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Physiological and Condition-Related Indicators of Environmental Stress in Fish

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Introduction

Stress has been defined as "the nonspecific response of the body to any demand made upon it" (Selye 1973). Brett (1958) extended Selye's definition in a fisheries context to include that the response to stress causes an extension of a physiological condition beyond its normal resting state to the point that the chances of survival may be reduced. This is a useful working definition as it incorporates both the notion of a physiological change occurring within the organism in response to a stimulus (stressor) and the idea that, as a result, some aspect of fish performance may be compromised. Long-term exposure to environmental stressors is a concern to biologists and managers because of the possible detrimental effects on important fish performance features such as metabolism and growth, disease resistance, reproductive capacity, and, ultimately, the health, condition, and survival of fish populations. Despite general acceptance by biologists and managers that stress can affect fish adversely, the phenomenon of stress is still poorly understood. Nevertheless, a common theme exists among widely ranging perceptions about stress, that there is a suite of nonspecific biological responses to a stimulus at different levels of organization.

A common misconception is that stress, in itself, is detrimental to the fish. This is, however, not necessarily the case. The response to stress is an adaptive mechanism that allows the fish to cope with real or perceived stressors in order to maintain its normal or homeostatic state (Figure 1). Quite simply, stress can be considered as a state of threatened homeostasis that is reestablished by a complex suite of adaptive responses (Chrousos 1998).

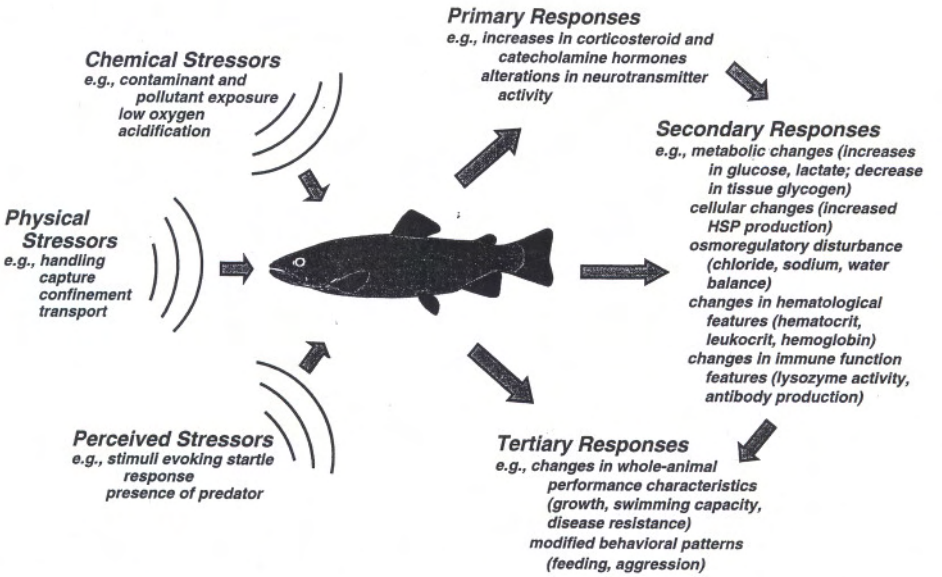

Figure 1. Physical, chemical, and other perceived stressors act on fish to evoke physiological and related effects, which are grouped as primary, secondary, and tertiary or whole-animal responses. In many instances, the primary and secondary responses, in turn, may directly affect secondary and tertiary responses, respectively, as indicated by the arrows (see text).

Also, studies have shown that repeated exposure to mild stressors can habituate fish and attenuate the neuroendocrine and metabolic responses to subsequent exposure to stressors (Iwama et al. 1998; Reid et al. 1998).

If the intensity of the stressor is overly severe or long-lasting, however, physiological response mechanisms may be compromised and can become detrimental to the fish's health and well-being, or maladaptive, a state associated with the term "distress" (Selye 1974; Barton and Iwama 1991). This view of an organism's ability or inability to adjust to a disturbance is consistent with the general adaptation syndrome (GAS) paradigm of Selye (1950), which considers that an organism passes through three stages in response to stress: (1) an alarm phase consisting of the organism's perception of the stimulus and recognition of it as a threat to homeostasis, (2) a stage of "resistance" during which the organism mobilizes its resources to adjust to the disturbance and maintain homeostasis, and (3) a stage of "exhaustion" that follows if the organism, in spite of mounting a stress response, is incapable of coping with the disturbance. The first two stages are usually manifested by measurable physiological changes at different levels of organization, particularly at the lower levels, whereas the final stage is the maladaptive phase normally associated with the development of pathological states, which can alter the health and condition of fish, eventually resulting in mortality.

Rand and Petrocelli (1985) defined acute as "having a sudden onset, lasting a short time; of a stimulus, severe enough to induce a response rap-

idly," whereas chronic was defined as that "involving a stimulus that is lingering or continues for a long time; often signifies periods from several weeks to years." In accordance with these definitions, acute aquatic toxicity tests are generally 4 d or less, but chronic tests are designed to study effects of continuous long-term exposures (Rand and Petrocelli 1985). Deciding seemingly arbitrary limits that differentiate acute and chronic stress at a sublethal level of response, however, is problematic. Researchers generally consider that acute exposures to stressors and subsequent responses to them are brief, ranging from seconds to minutes or possibly hours. Chronic disturbances are generally regarded as continuous or, at least, continual lasting from hours to weeks, months, or even throughout the fish's life, which is consistent with the view used in aquatic toxicology. However, it may be more useful to distinguish between acute and chronic in the context of stress responses from a functional perspective, such as described earlier. Thus, acute stress may be considered as that which usually occurs from brief or short-lived disturbances and results in physiologically adaptive responses to regain homeostasis. But responses that challenge compensatory mechanisms to the point that maladaptive or pathological conditions result are clearly chronic (Dhabhar and McEwan 1997) and can be either sublethal or eventually lethal depending on whether the fish's adaptive capacity is adequate to allow recovery from the stress.

Physiological responses to environmental stressors have been broadly grouped as primary and secondary (Figure 1). Primary responses, which involve the initial neuroendocrine responses, include the release of catecholamines from chromaffin cells (Randall and Perry 1992; Reid et al. 1998) and the stimulation of the hypothalamic-pituitary-interrenal (HPI) axis culminating in the release of corticosteroid hormones into circulation (Donaldson 1981; Wendelaar Bonga 1997; Mommsen et al. 1999). Secondary responses include changes in plasma and tissue ion and metabolite levels and hematological features, all of which relate to physiological adjustments such as in metabolism, respiration, acid–base status, hydromineral balance, and immune function (Pickering 1981; Iwama et al. 1997; Mommsen et al. 1999). Additionally, tertiary responses occur (Figure 1), which refer to aspects of whole-animal performance such as changes in growth, condition, overall resistance to disease, metabolic scope for activity, behavior, and ultimately survival (Wedemeyer and McLeay 1981; Wedemeyer et al. 1990). This grouping is simplistic, however, as stress, depending on its magnitude and duration, may affect fish at all levels of organization from molecular and biochemical to population and community (Adams 1990). Moreover, responses to stress at different levels of organization are not only interrelated functionally to each other, but often interregulated as well. It is useful to consider responses of fish in an integrated or holistic sense rather than simply observe isolated physiological phenomena in order to appreciate the animal's response at the population level to environmental stressors. This holistic

view of stress becomes especially important for the development and validation of subcellular and molecular indicators as biomarkers for stress as they relate to biologically significant effects at higher levels of organization. Thus, including physiological and condition-based indices as components of more comprehensive bioassessment programs would be prudent.

