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1

THE CONCEPT OF STRESS IN FISH

CARL B. SCHRECK
LLUIS TORT

The general physiological response of fish to threatening situations, as with all vertebrates, is referred to as *stress*. A stress response is initiated almost immediately following the perception of a stressor. Mildly stressful situations can have beneficial or positive effects (eustress), while higher severities induce adaptive responses but also can have maladaptive or negative consequences (distress). The stress response is initiated and controlled by two hormonal systems, those leading to the production of corticosteroids (mainly cortisol) and catecholamines (such as adrenaline and noradrenaline and their precursor dopamine). Together these regulate the secondary stress response factors that alter the distribution of necessary resources such as energy sources and oxygen to vital areas of the body, as well as compromise hydromineral imbalance and the immune system. If fish can resist death due to a stressor, they recover to a similar or somewhat similar homeostatic norm. Long-term consequences of repeated or prolonged exposures to stress are maladaptive by negatively affecting other necessary life functions (growth, development, disease resistance, behavior, and reproduction), in large part because of the energetic cost associated with mounting the stress response (allostatic load).

There is considerable variation in how fish respond to a stressor because of genetic differences among different taxa and also within stocks and

Biology of Stress in Fish: Volume 35
FISH PHYSIOLOGY

DOI: http://dx.doi.org/10.1016/B978-0-12-802728-8.00001-1

species. Variations within the stress response are introduced by the environmental history of the fish, present ambient environmental conditions, and the fish's present physiological condition. Currently, fish physiology has progressed to the point where we can easily recognize when fish are stressed, but we cannot always recognize when fish are unstressed because the lack of clinical signs of stress does not always correspond to fish being unstressed. In other words, we need to be aware of the possibility of false negatives regarding clinical signs of stress. In addition, we cannot use clinical data to precisely or accurately infer severity of a stressor.

s0005

1. INTRODUCTION

p0050 A fish's life is filled with overcoming, coping with, and recovering from threatening challenges. Threats to the well-being of vertebrates generally result in a physiological cascade of events that helps the organism react to and hopefully recover from or cope with stressors. This concept of how animals respond physiologically to such challenges was termed *stress* by Selye (1950). The response, he proposed, was similar irrespective of the nature of the stressor and referred to by him as the general adaptation syndrome (GAS). The GAS consists of a hormonal cascade that produces all the other responses to a stressor. He goes as far as to suggest that the complete absence of stress is death. The general contentions suggested by Selye (1976) are appropriate for fish but, as with other vertebrates, come with numerous caveats. Of course, the physiological stress response has consequences to essentially all physiological systems. Consequently, understanding the biology of stress in fish will provide a fundamental understanding important for the science of fish biology as well as fish research, management, and husbandry.

p0055 This book provides an detailed account of the state-of-the-art of stress physiology in fishes. It builds upon and expands the seminal publication by Pickering (1981) on *Stress and Fish*. While providing a holistic review of stress, it does not focus on any particular stressor or environmental tolerance limits. Instead, we recognize that many environmental factors (eg, temperature, pH, turbidity, toxicants, pathogens, predators, handing by people, etc.) can lead to stress and dysfunction in the fish if they are encountered at levels approaching or beyond the normal tolerance capacity of fish.

p0060 The intent of this chapter is to provide an overview of the stress concept as it relates to fish. We discuss stress in fish in general terms; the other chapters will provide the necessary detail regarding what is known about

their respective topics. Understanding the limits of our knowledge is also important from both a basic and applied perspective; the mission of this book is to also provide this insight.

s0010 ## 1.1. What Is Stress?

p0065 The word "stress" is surprisingly difficult to define. It is a physiological response of the organism. It is not the environmental variable that causes the response; that would be referred to as the *stressor*.

s0015 ### 1.1.1. Stress Definition

p0070 The word stress has its roots in the physiological definition proposed by Selye (1950, 1973): "stress is the nonspecific response of the body to any demand placed upon it." However, the definition we prefer and the one used throughout this book is "The physiological cascade of events that occurs when the organism is attempting to resist death or reestablish homeostatic norms in the face of insult" (Schreck, 2000).

