent on the degree of the stressor. Evidence for this comes from studies using the primary culture of hepatocytes from salmonids exposed to various environmental contaminants (Vijayan *et al.,* 1998), as well as from the study of Currie and Tufts (1997) on rainbow trout erythrocytes exposed to heat shock in tonometers.

Stress-induced increases in hsps in fish may occur in a threshold manner, rather than in a graded way dependent on the degree of the stressor. Evidence for this comes from studies using the primary culture of hepatocytes from salmonids exposed to various environmental contaminants (Vijayan *et al.,* 1998), as well as from the study of Currie and Tufts (1997) on rainbow trout erythrocytes exposed to heat shock in tonometers. However, those studies were not designed to specifically investigate that response.

The studies on the desert topminnows by White *et al.* (1994) and Norris *et al.* (1995) suggest that the combination of high hsc70 and the ability to quickly induce hsp70 expression are vital to the tolerance of high temperatures in the studied species of that genus. Although there are many studies showing a correlation between the induction temperatures for hsps and various aspects of thermal tolerance or thermal preference in fish, none of them unequivocally show that hsps cause the observed thermotolerance or influence geographic distribution.

CONCLUDING REMARKS

Like all other organisms, fish face the challenges of maintaining a constant "milieu intérieur" in the face of environmental change, at all levels of biological organization. The generalized stress response at the cellular level as well as at the whole animal level share some common properties, and comprise the attempt to maintain and reestablish homeostasis in the face of any stressor. A wide range of abiotic and biological stressors elicits these responses. They both have genetic components but are modified by environmental factors. Current evidence points to a complex relationship between the cellular and neuroendocrine stress responses in fish, as is the case for mammals. While there are similarities between the cellular stress responses of fish and other vertebrates, there are notable differences that have to be reconciled. Why does restraint stress increase hsp levels in mice, but handling stress does not cause a change in fish tissues? Clearly, further research is needed to study the nature of the relationship between these two stress responses as well as between vertebrate groups.

There are many possible applications of measuring the stress response in fishes, and other aquatic organisms. They range from being able to resolve the generalized stress response in our experimental animals, separate from treatment-specific effects, to the monitoring of the quality of the aquatic environment through the stressed states of the organisms that live there. However, these applications can only be developed if there is unequivocal evidence for a relationship between the stressed state of the animal and the cellular stress response. The fact that stressors cause the induction of specific proteins offers the possibility of developing diagnostic probes for monitoring the con-

dition of fish and their environment. The evidence showing that increased levels of hsps induce tolerance of cells, tissues, and whole fish to subsequent stressors suggests that it may be possible to develop strategies to enhance tolerance to stressors by inducing the cellular stress response.

REFERENCES

Airaksinnen, S., C. M. I. Råbergh, L. Sistonen, and M. Nikinmaa. 1998. Effects of heat shock and hypoxia on protein synthesis in rainbow trout (*Oncorhynchus mykiss*) cells. J. Exp. Biol. 201:2543–2551.

Balm, P. H. M., P. Pepels, S. Helfrich, M. L. M. Hovens, and S. E. Wendelaar Bonga. 1994. Adrenocorticotropic hormone in relation to interrenal function during stress in tilapia (*Oreochromis mossambicus*). Gen. Comp. Endocrinol. 96:347–360.

Barton, B. A. 1997. Stress in finfish: Past, present, and future—a historical perspective. *In* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck (eds.), *Fish stress and health in aquaculture,* pp. 1–34. Cambridge University Press, Cambridge.

Barton, B. A. and G. K. Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. of Fish Diseases. 1:3–26.

Blake, M. J., R. Udelsman, G. J. Feulner, D. D. Norton, and N. J. Holbrook. 1991. Stress-induced HSP70 expression in adrenal cortex: a glucocorticoid-sensitive, age-dependent response. Proc. Natn. Acad. Sci. U.S.A. 88:9873–9877.

Blake, M. J., T. S. Nowak, and N. J. Holbrook. 1990. In vivo hyperthermia induces expression of HSP70 mRNA in brain regions controlling the neuroendocrine response to stress. Mol. Brain Res. 8:89–92.

Bohen, S. P. and K. R. Yamamoto. 1994. Modulation of steroid receptor signal transduction by heat shock proteins. *In* R. I. Morimoto, A. Tissieres, and C. Georgopoulos, (eds.), *The biology of heat shock proteins and molecular chaperones,* Cold Spring Harbor Monograph Series, Vol. 26, pp. 313–334. Cold Spring Harbor Laboratory Press, Plainview, N.Y.

Brown, M. A., R. P. Upender, L. E. Hightower, and J. L. Renfro. 1992. Thermoprotection of a functional epithelium: heat stress effects on transepithelial transport by flounder renal tubule in primary monolayer culture. Proc. Natl. Acad. Sci. U.S.A. 89:3246–3250.