Much of our present knowledge about physiological responses of fish to stress has been gained from studying the primary responses of the HPI axis and the brain-chromaffin axis to stressors and the subsequent or secondary effects associated with neuroendocrine stimulation on metabolism, reproduction, and the immune system (Randall and Perry 1992; Pickering 1993; Iwama et al. 1997; Reid et al. 1998; Mommsen et al. 1999). The investigation of heat-shock or stress proteins (HSP) in fish as a general indicator of the cellular response to various stressors is a recent and rapidly emerging field (Iwama et al. 1998, 1999). Also, studies have shown that HSP induction can be used as a sensitive and reliable indicator of cellular effects associated with acute and chronic exposure to stressors (Vijayan et al. 1997a, 1998). However, most research in this area is descriptive at this point and elucidation of possible functional relationships between the cellular responses to stress and neuroendocrine, immune, and other physiological systems may provide valuable information on the mechanisms involved in the stress tolerance process in fish. The majority of previous research on stress physiology in fish during the last few decades has focused on aquaculture. Many reviews have been published in that context (e.g., Barton and Iwama 1991; Iwama et al. 1997; Pickering 1998), and a few have dealt with the nature of stress responses and manipulations of stress-hormone levels in fish (Mazeaud et al. 1977; Pickering 1981; Gamperl et al. 1994; Wendelaar Bonga 1997; Mommsen et al. 1999; Schreck 2000). Comparatively less information is available on physiological and condition-related responses of fish to environmental perturbations associated with natural or anthropogenic stressors (e.g., Cairns et al. 1984; Adams 1990; Niimi 1990; Brown 1993; Hontela 1997).

Physiological Stress Indicators

Primary Indicators

When fish are exposed to a stressor, the physiological stress response is initiated by the recognition of a real or perceived threat by the central nervous system (CNS). The sympathetic nerve fibers, which innervate the chromaffin cells (adrenal medulla homologue), stimulate the release of catecholamines via cholinergic receptors (Reid et al. 1996, 1998). Because catecholamines, predominantly epinephrine in teleostean fishes, are stored in the chromaffin cells, their release is rapid and the circulating levels of these hormones increase immediately with stress (Mazeaud et al. 1977; Randall and Perry 1992; Reid et al. 1998). Therefore, it is very difficult to measure resting levels of

catecholamines unless the fish are cannulated, resulting in little application of this approach to measure stress in fish (Gamperl et al. 1994).

The release of cortisol in teleostean and other bony fishes is delayed relative to catecholamine release. The pathway for cortisol release begins in the HPI axis with the release of corticotropin-releasing hormone (CRH), or factor (CRF), chiefly from the hypothalamus in the brain, which stimulates the corticotrophic cells of the anterior pituitary to secrete adrenocorticotropin (ACTH). Circulating ACTH, in turn, stimulates the interrenal tissue (adrenal cortex homologue) located in the kidney to synthesize and release corticosteroids into circulation for distribution to target tissues. Cortisol is the principle corticosteroid in teleostean, other neopterygian, and chondrostean fishes (Sangalang et al. 1971; Idler and Truscott 1972; Hanson and Fleming 1979; Barton et al. 1998) whereas 1α-hydroxycorticosterone is the major corticosteroid in elasmobranchs (Idler and Truscott 1966, 1967). Cortisol synthesis and release from interrenal cells has a lag time of several minutes, unlike chromaffin cells, and, therefore, proper sampling protocol can allow measurement of resting levels of this hormone in fish (Wedemeyer et al. 1990; Gamperl et al. 1994). As a result, the circulating level of cortisol is commonly used as an indicator of the degree of stress experienced by fish (Barton and Iwama 1991; Wendelaar Bonga 1997). Control of cortisol release is through negative feedback of the hormone at all levels of the HPI axis (Fryer and Peter 1977; Donaldson 1981; Bradford et al. 1992; Wendelaar Bonga 1997). Regulation of the HPI axis is far more complicated than this description implies, however, and is beyond the scope of this chapter. For additional details, Sumpter (1997), Hontela (1997), and Wendelaar Bonga (1997) have provided more complete descriptions of the endocrine stress axis in fish.

Other hormones can become either elevated or suppressed during stress, including, notably, thyroxine, prolactin, somatolactin, gonadotropins, and reproductive steroids in circulation (Barton 1997; Wendelaar Bonga 1997); however, these other hormones have not yet been demonstrated to be useful stress indicators and, therefore, are not discussed in this chapter.

Interest has focused recently on the responses of central brain monoamines, specifically the catecholamines (dopamine and derivatives) and indoleamines (serotonin and derivatives) in response to stress (Winberg and Nilsson 1993a). Serotonin, in particular, has been implicated in both epinephrine and cortisol regulation in fish during stress (Fritsche et al. 1993; Winberg and Nilsson 1993a; Winberg et al. 1997). These neurotransmitters are directly involved in behavioral changes associated with feeding activity, aggressive interactions among individuals, and establishment of social hierarchies (Winberg et al. 1992a; Winberg and Nilsson 1993b; Alanärä et al. 1998; Winberg and LePage 1998). Winberg et al. (1992b), for example, found that handling Arctic char *Salvelinus alpinus* daily for 4 weeks not only increased serotonin's metabolite, 5-hydroxyindoleacetic acid (5-HIAA), but also

5-HIAA/serotonin (5-HT) ratios, both of which are direct indicators of increased brain serotonergic activity. Increased levels of 5-HIAA and 5-HIAA/5-HT ratios are evident in subordinate low-ranking fish in dominance hierarchies (Winberg et al. 1992a; Winberg and Nilsson 1993b), which may result from social stress. Changes in brain monoamines, notably those in serotonergic activity, have also been reported in fish exposed to environmental stressors including ammonia (Atwood et al. 2000) and copper (DeBoeck et al. 1995). Moreover, such changes were correlated with growth suppression in a dose-dependent fashion following chronic ammonia exposure (Atwood et al. 2000). Taken together, these correlative results suggest the potential of using brain monoamine levels as indicators of tertiary changes associated with chronic stress in fish, such as growth and behavior; however, more study is needed to substantiate these relationships.

Secondary and Tertiary Indicators

Coping with stress is an energy-demanding process and, therefore, the physiological adjustments to stress are geared toward increased tissue oxygen availability and metabolic energy substrate mobilization and utilization in order to cope with the increased energy demand. The primary response, the release of corticosteroids and catecholamines, can directly or indirectly affect the secondary stress responses (Figure 1) including metabolism; hydromineral balance; cardiovascular, respiratory, and immune functions; and those related to hematology (e.g., circulating erythrocytes and leukocytes, differential leukocyte ratios, hemoglobin).

A major role of catecholamines is to modulate cardiovascular and respiratory functions, thereby maintaining adequate oxygen supply to the tissues (Randall and Perry 1992). This homeostatic mechanism includes increased blood flow to the gills, gill permeability, and lamellar perfusion to enhance oxygen uptake (Wendelaar Bonga 1997; Reid et al. 1998; Cech 2000). Accompanying the increased gill permeability, however, is an increase in water and ion transfer across the gill membrane, which can cause a temporary hydromineral imbalance in the fish. In freshwater fishes, this can be manifested during acute stress as a loss in major blood ions, such as sodium and chloride, and total osmolality, and an uptake of water (Eddy 1981; Wendelaar Bonga 1997; Cech 2000). Marine fishes during stress, however, may exhibit an increase in ion influx and osmolality and a loss of water across the gills (Eddy 1981; Wendelaar Bonga 1997; Cech 2000).

Catecholamines are also important for energy substrate mobilization, which is necessary to cope with the increased energy demand from metabolic activity associated with stress in fish (Barton and Schreck 1987a; Davis and Schreck 1997; Fabbri et al. 1998). One of the well characterized effects of catecholamines, especially epinephrine, is the stimulation of glycogenolysis, the conversion of stored tissue glycogen to glucose, in response to increased

metabolic demands during stress (Mazeaud and Mazeaud 1981; Randall and Perry 1992; Gamperl et al. 1994; Wendelaar Bonga 1997). This effect of epinephrine is very rapid as it is mediated by the beta-receptors and includes the activation of cyclic adenosine monophosphate (cAMP) and phosphorylation of glycogen phosphorylase in fish hepatocytes (Randall and Perry 1992; Fabbri et al. 1998). As this pathway can be rapidly activated, the stressor-induced increase in circulating glucose levels, at least immediately after stress, is mediated primarily by catecholamine stimulation of glycogenolysis (Vijayan and Moon 1994; Vijayan et al. 1994a; Fabbri et al. 1998).