p0075 Our preference is dictated in part by Selye's unfortunate selection of the word "stress" with reference to the GAS, which has led to some confusion in clearly defining the term. Classically, in terms of physics, stress refers to a force to which an object is exposed (the stressor in GAS). *Strain* would have been a better choice for GAS, being the distortion of an object due to a force (ie, the response to the stressor). To be complete, we list many numerous, differing definitions of the word "stress" that have appeared in the literature:

u0040 • *A state produced by an environmental or other factor that extends the adaptive responses beyond the normal range* (Brett, 1958).

u0045 • *The sum of all physiological responses that occur when animals attempt to establish or maintain homeostasis* (Wedemeyer and McLeay, 1981).

u0050 • *The alteration of one or more physiological variables to the point that long-term survival may be impaired* (Bayne, 1985).

u0055 • *Stress is a state caused by a stress factor, or stressor, that deviates from a normal resting or homeostatic state* (Barton and Iwama, 1991).

u0060 • *The cascade of biological events that occur when the organism faces a challenge out of the normal range and the attempt to reestablish homeostatic values* (Barton, 1997).

u0065 • *A state of threatened homeostasis that is re-established by a complex suite of adaptive responses* (Chrousos, 1998).

u0070 • *The reaction of the organism aimed at regaining homeostasis* (Chrousos, 2009).

u0075 • *Stress is a condition where an environmental demand exceeds the natural regulatory capacity of an organism* (Koolhaas et al., 2011).

p0120 A general theme does run through all definitions: stress is the physiological response to a stressor. Some definitions restrict the stress response to imply a neuroendocrine-induced cascade. Irrespective of definition, this cascade tends to be nonspecific, being qualitatively similar irrespective of the nature, type, and severity of stressor. That said, the quantitative magnitude of the stress response differs widely for a variety of reasons that are discussed and reviewed in detail in Chapter 2, Variation in the Neuroendocrine Stress Response. A general discussion of the definition of stress can be found in Levine (1985), while McEwen and Lasley (2002) provide a very readable, more contemporary discussion of what constitutes stress.

p0125 Stressors can range anywhere from very brief (acute)—for example, being caught in a net or escaping a predator—to those that are prolonged and even more or less permanent (chronic)—for example, being overcrowded in a tank or at the bottom of a social hierarchy. The terms "acute" and "chronic" are context-dependent and so not easily defined. When considering whether something results in an acute or chronic stress, it should not be based on the duration of the stressor but rather on "… the duration of its consequences on the physiology of the animal" (Boonstra, 2013). Stressors also vary in severity.

s0020 1.1.2. The Physiological Stress Response

p0130 The GAS concept (Selye, 1950) embraced the notion that stress is a generalized response. We describe this physiological stress response for fishes in the following, and others expand on this in other chapters of this book. While in a very general sense the GAS concept is a good way to think about the stress response, we emphasize that the response is not in actuality all that general, something Schreck (1981) pointed out for fish many years ago. For example, fish can be killed by certain toxicants, such as cadmium, or by anesthesia without evoking a GAS-like response. Different stressors and different severities of stressors also result in considerably different response dynamics of elements of the GAS (see chapter: Variation in the Neuroendocrine Stress Response).