Cho, W., S. Cha, J. Do, J. Choi, J. Lee, C. Jeong, K. Cho, W. Choi, H. Kang, H. Kim, and J. Park. 1997. A novel 90-kDa stress protein induced in fish cells by fish rhabdovirus infection. Biochem. Biophys. Res. Comm. 233:316–319.

Currie, S. and B. L. Tufts. 1997. Synthesis of stress protein 70 (Hsp70) in rainbow trout (*Oncorhynchus mykiss*) red blood cells. J. Exp. Biol. 200: 607–614.

Deane, E. E., S. P. Kelly, C. K. M. Lo, and N. Y. S. Woo. 1999. Effects of GH, prolactin and cortisol on hepatic heat shock protein 70 expression in a marine teleost *Sparus sarba.* J. of Endocrinology. 161:413–421.

Dietz, T. J. and G. N. Somero. 1992. The threshold induction temperature of the 90 kDa heat shock protein is subject to acclimatization in eurythermal goby fishes (genus *Gillichthys*). Proc. Natl. Acad. Sci. U.S.A. 89:3389–3393.

Dietz, T. J. and G. N. Somero. 1993. Species- and tissue-specific synthesis patterns for heat-shock proteins HSP 70 and HSP 90 in several marine teleost fishes. Physiol. Zool. 66:863–880.

Dyer, S. D., K. L. Dickson, and E. G. Zimmerman. 1991. Tissue-specific patterns of synthesis of heat-shock proteins and thermal tolerance of the fathead minnow (*Pimephales promelas.*) Can. J. Zool. 69:2021–2027.

Forsyth, R. B., E. P. M. Candido, S. L. Babich, and G. K. Iwama. 1996. Stress protein expression in coho salmon with bacterial kidney disease. J. Aquatic Animal Health 9:18–25.

Gedamu, L., B. Culham, and J. J. Heikkila. 1983. Analysis of the temperature-dependent temporal pattern of heat-shock protein synthesis in fish cells. Biosci. Rep. 3:647–658.

Heikkila, J. J., G. A. Schultz, K. Latrou, and L. Gedamu. 1982. Expression of a set of fish genes following heat or metal ion exposure. J. Biol. Chem. 257:12000–12005.

Hightower, L. E. 1991. Heat shock, stress proteins, chaperones and proteotoxicity. Cell 66:191–197.

Iwama, G. K., P. Thomas, M. M. Vijayan, and R. Forsyth. 1998. Stress protein expression in fish. Reviews in Fish Biology and Fisheries. 8:35–56.

Iwama, G. K., M. M. Vijayan, and J. D. Morgan. 1999. The stress response in fish. *In* B. G. Kapoor (ed. of "Felicitation Volume"), *Advances in ichthyological research.* Oxford and IBH Publishing Co. Ltd. New Delhi. (In Press)

Iwama, G. K., J. C. McGeer, and N. Bernier. 1992. The effects of stock and rearing history on the stress response in juvenile coho salmon (*Oncorhynchus kisutch*). ICES Mar. Sci. Symp. 194:67–83.

Kikuchi, K., S. Watabe, Y. Suzuki, K. Aida, and H. Nakajima. 1993. The 65-kDa cytosolic protein associated with warm temperature acclimation in goldfish, *Carassius auratus.* J. Comp. Physiol. B 163:349–354.

Koban, M., A. A. Yup, L. B. Agellon, and D. A. Powers. 1991. Molecular adaptation to environmental temperature: heat-shock response of the eurythermal teleost *Fundulus heteroclitus.* Mol. Mar. Biol. Biotech. 1:1–17.

Kothary, R. K. and E. P. M. Candido. 1982. Induction of a novel set of polypeptides by heat shock or sodium arsenite in cultured cells of rainbow trout, *Salmo gairdneri.* Can. J. Biochem. 60:347–355.

Kothary, R. K., E. A. Burgess, and E. P. M. Candido. 1984*a*. The heat-shock phenomenon in cultured cells of rainbow trout: hsp 70 mRNA synthesis

and turnover. Biochim. Biophys. Acta 783:137–143.

Kothary, R. K., D. Jones, and E. P. M. Candido. 1984*b*. 70-kilodalton heat shock polypeptides from rainbow trout: characterization of cDNA sequences. Mol. Cell. Biol. 4:1785–1791.

Misra, S., M. Zafarullah, J. Price-Haughey, and L. Gedamu. 1989. Analysis of stress-induced gene expression in fish cell lines exposed to heavy metals and heat shock. Biochim. Biophys. Acta 1007: 325–333.