Cortisol has been shown to have several functions in fish including in metabolism, osmoregulation, and immune function (see Mommsen et al. 1999 for review). The metabolic role of cortisol has focused mainly on the gluconeogenic actions of the hormone (Vijayan et al. 1994b, 1996, 1997b). Cortisol treatment has been shown to increase plasma glucose concentration and liver gluconeogenic capacity including the activities of several key enzymes in intermediary metabolism (Mommsen et al. 1999). In addition, using RU486, a cortisol receptor antagonist, Vijayan et al. (1994b) showed that cortisol has a direct stimulatory effect on gluconeogenesis from alanine. As amino acids are preferred substrates for piscine gluconeogenesis (Suarez and Mommsen 1987), it is likely that cortisol's effect on gluconeogenesis is mediated via cortisol-induced peripheral proteolysis. Although no direct effect of cortisol on fish muscle proteolysis is apparent, studies have shown that cortisol treatment elevates plasma amino acid concentration and the activities of hepatic enzymes involved in amino acid catabolism (Vijayan et al. 1996, 1997b). These studies argue for a role for cortisol in the stress-induced elevation of plasma glucose concentration to cope with the increased energy demand (Mommsen et al. 1999). In addition to increasing plasma glucose concentration, cortisol may also be channeling carbon substrates for liver glycogen repletion after stress has occurred in fish (Vijayan et al. 1994a, 1997b). Therefore, it is thought that the immediate glucose elevation after stress is due to catecholamine stimulation, whereas the longer-term maintenance of glucose, in the absence of glycogen depletion after stress, is mediated by cortisol-induced gluconeogenesis (Vijayan et al. 1994a, 1997b; Mommsen et al. 1999). The effect of cortisol on in vivo hepatic glycogenolysis is less clear with studies showing an increase, decrease, or no change in liver glycogen content with cortisol treatment in fish (see Mommsen et al. 1999). Several factors, including species differences, nutritional status, and prior rearing history of the fish, may influence the differing liver glycogen response to cortisol treatment (Mommsen et al. 1999).

Circulating lactate, or lactic acid, concentrations often increase during stressful conditions as a result of increased muscular activity associated with the stress (Driedzic and Hochachka 1978). Plasma lactate increases in fish that follow physical disturbances have been well documented and were used extensively to monitor various types of physical stressors in fish before

the advent of techniques to measure hormonal responses (Barton 1997). The role of stress hormones in the lactate response to stress is not clear, although studies have shown that cortisol increases gluconeogenic rates from lactate in isolated hepatocytes or liver slices (Mommsen et al. 1999).

Heat-shock or stress proteins are also being used increasingly as an indicator of cellular stress in fish (see Bradley 1993; Bradley et al. 1994; Iwama et al. 1998, 1999). There are several proteins that belong to the HSP family. The 70-kDa protein (HSP70) has been the most widely studied in fish, although HSP90 appears to be more directly involved in corticosteroid receptor activation (Pratt 1997). A recent study showed that cortisol modulates heat-shock-induced HSP90 transcription in fish hepatocytes, suggesting a possible link between the endocrine stress axis and the cellular stress response in fish (Sathiyaa et al. 2001). Heat-shock protein is expressed in response to stressors that seem to affect the protein machinery, and a recent study showed that a handling disturbance alone did not induce the expression of HSP70 in trout liver (Vijayan et al. 1997a). However, contaminants at sublethal concentrations, including bleached-kraft pulp mill effluent, induce the expression of HSP70 in salmonids (Vijayan et al. 1998) and white suckers *Catostomus commersoni* (Janz et al. 1997). Similarly, coho salmon *Oncorhynchus kisutch* chronically infected with bacterial kidney disease exhibited induction of HSP70 in their tissues (Forsyth et al. 1997). Therefore, these proteins may be sensitive indicators of adaptive cellular responses to stressors, especially as the onset of rainbow trout *O. mykiss* mortality with contaminant exposure was coincident with a lack of liver HSP70 expression (Vijayan et al. 1998). Studies on the role of stress hormones in the regulation of HSP expression are currently underway. Cortisol has been shown to modulate HSP70 expression (M. Vijayan, C. Pereira, and G. Iwama, University of Waterloo, personal communication) and HSP90-mRNA expression (Sathiyaa et al. 2001) in primary cultures of trout hepatocytes, whereas, the effect of cortisol on HSP expression in vivo is not clear (Deane et al. 1999). No information is available about the role of catecholamines on HSP expression in fish.

Both the corticosteroids and catecholamines can directly or indirectly affect aspects of tertiary responses or performances of particular concern to biologists including disease resistance (see Chapter 6), scope for growth, feeding and avoidance behavior (see Chapter 11), and reproductive capacity (Randall and Perry 1992; Iwama et al. 1997; Mommsen et al. 1999; see Chapter 9). Some of these tertiary responses, such as growth, condition, and general health, are reflected in changes in the various indices described later in this chapter. The secondary physiological changes that occur tend to take longer to manifest themselves in circulation than primary responses, from minutes to hours (e.g., glucose, lactate, chloride), but often remain altered for more extended periods. Timing of changes in tertiary or whole-animal performance characteristics may be variable; for example, swimming stamina may be affected relatively quickly, whereas, alterations in the immune sys-

tem or reproductive function may not appear for hours or days or even weeks. Nevertheless, most research tends to support the notion that the magnitude and duration of the response reflect the severity and duration of the stressor. Thus, many of the primary and secondary physiological stress responses documented in fish have become well established as useful monitoring tools to assess the degree of stress experienced by fishes. It is emphasized, however, that functional relationships between sublethal physiological changes and mortality are not yet very well understood (Davis et al. 2001).

Factors Influencing Physiological Responses

The physiological responses of fish to stressful encounters are influenced by nonstress factors that affect both the magnitude of the response and recovery from it. These factors are primarily genetic, developmental, nutritional, and environmental. Fishes exhibit a wide variation in their responses to stress, particularly endocrine responses (Barton and Iwama 1991; Gamperl et al. 1994), ranging as much as two orders of magnitude in response to the same stressor (Table 1). The cause for such major differences between and among taxa is likely genetic. It is unknown, however, whether fishes that display relatively low corticosteroid stress responses are actually "less stressed"

Table 1. Examples of mean (± SE) plasma cortisol concentrations in selected juvenile freshwater fishes before and 1 h after being subjected to an identical 30-s aerial emersion (handling stressor). All species were acclimated to their respective environmental preferenda and are listed in increasing order of the magnitude of the 1-h poststress cortisol response (from Barton and Zitzow 1995; Barton and Dwyer 1997; Barton et al. 1998, 2000; Barton 2000; and personal communications for Arctic grayling [B. Barton and W. Dwyer], common carp [N. Ruane and J. Komen], and yellow perch [A. Haukenes and B. Barton]).