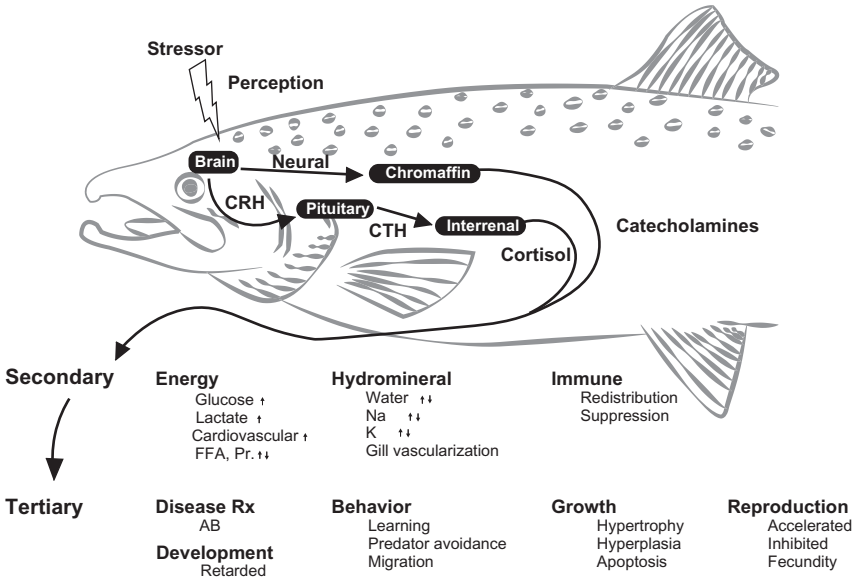
p0135 Events that extend the normal daily bounds of a fish's experiences can lead to our perception that a fish is in some way in danger, which then leads to a stress response. Detection of a real or perceived stressor is essential to initiate the stress response (Schreck, 1981). The classical stress response is not initiated if the fish does not perceive a real life-threatening stressor (Schreck, 1981). Therefore, the psychogenic aspect of the stress response is extremely important to the animal's well-being, as proposed by Ellis et al. (2012).

p0140 There are three main stages to the stress response: alarm, resistance, and either compensation or exhaustion (death) (Selye, 1950; Schreck, 2000).

The nature (magnitude and duration) of the stress response is dependent on the severity and duration of the stressor. In essentially all cases the alarm phase consists of the upregulation of systems involved in flight, fight, and importantly, coping. During the resistance stage the fish either (1) fully overcomes the stressor, allowing for reestablishment of homeostatic norms, (2) sufficiently overcomes the stressor to allow it to nearly recover (compensate), or (3) starts down a trajectory leading to death. As we discuss more fully later, very low levels of stress (eustress) are actually adaptive, while higher levels of stress (distress, a term also difficult to define precisely; Holde[illegible]000) have maladaptive and adaptive elements. Here we are considering stress from the perspective of distress.

p0145 Basically, the primary reaction to the perception of a stressor involves the induction of a neuroendocrine cascade response involving the secretion and synthesis of the corticosteroid hormone (cortisol and related compounds) and the catecholamines (mainly adrenaline and noradrenaline, also called epinephrine and norepinephrine). The endocrinology of stress has been reviewed by Wendelaar Bonga (1977), Sumpter (1997), and recently by Pankhurst (2011). The main corticosteroid responding to stress is 11-deoxycortisol in lamprey (Close et al., 2010; Roberts et al., 2014), while in elasmobranchs it is 1α-hydroxycorticosterone (Idler and Truscott, 1966; Idler et al., 1967a) and cortisol and other related steroids in chondrosteans (Idler and Sangalang, 1970; Webb et al., 2007). There are also large circulating concentrations of cortisone, a cortisol metabolite, consequent to stress in fish (Patino et al., 1987). It is thought that this conversion to cortisone serves to downregulate cortisol into a nonactive metabolite, but this is a subject little studied. It is evident that fish also can produce a host of other corticosteroids in response to stress; often these are at low concentrations, but we know almost nothing about their function or potency. A main role of these hormones is to make energy available for systems involved in fight, flight, or coping. Fig. 1.1 depicts the sequence of events that comprise the stress response. Chapter 3, The Endocrinology of the Stress Response in Fish provides a contemporary discussion of the endocrine stress axis and control of the stress response.

p0150 These secondary responses include the cardiovascular and respiratory responses (see chapter: The Stress and Stress Mitigation Effects of Exercise: Cardiovascular, Metabolic, and Skeletal Muscle Adjustments), which increase distribution of oxygen as well as energy substrates that are liberated into the circulation also as a result of the stress response. Other accompanying secondary responses include a hydromineral dysfunction because adrenaline alters the gill blood flow patterns and gill permeability, both of which favor water flowing down its osmotic gradient, either in or out of the fish depending on environmental salinity. Thus, a logical role of



f0005 **Figure 1.1.** The primary (depicted inside fish), secondary, and tertiary responses of fish during distress. CRH, corticotropin releasing hormone; CTH, corticotropic hormone; FF, free fatty acids; P, proteins; AB, antibodies.