Morimoto, R. I., P. E. Kroeger, and J. J. Cotto. 1996. The transcriptional regulation of heat shock genes: A plethora of heat shock factors and regultory conditions. *In* U. Feige, R. I. Morimoto, I. Yahara, and B. Polla (eds.), *Stress-inducible cellular responses,* pp. 139–164. Birkhauser, Basel. Pp 139–164.

Mosser, D. D. and N. C. Bols. 1988. Relationship between heat-shock protein synthesis and thermotolerance in rainbow trout fibroblasts. J. Comp. Physiol. (B) 158:457–467.

Mosser, D. D., J. J. Heikkila, and N. C. Bols. 1986. Temperature ranges over which rainbow trout fibroblasts survive and synthesize heat-shock proteins. J. Cell Physiol. 128:432–440.

Norris, C. E., P. J. diIorio, R. J. Schultz, and L. E. Hightower. 1995. Variation in heat shock proteins within tropical and desert species of Poeciliid fishes. Mol. Biol. Evol. 12:1048–1062.

Pottinger, T. G. and A. D. Pickering. 1997. Genetic basis to the stress response: Selective breeding for stress-tolerant fish. *In* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck (eds.), *Fish stress and health in aquaculture,* pp. 171–193. Cambridge University Press, Cambridge.

Pratt, W. B. 1993. The role of heat shock proteins in regulating the function, folding, and trafficking of the glucocorticoid receptor. J. Biol. Chem. 269: 21455–21458.

Randall, D. J. and S. F. Perry. 1992. Catecholamine. *In* W. S. Hoar, D. J. Randall, and T. P. Farrell (eds.), *Fish physiology,* Vol. XII B, pp. 255–300. Academic Press, New York.

Reid, S. G., M. M. Vijayan, and S. F. Perry. 1996. Modulation of catecholamine storage and release by the pituitary-interrenal axis in the rainbow trout (*Oncorhynchus mykiss*). J. Comp. Physiol. B. 165:665–676.

Renfro, J. L., M. A. Brown, S. L. Parker, and L. E. Hightower. 1993. Relationship of thermal and chemical tolerance to transepithelial transport by cultured flounder renal epithelium. J. Pharmacol. and Exp. Therap. 265:992–1000.

Sussman-Turner, C. and J. L. Renfro. 1995. Heat-shock-stimulated transepithelial daunomycin secretion by flounder renal proximal tubule primary cultures. Am. J. Physiol. 267:F135–F144.

Udelsman, R., M. J. Blake, C. A. Stagg, and N. Holbrook. 1994*a*. Endocrine control of stress-induced heat shock protein 70 in vivo. Surgery 115:611–616.

Udelsman, R., D. Li, C. A. Stagg, C. B. Gordon, and R. Kvetnasky. 1994*b*. Adrenergic regulation of adrenal and aortic heat shock protein. Surgery 116:177–182.

Vijayan, M. M. and T. W. Moon. 1992. Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 49:2260–2266.

Vijayan, M. M. and T. W. Moon. 1994. The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. Can. J. Zool. 72:379–386.

Vijayan, M. M., C. Pereira, R. B. Forsyth, C. J. Kennedy, and G. K. Iwama. 1997*b*. Handling stress does not affect the expression of the hepatic heat shock protein 70 and conjugation enzymes in rainbow trout treated with β-naphthoflavone. Life Sci. 61:117–127.

Vijayan, M. M., C. Pereira, E. G. Grau, and G. K. Iwama. 1997*a*. Metabolic responses associated with confinement stress in tilapia: The role of cortisol. Comp. Biochem. Physiol. 116C:89–95.

Vijayan, M. M., C. Pereira, G. Kruzynski, and G. K. Iwama. 1998. Sublethal concentrations of contaminant induce the expression of hepatic heat shock protein 70 in two salmonids. Aquat. Toxicol. 40: 101 -108.

Welch, W. J. 1993. How cells respond to stress. Sci. Am. 269:56–64.

Wendelaar Bonga, S. E. 1997. The stress response in fish. Physiol. Rev. 77:591–625.

White, C. N., L. E. Hightower, and R. J. Schultz. 1994. Variation in heat-shock proteins among species of desert fishes (Poeciliidae, *Poeciliopsis*). Mol. Biol. Evol. 11:106–119.

Wilson, J. M., M. M. Vijayan, C. J. Kennedy, G. K. Iwama, and T. W. Moon. 1998. β-napthoflavone abolishes interrenal sensitivity to ACTH stimulation in rainbow trout. J. Endocrinol. 157:63–70.

Yamashita, M., N. Ojima, and T. Sakamoto. 1996. Induction of proteins in response to cold acclimation of rainbow trout cells. Fed. Eur. Biochem. Soc. Letters 382:261–264.