Species	Cortisol (ng/mL)	
	Prestress	Poststress
Pallid sturgeon *Scaphirhynchus albus*	2.3 ± 0.3	3.0 ± 0.3
Hybrid sturgeon *S. albus* × *platorynchus*	2.2 ± 0.4	3.2 ± 0.3
Paddlefish *Polyodon spathula*	2.2 ± 0.6	11 ± 1.8
Arctic grayling *Thymallus arcticus*	1.1 ± 0.3	26 ± 4.4
Rainbow trout *Oncorhynchus mykiss*	1.7 ± 0.5	43 ± 3.5
Common carp *Cyprinus carpio*	7.4 ± 2.9	79 ± 14
Brook trout *Salvelinus fontinalis*	4.0 ± 0.6	85 ± 11
Yellow perch *Perca flavescens*	3.4 ± 1.1	85 ± 12
Bull trout *Salvelinus confluentus*	8.1 ± 1.2	90 ± 11
Brown trout *Salmo trutta*	1.0 ± 0.3	94 ± 11
Lake trout *Salvelinus namaycush*	2.8 ± 0.4	129 ± 11
Walleye *Stizostedion vitreum*	11 ± 4.4	229 ± 16

than others or are as "stressed" but have a different capacity to respond to stress. Barton et al. (1998, 2000), for example, observed that low poststress changes also occurred among secondary physiological indicators measured in chondrosteans in addition to cortisol, suggesting a reduced response overall to the stressors. Consistent response differences to stress are not only evident among fish species (Barton and Iwama 1991; Vijayan and Moon 1994; Ruane et al. 1999; Barton 2000) but also among strains or stocks within the same species (Woodward and Strange 1987; Iwama et al. 1992) and even within the same population (Pottinger et al. 1992), a trend that appears to be at least partially heritable (Pottinger et al. 1994). Differences in physiological mechanisms that would account for these variations remain largely unexplored, but Pottinger et al. (2000) found that very high poststress cortisol levels in European chub *Leuciscus cephalus* were associated with low corticosteroid receptor affinity.

The developmental stage of the fish can also affect its responsiveness to stress. A fish's ability to respond to a disturbance develops very early in life, for example, as early as two weeks after hatching in some fishes (Barry et al. 1995; Stephens et al. 1997) and even earlier in cyprinids (Stouthart et al. 1998). Little evidence exists to suggest that fish show a consistent increase in stress responses as they develop, but they do appear to have heightened responses during periods of metamorphosis. Juvenile anadromous salmonids, for example, appear to be especially sensitive to certain stressors, particularly physical disturbances, during the period of parr–smolt transformation (Barton et al. 1985a; Maule et al. 1987). As fish mature, primary stress responses may actually decrease in magnitude, possibly as a result of a reduced threshold for regulatory feedback with the onset of maturity (Pottinger et al. 1995).

Almost all environmental nonstress factors examined to date can influence the degree to which fish respond to stress including acclimation temperature, external salinity, nutritional state, water quality, time of day, light, fish density, and even background color (Barton and Iwama 1991; Barton 1997). An awareness of the extent to which these nonstress factors modify responses is important to both researchers and managers wishing to interpret experimental results and compare them with published values. For example, the fish's acclimation temperature or nutritional state is likely to have an appreciable effect on the magnitude of poststress elevations of cortisol and glucose, particularly the latter (Davis et al. 1984; Barton and Schreck 1987b; Barton et al. 1988; Davis and Parker 1990; Vijayan and Moon 1992, 1994). In certain instances, stress-modifying factors that are themselves chronically stressful, such as poor water quality or toxicants, can actually exacerbate (Barton et al. 1985b) or attenuate (Pickering and Pottinger 1987; Hontela 1997; Wilson et al. 1998) the cortisol response to a second stressor.

The length of time between discrete stressors, the effect of multiple stressors, and the severity of continuous stressors are important factors that influ-

ence how fish will respond to stress. Schreck (2000) describes conceptually how fish can respond to (1) sequentially applied separate stressors, with and without sufficient recovery intervals for compensation to occur; and (2) single or multiple continuous concurrent stressors. If a stressor is persistent, the fish will either compensate eventually for the disturbance and become acclimated or habituated to it or die (Schreck 2000)—responses that are consistent with Selye's GAS model. Thus, it is important for investigators and managers to also know whether fish from experimental or monitored populations are naive or have been exposed to other stressors in their environment prior to study.

Condition-Based Stress Indicators

Condition Indices

Tertiary or whole-animal responses to stress, including changes in growth, condition, and health, indicate the extent to which stress may affect fish performance and provide a basis for understanding the effects of environmental perturbations on fish populations. While assessment of physiological stress indicators certainly provides useful data on the status of fish populations, some of the sophisticated methods employed for measurement are not practical in field bioassessment situations. Although sample kits are available for some physiological parameters (Iwama et al. 1995; Wells and Pankhurst 1999), most methods are used mainly in research and are often beyond the resources or expertise available to the monitoring agency. Thus, the use of physiological test kits for assessing stressed states in fish may be accompanied by recommendations for determining a variety of relatively simple indices related to higher level responses, such as the fish's health and condition, which occur following manifestation of primary and secondary responses (Morgan and Iwama 1997). These indices include a number of organosomatic indices, types of condition factors, and growth, and have been reviewed previously (Anderson and Gutreuter 1983; Busaker et al. 1990; Goede and Barton 1990; Anderson and Neumann 1996). Condition-based indicators, by nature, appear to be relatively insensitive to environmental stressors compared with most specific measurements of blood chemistry or other physiological indicators. Nevertheless, data on fish condition are easy to collect and can contribute appreciably toward understanding long-term trends in fish populations exposed to chronic environmental stressors.

Condition Factor and Length–Weight Relationship

Fulton's condition factor is a measure of robustness and is a function of fish weight divided by the cube of the length and approaches unity with a scaling constant. Condition factor (K, or often CF) is usually expressed in metric units (e.g., $K = g/mm^3 \times 10^5$; Anderson and Neumann 1996), but its calcula-

tion in English units (e.g., C = in/lb × 10^4) is still used in many aquacultural applications (Piper et al. 1982). Calculated values for K and C assume a constant length exponent of 3, but, in actuality, this exponent normally ranges between 2.5 and 3.5 (Carlander 1969) depending on the species' body shape and its life stage. For fusiform fishes, such as salmonids, the length exponent as determined from the length–weight relationship is usually within 2.8 and 3.2 (Barton 1996). Determination of length–weight relationships (i.e., log[weight] = a + b·log[length]) for specific populations or year-classes by linear regression of the two variables has also been used extensively to compare fish populations, particularly in ecological studies and for management use (Anderson and Neumann 1996). Published K values and length–weight relationships for many freshwater populations of the most important North American species are available in three volumes by Carlander (1969, 1977, 1997).

Changes in condition factor or the length–weight relationship can reflect the nutritional or energy status of the fish (Lambert and Dutil 1997; Grant et al. 1998; Grant and Brown 1999) as the fish's weight relative to length will change with storage or mobilization of energy reserves such as proteins, carbohydrates, or lipids. Declines in reserves, such as liver glycogen or visceral fat deposits, may indicate a change in feeding behavior and food consumption, whereas muscle wasting (proteolysis) will indicate a starvation response. Moreover, changes in condition factor resulting from changes in energy reserves and tissue biochemistry may be accompanied by concomitant changes in body water content (Adams et al. 1985; Cunjak and Power 1986; Lambert and Dutil 1997). These changes may be natural occurrences relating to season (e.g., overwintering) or life history stages (e.g., parr–smolt transformation in salmonids), or may result from the presence of disease or exposure to stressors.

Relative Weight Index

The length–weight relationship changes during a fish's life history, which complicates population assessments that compare different year-classes, or cohorts. Wege and Anderson (1978) developed the relative weight index (W_r) to accommodate these allometric growth differences over a wide range of sizes. The W_r is a comparison of the mean weight of the population in question (W) with a standard weight (W_s) and is calculated as follows: $W_r = W/W_s \times 100$, after first determining a W_s value from: $\log(W_s) = a + b\cdot\log(L)$, where L is the mean length of the population with mean weight W and intercept a and slope b are empirically based, published standard values (Anderson and Neumann 1996).