cortisol in this regard would be the restoration of osmotic equilibrium (see chapter: Homeostatic Responses to Osmotic Stress). Another response to cortisol, and perhaps other control factors, is immunosuppression. While the hormones ultimately have a positive effect on the organism by making energy available where it is needed during exposure to a stressor, many of the tertiary responses, those at the whole animal level, are maladaptive. For example, health and disease resistance (see chapter: Stress and Disease Resistance: Immune System and Immunoendocrine Interactions), reproduction (see chapter: Reproduction and Development), growth (see chapter: Stress and Growth), learning, and other behaviors such as predator avoidance (see chapter: Cognition, Learning, and Behavior) are all impaired.

s0025 ### 1.1.3. Stress and Homeostasis

p0155 The stress response is clearly a necessary mechanism of fish to overcome severe challenges and, if possible, restore homeostasis. All the same, it is equally important to understand that under normal, nonstressful conditions a low circulating level of stress hormones is essential for maintenance of routine life functions such as growth (Schreck, 1992), the immune system

AU:2 (Schreck, 1996; Yada and Nakanishi, 2002; Dhabhar, 2008; Verburg-Van

Kemenade et al., 2009; Tort, 2011; Dhabhar and McEwen, 2001), development (Schreck, 1981), and learning (Sorensen et al., 2013; Martins et al., 2013). That is, the effects of levels of cortisol and catecholamines at low severities of stress are positive on routine life functions, while the effects become negative at higher and more prolonged elevations in concentration (Schreck, 1992). In other words, the individual elements involved in the stress response are part of homeostasis.

p0160 "Homeostasis" (Cannon, 1926, 1932) is another difficult word to define and concept to describe. The word is derived from the Greek roots *homeo* meaning "like" or "similar" used as a prefix and *stasis*, meaning "stability" or "standing still," and is used to imply the relative constancy of the internal environment of an organism "despite continual changes in the organisms' external environment." This condition of stasis is maintained by feedback systems (Tortora and Grabowski, 2000; Guyton and Hall, 2011). Thus, homeostasis is a dynamic process and so it is very difficult to recognize a stable zone, which adds difficulty to the definition. Selye (1973) recognized this difficulty in terms of describing stress within a concept related to homeostasis. He suggested the term *heterostasis*, meaning that a new steady state is established by exogenous factors stimulating adaptive (nongenetic, or compensatory) mechanisms. This happens via development and maintenance of defensive tissue reactions that heretofore were dormant. The fluctuations experienced by the internal environment of a fish are small in contrast to the fluctuations resultant from stress. So, we can conclude that fish with elements of their internal environments beyond the ranges experienced when in homeostasis would suggest that the fish are under stress, which fits our definition. However, just as the variations experienced by a fish's internal environment during stress are quite large, there can be considerable variation as well during normal times when the fish is at homeostasis if we considers fish only during this state, excluding stress and other challenges that push the fish beyond this scope.

p0165 Stress results when a fish's system experiences conditions that result in homeostatic overload, the situation where routine physiological mechanisms cannot maintain a fish's internal environment within normal bounds. A conceptual integration of concepts involving stress, allostasis (see Section 1.1.4), and homeostasis led Romero et al. (2009) to propose a "reactive scope model" as a way of considering the physiological bounds between lack of stress and stress.

p0170 Recovery from stress returns the internal environment to within homeostatic ranges. If the stressor is acute and not overly injurious, full recovery can occur. However, in the face of a prolonged, chronic stressor or a very severe acute stressor, the fish may never reestablish the exact same characteristics of homeostasis, a condition referred to as compensation