The W_r index can, thus, be a very useful parameter for assessing fish population status, general fish health, fish stocking, and other management programs; these uses and the underlying theory are reviewed extensively by Murphy et al. (1990) and Blackwell et al. (2000). However, W_r has two draw-

backs. First, W_s is calculated from the intercept (a) and slope (b) values provided from a compilation of as many populations as possible with the assumption that the more populations that are included in the calculation, the closer the regression parameters (and, thus, W_s) will be to a hypothetical ideal standard (Cone 1989). Second, W_s regression parameter values (a and b) are not published for all species, but mainly for freshwater fishes important to managers. Despite these shortcomings, W_r provides another quantitative tool for evaluating fish condition, at least for the approximately 56 freshwater species for which published regression parameters for W_s equations are available (Anderson and Neumann 1996; Bister et al. 2000; Blackwell et al. 2000; Hyatt and Hubert 2001).

Organosomatic Indices

Ratios of the mass of particular organs or tissues relative to total body mass can be used as indices of change in nutritional and energy status. Commonly used organosomatic indices include: hepatosomatic index (HSI, liver:body weight), gonadosomatic index (GSI, gonads:body weight), viscerosomatic index (VSI, entire viscera:body weight), and splenosomatic index (SSI, spleen:body weight) (Anderson and Gutreuter 1983; Goede and Barton 1990).

Necropsy-Based Indicators

If the stress experienced by fish is overly severe or long-lasting, then pathological conditions may ensue as compensatory mechanisms are exceeded (Moberg 1985). The necropsy-based condition assessment method described by Goede and Barton (1990) is based on that assumption. This approach was developed to meet the needs of field biologists and hatchery managers for a simple and inexpensive method to monitor and detect trends in their fish stocks. This system is useful for establishing long-term databases on the health and condition of fish populations, but, it is not designed to be a post hoc diagnostic tool. The system can be used alone or in combination with other physiological and hematological measurements (Iwama et al. 1995). As stated by Goede and Barton (1990), this method requires the following assumptions: (1) in fish under stress, tissue and organ function will change in order to maintain homeostasis; (2) if a change in function persists in response to continuous stress, there will be a gross change in structure of organs or tissues; (3) if the appearance of all organs and tissues is normal according to the necropsy criteria, there is a good likelihood that the fish is normal; and (4) if the appearance of an organ or tissue system departs from the normal or control condition, the fish is responding to changes brought about by the environmental stressor.

During the past decade, the necropsy-based assessment method or health condition profile (HCP) has been used in its original form mainly for monitoring hatchery stocks (Novotny and Beeman 1990; Wagner et al. 1996). This

protocol has also been modified to provide a quantitative index that can be compared statistically. Adams et al. (1993) assigned numerical values to the necropsy categories, which could then be summarized in a health assessment index (HAI). The HAI has been used to evaluate wild fish populations subjected to various environmental perturbations (Adams et al. 1993; Barton 1994; Schlenk et al. 1996; Bergstedt and Bergerson 1997; Raymond and Shaw 1997; Steyermark et al. 1999; Sutton et al. 2000; McKinney et al. 2001) and, in some instances, modified or extended to incorporate additional site-specific observations (Steyermark et al. 1999; McKinney et al. 2001). Other external indices, such as skin ulceration (e.g., Barton 1994; Noga et al. 1998) or tumor incidence (e.g., Baumann 1992), can be used separately to evaluate fish populations or incorporated into a standardized examination system. Specific protocols for investigating fish kills are available (Meyer and Barclay 1990) and should be followed in such instances, especially where a legal chain of custody of data is required.

Measuring and Interpreting Stress Responses

Physiological Indicators

As judged by the prevalence in the literature and inferred earlier from numerous studies, the most popular approaches for evaluating physiological responses of fish to environmental disturbances are measurements of plasma cortisol, glucose, lactate, chloride (and other ions), and osmolality, and various hematological features. Typical ranges for resting and poststress-elevated values for commonly measured primary and secondary physiological responses are listed in Table 2. However, readers should note that these are approximate ranges of values that serve as a guideline only and have limited diagnostic value because stress responses are highly variable depending on genetic makeup, early life history, nutritional status, and the fish's environment. Detailed summaries of endocrine changes in fish following exposure to both physical and chemical disturbances are found in Barton and Iwama (1991), Brown (1993), Gamperl et al. (1994), and Hontela (1997). Folmar (1993) provides an extensive compilation of chemical contaminant effects on a number of blood chemistry features in fishes including hormone levels, secondary physiological parameters, and hematology. Species-specific blood chemistry summaries can also serve as useful guidelines for interpreting stress-induced physiological changes (e.g., Hille 1982; Roche and Bogé 1996; Noga et al. 1999). Extensive data indicating the point at which certain secondary physiological features may actually indicate tertiary changes or a life-threatening situation are not available, but, plasma chloride and osmolality concentrations less than 90 meq/L and 200 mOsm/kg, respectively, have been suggested as indicative of compromised osmoregulatory ability in salmonids (Wedemeyer 1996).

Table 2. Ranges of typical resting and stress-elevated values for primary and secondary physiological parameters used as indicators of stress in fish (compiled from Wedemeyer et al. 1990; Barton and Iwama 1991; Folmar 1993; Gamperl et al. 1994; and authors' unpublished data). However, considerable variation among these values and many exceptions outside of these ranges exist depending on species, genetic background, rearing history, and environmental conditions (see text and cited reviews).

Physiological parameter	Resting	Poststress
plasma epinephrine (nmoles/L)	1–6	5–200
plasma norepinephrine (nmoles/L)	1–14	10–100
plasma cortisol (ng/mL)	2–50	30–300
plasma glucose (mg/dL)	50–150	100–250
plasma lactate (mg/dL)	20–30	40–80
plasma chloride (meq/L)	100–130	≈10% ↑ or ↓ [a]
plasma sodium (meq/L)	140–170	≈10% ↑ or ↓ [a]
plasma potassium (meq/L)	2–6	≈10% ↑ or ↓ [a]
plasma osmolality (mOsm/kg)	290–320	≈10% ↑ or ↓ [a]
hemoglobin (g/dL)	5–9	< 4
hematocrit (% packed cell volume)	25–40	40–50+

[a] Blood ions and other features related to hydromineral status will fluctuate upward or downward depending on whether fish is marine or freshwater species, respectively.

Characteristic resting and stress-altered levels of important central monoamines in fishes are summarized in Table 3. Previous studies have demonstrated that these neurotransmitters and their metabolites appear to be concentrated in the telencephalon, the hypothalamus, and the brain stem. Analysis of whole brains would be expected to yield values lower than in specific brain areas because of the dilution effect of other tissues in the preparation. Most studies have examined teleost brains, but Sipiorski (2000) found that chondrostean brain tissue contained monoamine concentrations within the

Table 3. Ranges of characteristic levels of important brain monoamine neurotransmitters in bony fishes (summarized from published data compiled by Sipiorski 2000). All values are in pg/mg brain tissue and include both pre- and poststress values as considerable overlap exists among both species and experiments (5-HT: serotonin, or 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid; DA: dopamine; DOPAC: 3,4-dihydroxyphenylacetic acid).

Brain region	5-HT	5-HIAA	DA	DOPAC
Telencephalon	210–1,000	25–270	180–300	
Brain stem	200–1,800	33–200	125–180	
Hypothalamus	290–1,500	50–360	285–610	
Whole brain	30–500	40–210	31–280	10–109

same order of magnitude as those in teleosts but at the low end or somewhat lower than the ranges reported in Table 3.

Methods of physiological stress assessment in fish have been described (Wedemeyer et al. 1990; Iwama et al. 1995) and include simple assay kits (e.g., glucose, lactate) and easy-to-use meters (e.g., chloride, osmolality) for many of the physiological features of general interest. Measuring hormones such as cortisol is more complicated and usually involves a radioimmunoassay or enzyme-linked immunosorbent assay (ELISA) technique; a number of clinical kits for those assays are now available but should be calibrated and verified for the taxonomic group being studied. Detection methods for HSPs have been described previously and usually involve gel electrophoresis followed with Western blotting or ELISA, or both (Forsyth et al. 1997; Vijayan et al. 1997a; Iwama et al. 1998). Determination of neurotransmitters, such as the brain indoleamines and catecholamines, requires high performance liquid chromatography (HPLC). Inexpensive, readily available, portable meters, such as those used clinically for glucose, hemoglobin, plasma protein, and other features, have been tested for their efficacy in fish stress assessment (Iwama et al. 1995; Morgan and Iwama 1997; Wells and Pankhurst 1999) and such approaches show promise as future useful tools for field monitoring programs. Such kits should be compared with proven laboratory methods for accuracy and reproducibility before use.

Interpreting the physiological variables can be more problematic than measuring the actual responses for three major reasons. As discussed earlier, other nonstress factors such as the various genetic, developmental, and environmental factors can have a modifying effect on the magnitude and duration of the stress response. Without knowing the extent to which other nonstress factors may have altered the response, it is difficult to interpret the biological significance of that response in a relative context. A second factor complicating data interpretation is the variation and apparent inconsistency among fishes in the responses of different blood chemistry characteristics. For example, a species that shows the greatest endocrine response increase (e.g., plasma cortisol) compared with other taxa may not be the same species that elicits the greatest increase in a secondary response, such as glucose or lactate, when subjected to the identical stressor. Thus, a species or group that appears "most stressed" as indicated by one particular level of response may not necessarily reflect that same degree of stress if measured by another level of response (Barton 2000). Such discrepancies among different physiological indicators emphasize the importance of not relying on a single indicator but multiple indicators and also the need for appropriate controls in stress assessment. A third complicating factor is the nature of the stress response itself. The response to stress is a dynamic process and physiological measurements taken during a time course are only representative instantaneous "snapshots" of that process. A significant delay, depending

on the level and type of response, can occur from initial perception of the stressor by the CNS to the time when the physiological feature of interest reaches a peak level of response. Thus, the measurement of a particular stress indicator may not necessarily reflect the degree of stress experienced by the fish at that instant but more likely be representative of the extent of the earlier or initial response. This time lag between perception of the stressor and manifestation of a measurable response can complicate interpretation of results.

Physiological measurements provide a useful approach to evaluate responses of fish to acute stressors but may not necessarily be so for monitoring fish experiencing sublethal chronic stress. Unless the stressors, singularly or in combination, are severe enough to challenge the fish's homeostatic mechanisms beyond their compensatory limits or permanently alter them, which ultimately may cause death, physiological processes generally adapt to compensate for the stress (Schreck 1981, 2000). In these cases, blood chemistry parameters, such as cortisol titer, may appear normal and other approaches may be needed to determine the fish's physiological status.

As one alternative approach for evaluating the stressed states in fish, increased continued activity of the HPI axis, which implies a continuous response to a stressor, can be determined from tissue histology. Continued synthetic and secretory activity of the interrenal tissue during chronic stress can result in hypertrophy (increase in size) and hyperplasia (increase in abundance) of the interrenal cells. Using standard tissue histological preparation and examination techniques, interrenal cells can be measured and counted, and, thus, quantified. This approach has been used to assess chronically stressed states in fish subjected to social stressors including rearing density (Noakes and Leatherland 1977; Fagerlund et al. 1981) and exposure to acidification (Brown et al. 1984; Tam et al. 1988), heavy metals (Norris et al. 1997), and other environmental pollutants (Ram and Singh 1988; Servizi et al. 1993). Continual interrenal activity, however, will also downregulate the HPI axis as a result of negative feedback by cortisol. Thus, when a second acute stressor subsequently challenges fish exposed to a chronic stressor, the corticosteroid response to the additional stress may be reduced considerably relative to controls (Hontela 1997). Impaired interrenal function from chronic stress has been demonstrated in vivo by subjecting fish to an acute physical disturbance after being exposed to various contaminants (Hontela et al. 1992; Wilson et al. 1998; Norris et al. 1999; Laflamme et al. 2000).

Another approach for determining the effect of chronic stress on the HPI axis in fish is by assessing the functional integrity of the interrenal tissue in vitro. Brodeur et al. (1997) developed a relatively simple perifusion bioassay protocol to measure the corticosteroidogenic capacity of ACTH-stimulated interrenal tissue removed from chronically stressed fish. More recently, Leblond and Hontela (1999) and Leblond et al. (2001) described a method of preparing and using interrenal cell suspensions for quantifying the extent of in vitro steroidogenic inhibition at the cellular level. These and similar approaches

have been used by this group of investigators and others to evaluate the mechanisms involved in the depression of interrenal capacity following exposure to contaminants including heavy metals and organochlorine compounds (Brodeur et al. 1998; Girard et al. 1998; Wilson et al. 1998; Leblond and Hontela 1999; Benguira and Hontela 2000; Laflamme et al. 2000; Leblond et al. 2001).

A summary of advantages and disadvantages of the physiological indices commonly used for measuring stressed states in fish is presented in Table 4.

Condition-Based Indicators

Condition Factor and Length–Weight Relationship

Condition factors are often used in stress assessment studies as they are derived from easily obtained length and weight measurements (Anderson and Neumann 1996). As mentioned previously, declines in condition factor or changes in the length–weight relationship can indicate a change in nutritional or energy status, as reflected by depletions in liver glycogen and body fat deposits. These declines in energy reserves and, thus, condition factors may be caused by external stressors including high rearing densities in hatcheries, acidification, and other adverse environmental conditions (see Goede and Barton 1990). Such stressors may directly affect feeding behavior and food intake, alter metabolic rates, or divert energy away from storage reserves to cope with the stress. However, declines in condition factors in fish and changes in their length–weight relationships may also occur for reasons other than stress. These include seasonal and developmental changes such as natural fluctuations in food availability (Adams et al. 1982), sexual maturation and gonad development (Medford and Mackay 1978), and parr–smolt transformation in juvenile anadromous salmonids (Vanstone and Markert 1968). Furthermore, condition factors may not change in situations where other condition-based biomarkers indicate problems with fish health (Steyermark et al. 1999). Due to their ease of measurement, condition factors will undoubtedly continue to be used in stress assessment studies as indicators of general health. However, the results should be interpreted with caution for the above reasons.

Organosomatic Indices

Organosomatic indices such as HSI, GSI, VSI, and SSI are also relatively easy to measure in the laboratory and field and have been used in a number of stress assessment studies. The assumption generally made with some of these indices (i.e., HSI, GSI, VSI) is that lower than normal values indicate a diversion of energy away from organ or tissue growth in order to combat a stressor of some type. The HSI, sometimes referred to as the liver-somatic index (LSI), is the most frequently used organ mass ratio (Goede and Barton 1990).

Table 4. Physiological responses and their advantages and disadvantages as potential indicators of the degree of stress experienced by fish (see text for details).

Feature	Uses or functions	Advantages	Disadvantages
Heat-shock proteins (HSPs)	Indicator of the cellular stress response to various types of stressors	Sensitive indicator of the adaptive cellular response to acute and chronic stress Can be used as an indicator of both exposure and cellular effects (at the level of cell function) associated with stress	Still considered as a research area; the nonspecificity of the HSP70 response may limit its use as an indicator of stress Functional relationships with the endocrine responses not well established Most of the studies have used HSP70, and the response of other HSPs to different stressors needs to be established in order to validate their use as sensitive indicators Requires sophisticated methodology including Western blotting or ELISA
Brain neurotransmitters	Indicates central nervous system response to stress perception	May help explain mechanisms underlying changes in peripheral endocrine responses and certain behaviors associated with stress Can help establish neuroendocrine links between perception of the stressor and resultant physiological responses	Requires sophisticated HPLC analysis Requires rapid brain removal and instant freezing Whole brain analysis may yield lower values because of dilution effect Proper interpretation requires analysis of preparations from specific brain sites
Plasma catecholamines	Rapid endocrine response (primary) to most forms of disturbance Associated functionally with several physiological roles including tissue oxygen delivery and fuel mobilization for tissue metabolism	Very responsive to acute stress	Difficult to obtain samples from unstressed fish without cannulation because of the rapidity of response May require sophisticated analytical methods (HPLC)
Plasma cortisol	Primary endocrine response used commonly as an indicator of exposure to stressors in aquaculture systems Multiple functional roles including liver metabolism, immune function and osmo-	Predictable indicator of magnitude and duration of acute stress Useful endocrine indicator because of time lag between perception of the stressor and manifestation	Influenced by genetic, developmental and environmental nonstress factors Response may become desensitized or habituated with

Table 4. (continued.)