(Schreck, 2000). A good example of a temporary state of compensation in fish is acclimation to a new external environment; for example, the compensatory responses to thermal acclimation. However, there are two important distinctions: compensatory responses when acclimating are typically beneficial and reversible. Thus, while many clinical signs of stress will return to normal after an acute stressor, other physiological systems may be affected for a long time. For example, cortisol levels in a fish may return to normal within a few hours to a day following a brief (seconds to minutes) handling stressor, while the immune system can take at least a up to a week to do so (Maule et al., 1989). Consequently, the recovery process is very dynamic and not described by unimodal trajectories. There can be overshoots followed by corrections as systems return to unstressed conditions. In the example cited earlier, stress was followed by an immediate suppression of the acquired immune system followed the next day by an apparent enhancement over a prestress condition with a return to prestressed levels within a week (Maule et al., 1989). In addition, fish may never recover from severe acute or chronic stressors despite recovery of primary and secondary stress factors. Indeed, there can be a complete lack of concordance between clinical indicators (components of the primary and secondary stress responses) of stress that suggest recovery and the delayed mortality that starts to happen a week or two later (eg, see Davis et al., 2001 and Davis and Schreck, 2005).

p0175 Some of the earliest work in this regard found that forced activity across all species tested resulted in significant elevations in circulating lactic acid concentrations and that elevated concentrations correlated with the ability of the fish to perform under differing environmental conditions such as temperature (Black, 1957). Further, fish can experience delayed mortality following severe muscular activity that first becomes apparent about 30 min after the cessation of the activity, perhaps due to disturbance to acid–base balance (Black, 1958). While the classical GAS indicators of the stress response may not suggest that the fish are out of homeostatic ranges, the best predictor of impending death was actually not a measurement of something classically thought of as being part of the GAS but related to reflex actions of the fish (Davis and Ottmar, 2006; Davis, 2007, 2010). Chronic stressors may also lack concordance between concentrations of stress response factors such as cortisol and whether or not fish are stressed. It is the mechanisms associated with homeostasis and heterostasis that appear to cause lack of concordance between primary and tertiary stress response factors. For example, Patino et al. (1986) found that cortisol levels can be similar for individuals that are unstressed or under chronic moderate stress due to crowding, while the capacity of the latter to resist other stressors such as low oxygen levels, elevated salinity, or disease challenge is

severely lowered. This example of chronically stressed fish not having elevated plasma cortisol (termed a false negative) can be explained by the fact that they had elevated their cortisol clearance rates to manage the circulating concentrations down to a tolerable level. Chapter 13, Stress in Fish as Model Organisms describes indicators of stress and interpretation of data in detail.

p0180 The nonspecific stress response initiated by the central nervous system releases hormones that operate via highly specific receptors on or in the cells. Therefore, the specificity of target organs and cell types, and whether the effect is an up- or downregulation, is determined at the cellular level. For example, the nature of the receptor can determine the rates of reactions by a cell; membrane receptors mediate more rapid effects than those affecting genes located in the nucleus. Once set in motion, the stress response is regulated by various processes as the fish attempts to restore a homeostatic equilibrium:

u0080 1. Principally the negative feedback mechanisms that concern virtually all endocrine factors involved.

u0085 2. Hormoral up- or downregulation of their own receptors, thereby altering the magnitude of cellular responses.

u0090 3. Hormoral (or their end products) effects on metabolism and clearance of themselves. For example, induction of hepatic enzymes specific for each hormone can inactivate the hormone into a form that can be excreted, preventing prolonged exposure of tissues to extreme hormone concentrations that could eventually lead to death if allowed to persist.

u0095 4. Hormone binding plasma carrier proteins that are somewhat specific and reduce hormone availability for receptor binding at target tissues. Indeed, these carrier proteins may have a large binding capacity relative to the freely dissolved (ie, unbound) hormone in the blood, which is the case with cortisol, but the affinity for the steroid is relatively low when compared the comparable protein in mammals (Caldwell et al., 1991). The high capacity suggests that a high proportion of steroids such as cortisol that are bound to such proteins are not available to bind to their specific receptors; the consequence of this would be a reduction in effects of the hormone. However, the low affinity suggests that receptors could perhaps strip the hormones off of the proteins, hence reducing the regulatory efficiency of the proteins. While we know that they are present in elasmobranchs (Idler et al., 1967b; Idler and Freeman, 1968) and teleosts (Freeman and Idler, 1966; Idler and Freeman, 1968), there is a paucity of information on carrier proteins of hormones concerned with stress in fish. We thus do not know how important such a protein is in fish as a regulatory

mechanism for downregulating stress hormone action. Such hormone-specific binding proteins may also be protective of eggs being developed in females that are experiencing stress. Binding proteins could help keep elevated circulating levels of hormones such as the hydrophobic cortisol in the maternal circulation, greatly reducing how much could cross into lipophilic eggs (Schreck et al., 2001).