Feature	Uses or functions	Advantages	Disadvantages
	regulation	of the measurable response in circulation	chronic stress Requires RIA or ELISA for analysis An assay for 1α-hydroxycorticosterone in elasmobranchs is not readily available
Plasma glucose	Metabolic response to stress; indicative of catecholamine- and cortisol-stimulated mobilization of energy reserves	Useful indicator of acute and chronic stress in teleost fishes, especially with respect to chronic stress-induced food-deprivation; less responsive in chondrosteans Can be measured using portable inexpensive kits Lab method is simple with a spectrophotometer using commercial reagents	Fields kits require calibration with lab method to verify accuracy Readings influenced by species, rearing history and other environmental nonstress factors such as temperature and diet
Plasma lactate	Metabolic response to stress; by-product of anaerobic metabolism	Useful indicator of acute stress when associated with muscular activity Lab method is simple with a spectrophotometer using commercial reagents	Not a useful indicator when stressor does not involve or result in strenuous muscular activity
Tissue glycogen	Indicates metabolic reserves stored in liver and muscle	Depletion indicates mobilization and use of energy reserves possibly due to stress or muscular activity	Liver glycogen is very much dependent on the prior history of the animal; values may be high or low depending on when the animal fed and, as a result, any stress effect may be masked
Plasma chloride	Change indicates hydromineral imbalance suggesting possible osmoregulatory dysfunction	Easy to measure using chloridometer (chloride meter)	Not a particularly sensitive indicator in chondrosteans or euryhaline teleosts
Plasma sodium	Change indicates hydromineral imbalance suggesting possible osmoregulatory dysfunction	Standardized saltwater challenge test for salmonids has been developed for comparison of results	Not a particularly sensitive indicator in chondrosteans or euryhaline teleosts Sodium ion analyzer is relatively expensive
Plasma osmolality	Change indicates hydromineral imbalance suggesting possible osmoregulatory dysfunction	Easy to measure with osmometer (vapor pressure or freezing-point depression models)	Not a particularly sensitive indicator in chondrosteans or euryhaline teleosts Does not give indication of what ion is in imbalance

Table 4. (continued.)

Feature	Uses or functions	Advantages	Disadvantages
Plasma protein	Change indicates water imbalance suggesting possible osmoregulatory dysfunction	Very easy to measure using hand-held refractometer	Not a sensitive stress indicator
Hematocrit	A measure of the cellular fraction of blood; determined as packed cell volume Increases may indicate stress-induced splenic release of red blood cells	Easy to measure in blood collected with capillary tubes	Not a very sensitive stress indicator Difficult to ascertain whether differences result from changes in blood volume or red blood cell number A low value may also indicate anemia and, thus, possible disease presence Requires special hematocrit centrifuge to process samples
Leukocrit	An indicator of the fraction of white blood cells (i.e., all leukocytes)	Easy to measure in blood collected with capillary tubes; i.e., from hematocrit samples	Not a sensitive stress indicator High value indicates possible pathogen infection; low value may result from stress
Hemoglobin	A possible indicator of the oxygen-binding capacity of the blood	Easy to measure with commercial hand-held meter or commercial test kit	Not a sensitive stress indicator (see hematocrit)

The liver serves as a major storage site for glycogen and the HSI can, therefore, provide an indication of the nutritional state of the fish, as well as reflect seasonal changes in growth in fish populations (Adams and McLean 1985). The HSI values have been shown to decrease in fish stressed by adverse changes in water quality (Lee et al. 1983), altered water flows (Barnes et al. 1984), and repeated handling (Barton et al. 1987). It has also been demonstrated that HSI values increase after exposure to certain types of contaminants, particularly petroleum hydrocarbons (Fletcher et al. 1982; Fabacher and Baumann 1985; Baumann et al. 1991). This increase in liver mass is presumably due to an increase in liver cell number (hyperplasia) and size (hypertrophy) as a consequence of the induction of the mixed-function-oxidase system in the liver to detoxify the contaminants (Poels et al. 1980). It is important to note that the various organosomatic indices may vary naturally with food availability, state of sexual maturation, and life history stage, often in concert with the season. The GSI, in particular, will exhibit a dramatic decrease after spawning has occurred because of the loss of gonadal products, especially in female fish. These factors should be taken into account when attempting to use organosomatic indices in stress-related studies, especially in the case of the HSI and GSI (see Goede and Barton 1990).

The presence of parasites in the various organs may also confound the interpretive value of organosomatic indices (e.g., Steyermark et al. 1999).

Necropsy-Based Assessment

As mentioned previously, the necropsy-based condition assessment method is a rapid, easy, and inexpensive procedure to detect changes in the health and condition of fish populations. The necropsy method is based on a systematic examination of the condition of external and internal tissues and organs (Table 5). A brief description of the procedure is given below, and more details can be found in Goede and Barton (1990) and Goede (1991).

The desired sample size to establish a health condition profile (HCP) in any given treatment is 20 fish. The fish should be examined soon after capture to prevent discoloration of the various organs. It is convenient to lay out the fish in a row and begin with an assessment of the external features. Observations of fin damage, opercles, eyes, gills, pseudobranchs, and the thymus tissue are made according to the classification scheme and coding system outlined in Table 5. After the external observations are completed, the fish is opened up using a pair of dissecting scissors to expose the internal organs. A ventral cut from the anal vent forward to the pectoral girdle is usually the most efficient method to open the fish. The physical appearance of the liver, kidney, and spleen is evaluated, as well as the relative amount of mesenteric fat, hindgut inflammation, and bile in the gallbladder (Table 5). The necropsy data can be recorded on a standardized data sheet and summarized to provide the following information: (1) percentage of fish with normal and abnormal eyes, gills, pseudobranchs, thymus, spleens, hindguts, kidneys, and livers; (2) mean index values of damage to fins, thymus hemorrhage, mesenteric fat deposition, hindgut inflammation, and bile color. Data entry and calculations can also be performed in the field using a laptop computer and spreadsheet program (Goede and Houghton 1987).

In its original form, the necropsy-based assessment method was used to determine HCPs mainly for hatchery fish populations (Novotny and Beeman 1990; Iwama et al. 1995; Wagner et al. 1996). Iwama et al. (1995) determined HCPs on a hatchery population of juvenile Atlantic salmon *Salmo salar* and, while all fish sampled exhibited normal swimming and feeding patterns, one tank contained fish that were known to be infected with *Aeromonas salmonicida*, the causative agent of furunculosis. They found a higher proportion of abnormal gills, kidneys, and livers, and a greater incidence of fin damage in fish that were infected compared with healthy fish that were held in different tanks. Thus, the HCP may be useful as an early warning system to detect departures from normal before more sophisticated and expensive diagnostic techniques, such as the previously discussed physiological indicators, are employed, although the necropsy method is not meant to serve as a diagnostic tool.

Table 5. Description of variables and numerical ranking system used in the health condition profile (HCP) and health assessment index (HAI) fish necropsy systems (modified from Goede and Barton 1990 and Adams et al. 1993, and adapted from Morgan and Iwama 1997).