It should also be noted that because of all the regulatory mechanisms involved, that the response patterns of onset and duration for each stress response factor can be quite different from each other. Chapter 3, The Endocrinology of the Stress Response in Fish provides a more thorough review of this area.

s0030 ### 1.1.4. Stress and Energetics

p0210 The physiological response to stressors ultimately results in an effect on the entire body; all physiological processes are impacted to some extent largely because there is a need to provide energy needed to overcome stressors. Fight or flight (Cannon, 1926, 1932) requires additional energy for the acute nervous and muscular emergency responses, while chronic exposure to stressors that render energy unavailable for other life processes must be rectified. These costs can be viewed as allostasis, the price that a fish pays in recovering from or coping with a stressor (Schreck, 2010). Sterling and Eyer (1988) proposed the paradigm allostasis as "the ability to achieve stability through change." Others have elaborated on the process in endothermic animals (eg, McEwen, 1998; McEwen and Wingfield, 2003; Ashley and Wingfield, 2012), while Schreck (2010) discussed this concept for fish. Chapter 5, Stress and Growth on metabolism and Chapter 7, The Stress and Stress Mitigation Effects of Exercise: Cardiovascular, Metabolic, and Skeletal Muscle Adjustments on the physiological impacts related to energetics, as well as material in Chapter 7, The Stress and Stress Mitigation Effects of Exercise: Cardiovascular, Metabolic, and Skeletal Muscle Adjustments on swimming and cardiovascular responses, are also relevant to energetics.

p0215 The energetic cost of resistance to, recovery from, and coping (attempting to restore homeostasis) with a stressor can be energetically costly and quantified in terms of calories or another metabolic currency (eg, mg/L oxygen consumed/hour/kg of fish). There are two types of allostatic load, type 1 and type 2 (McEwen and Wingfield, 2003). Type 1 is more acute and is associate more with emergency-type of responses, such as fleeing from a predator, while type 2 refers more to the coping-type of responses, such as being in a constantly overcrowded rearing environment, and is more typical of chronic stressors (Schreck, 2010). Moreover, accommodation to a

stressor diverts energy from other life processes. Mathematically, the energetic cost of an allostatic load ($E_{\text{allostasis}}$) can be viewed as:

$$E_{\text{Somatic Growth}} + E_{\text{Gametic Growth Maturation}} + E_{\text{Activity}} = (E_{\text{Stored}} + E_{\text{Food}}) - (E_{\text{STD}} + E_{\text{SDA}} + E_{\text{Waste}} + E_{\text{Allostasis}})$$

with $E_{\text{Somatic Growth}}$ and $E_{\text{Gametic Growth/Maturation}}$ representing the energy used for the two types of growth, the body and reproduction (gonads and secondary and tertiary sex characteristics).

p0225 E_{Activity} includes energy needed for all general activities such as moving (eg, feeding, swimming, and those associated with reproduction).

p0230 ($E_{\text{Stored}} + E_{\text{Food}}$) represents mobilizable forms of energy available to the fish. It can be used for positive functions such as both types of growth, activity, and standard metabolism (STD). STD in the context used here is instantaneous standard metabolism, which can be thought of as the instantaneous standard metabolic rate (SMR). We use the label STD to avoid confusion that could arise by the use of SMR, which has a time component.

AU:

p0235 E_{STD} represents the instantaneous energetic costs associated with standard metabolism (the minimum amount of expenditures needed just to keep the fish alive such as for respiration, circulation, and osmoregulation).