Variable	Condition	HCP value	HAI value
Fins	No active erosion	0	0
	Light active erosion	1	10
	Moderate active erosion	2	20
	Severe active erosion with hemorrhaging	3	30
Opercles	No shortening	0	not
	Slight shortening	1	assigned
	Severe shortening, gills exposed	2	
Eyes	Normal	N	0
	Blind (one or both)	B	30
	Exophthlamic; swollen, protruding (one or both)	E	30
	Hemorrhaging (one or both)	H	30
	Missing one or both eyes	M	30
	Other	OT	30
Gills	Normal	N	0
	Frayed; erosion at tips of gills	F	30
	Clubbed; swelling at the end of gills	C	30
	Marginate; colorless margin along tips	M	30
	Pale in color	P	30
	Other	OT	30
Pseudobranchs	Normal; flat or concave in appearance	N	0
	Swollen and convex in aspect	S	30
	Lithic; white mineral deposits	L	30
	Swollen and lithic	S&L	30
	Inflamed; redness, hemorrhage	I	30
	Other	OT	30
Thymus	No hemorrhage	0	0
	Mild hemorrhage	1	10
	Moderate hemorrhage	2	20
	Severe hemorrhage	3	30
Mesenteric fat	No fat deposits	0	not
	Less than 50% coverage of pyloric caeca with fat	1	assigned
	50% of pyloric caeca covered with fat	2	
	More than 50% of caeca covered with fat	3	
	Pyloric caeca completely fat covered	4	

Table 5. (continued.)

Variable	Condition	HCP value	HAI value
Spleen	Normal; black, very dark red, or red	B	0
	Normal; granular, rough appearance of spleen	G	0
	Nodular; cysts or nodules in the spleen	D	30
	Enlarged; noticeably enlarged	E	30
	Other	OT	30
Hindgut	Normal; no inflammation	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal; firm, red color, lying flat against the backbone	N	0
	Swollen or enlarged	S	30
	Mottled; gray discoloration	M	30
	Granular; granular appearance and texture	G	30
	Urolithiasis; creamy white deposits in the kidney	U	30
	Other	OT	30
Liver	Normal; red or pink color	A	0
	Fatty or "coffee with cream" color	C	30
	Nodules or cysts in the liver	D	30
	Focal discoloration	E	30
	General discoloration in whole liver	F	30
	Other	OT	30
Bile	yellow color bile, gall bladder mostly empty	0	not assigned
	yellow bile, mostly full bladder	1	
	light green bile, full bladder	2	
	dark green to blue-green bile, full bladder	3	

Adams et al. (1993) modified the original field necropsy method to a health assessment index (HAI) system to provide a quantitative index so that statistical comparisons can be made between data sets. In this system, all variables are assigned numerical values to allow the calculation of a single HAI for each fish and, thus, mean values for each treatment (Table 5; Adams et al. 1993). During the past few years, the HAI and variations incorporating species-specific features have been used in several field studies involving contamination assessments (Barton 1994; Raymond and Shaw 1997; Steyermark

et al. 1999) and other environmental disturbances (Sutton et al. 2000; McKinney 2001). These studies indicate that the HAI shows promise in detecting health problems in fish populations exposed to environmental stressors, particularly in bottom-feeding species that are in contact with contaminated sediments, such as the ictalurids (e.g., Steyermark et al. 1999). If the HAI approach is used, the HCP-ranking descriptions should also be recorded for possible future use.

A summary of advantages and disadvantages of the condition indices described in this chapter are presented in Table 6.

Summary

Knowledge and understanding of what constitutes stress in fish has increased immensely in the past few decades, notably in the area of physiological mechanisms and responses that lead to changes in metabolism and growth, immune functions, reproductive capacity, and normal behavior. Many of these changes are now used routinely for assessing stressed states in fish by measuring the primary and secondary physiological responses in individuals and tertiary or whole-animal changes that can relate to stress-induced alterations in fish populations. Stress is mediated through neuronal and endocrine pathways, known as the primary response, following initial perception of the stressor, which in turn influence secondary physiological features and whole-animal performance characteristics in the fish. Initially, the stress response is considered adaptive, one designed to help the fish overcome the disturbance and regain a homeostatic or normal state. If the stressor is overly severe or long-lasting, however, the fish may no longer be able to cope with it and enters a maladaptive or distressed state leading to a pathological condition or possibly death.

Typical primary responses used for evaluating stress in fish include determining circulating levels of cortisol and, to a lesser extent, catecholamines. Secondary responses, which may or may not be caused directly by the endocrine response, include measurable changes in blood glucose, lactate or lactic acid, and major ions (e.g., chloride, sodium, and potassium), and tissue levels of glycogen and HSPs. Many other apparent nonstress factors, however, influence characteristic physiological stress responses in fish that biologists need to be aware of for properly interpreting data, including genetic (e.g., species, strain), developmental (e.g., life history stage), and environmental (e.g., temperature, nutrition, water quality) factors.

Some of the whole-animal or tertiary changes, such as growth, condition, and general health, are reflected in various indices that can be useful to describe stressed states in fish. Some indices for this purpose include K (or CF), the length–weight relationship, W_r, and organosomatic indices (ratios of organ masses to total body mass) such as HSI, GSI, VSI, and SSI. Additionally, using a necropsy-based index system can help develop useful data on the normal status of fish populations for possible future detection of changes

Table 6. Uses, advantages, and disadvantages of selected condition-based indicators used to evaluate organismal changes in fish resulting from stress (see text for details).

Indicator	Uses	Advantages	Disadvantages
Condition factor (K or CF)	A measure of robustness of the fish Can be used to indicate changes in energy storage and metabolism and, possibly, feeding activity	Length and weight data easy to collect without invasive procedures	Relatively insensitive to acute stressors Assumes constant length exponent of 3 Varies considerably with sexual maturation and reproduction, especially in female fish because of egg mass Also varies with season and developmental state Changes occurring in body-water content can confound interpretation
Length–weight relationship	A linear regression equation used for comparing fish populations	Length and weight data easy to collect without invasive procedures A more useful comparative index for wild populations than K as length exponent is empirically based rather than fixed	Relatively insensitive to acute stressors Comparison of different life history stages problematic; relationship can change because of allometric growth
Relative weight index (*Wr*)	An empirically based index for comparing fish populations in management studies	Accounts for allometric growth differences over fish's life history	Published values needed for calculating index only available for limited number of species Assumes empirical data used for calculating index are representative of the ideal standard
Hepatosomatic index (HSI) Gonadosomatic index (GSI) Viscerosomatic index (VSI) Splenosomatic index (SSI)	HSI: indicator of nutritional or energetic status; possible indicator of chronic exposure to specific toxicants GSI: useful indicator of status of gonads or reproductive state of the fish VSI: indicator of nutritional or energetic status SSI: indicator of hematopoietic capacity of the fish and blood transfer into and out of circulation	Relatively simple to obtain data and calculate index	Requires sacrificing fish to remove and weigh organ Not particularly sensitive to acute stress Can vary with life history stage and season

Table 6. (continued.)

Indicator	Uses	Advantages	Disadvantages
Health condition profile (HCP)	Provides a long-term data set on the normal status of the population	Method is simple and inexpensive Changes in HCP over time may reflect changes in health of the population May serve as an early warning system to detect departures from normal in the population	Parameters used in the HCP may not be particularly sensitive to acute stress
Health assessment index (HAI)	Extends HCP information by providing a quantitative index for the population	Method is simple and inexpensive Changes in HAI over time may indicate changes in the population resulting from chronic environmental perturbation	Parameters used may not be particularly sensitive to acute stress Assigned numerical values are not empirically or experimentally based

resulting from environmental stressors. Index data on fish condition and health are relatively easy to collect and can contribute appreciably toward understanding long-term trends in fish populations subjected to perturbations. Understanding whole-animal changes is important to managers as these condition-based indices often provide clues that help relate the physiological stress responses of individual fish and changes manifested at the population level of organization.

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