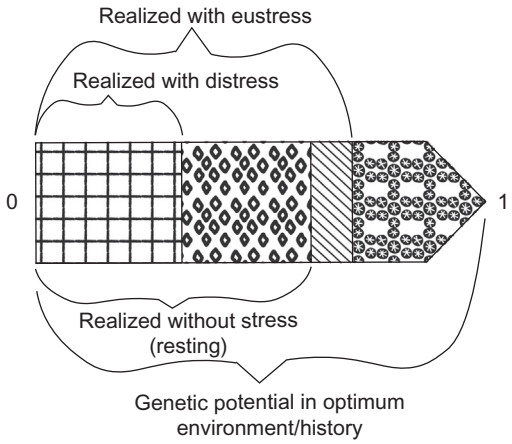
p0240 E_{SDA} represents the energy unavailable due to the energy cost of feeding and food processing. Metabolic inefficiencies as part of this can be thought of as entropy and is unavailable to the fish, being lost to the environment in the form of heat.

p0245 E_{Waste} represents energy in excreted wastes.

p0250 $E_{\text{Allostasis}}$ represents the gross energy needed to resist and recover from a stressor. That includes the energy that goes directly into processes of resistance and recovery as well as the energy lost due to specific dynamic action.

p0255 There is a surprising scarcity of literature regarding the energetic cost of stress in general. Most of the papers relate to stress due to infection and energy demands of the immune system (for reviews see Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000; Sandland and Minchella, 2003; Martin et al., 2003, 2008; Demas et al., 2012). Only a few papers discuss energetics of stress in fish, but we know that it is metabolically costly (Barton and Schreck, 1987). For example, stressors such as brief handling can cost between 12% and 30% of the entire scope for metabolic activity (Davis and Schreck, 1997). There are also a few papers that consider environmental stressors from an energetic perspective (eg, Glencross and Bermudes, 2011; Ogoshia et al., 2012).

p0260 The capacity of fish to perform any of life's tasks such as growth, development, reproduction, disease resistance, and general activities are ultimately determined by the animal's genetics. However, a fish's realized performance capacity, the phenotype that it expresses, is delimited by its ambient environment (how near or far it is from optimum conditions for each of these activities) and the environments the fish has had earlier in its life, such as nutritional history (Schreck, 1981; Schreck and Li, 1991) or behavioral experiences (Schjolden et al., 2005). Eustress can increase and distress can decrease this performance scope (Fig. 1.2). This conceptualization of energetic cost associated with stress allows us to suggest that the $E_{\text{allostasis}}$ needed during acutely stressful situations may not by sufficiently great enough to interfere to any large extent with normal life processes such as growth, reproduction, migration, and the like. Indeed, during acute stress



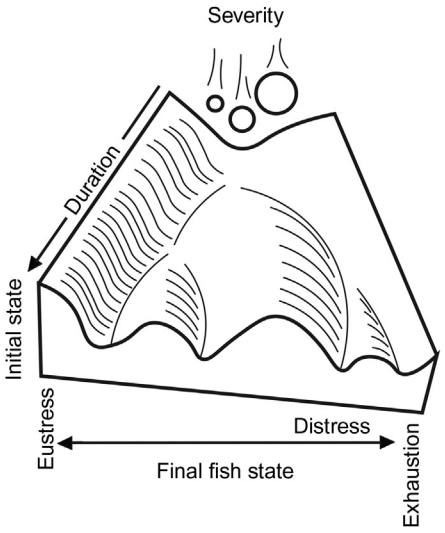
f0010 **Figure 1.2.** Conceptual depiction of the performance capacity of a fish as represented by a vector that ranges from death, or 0 capacity, to the maximum capacity that a fish could achieve based on its genetics and if it lived in an optimum environment in every way, valued at 1. This vector could describe any performance trait. The concept illustrates that the maximum performance can be viewed as a magnitude ranging from no performance (0) to the maximum potentially achievable performance (1), determined by the organism's genetics in a perfect environment. But given that the environment is never perfect, in the absence of stress (realized without stress) the actual performance realized is less than the maximum potential performance. The realized performance can be viewed as the scope to actually perform work. A small amount of stress (eustress) would increase the magnitude of the actual performance capacity while more severe stress (distress) would diminish it. Therefore, given that the environment and magnitude of a stressor can change through time, so does the realized performance capacity. The fish's realized capacity in the absence of any stressor is delimited by the fish's environment and history (and epigenetics). Stress further increases or decreases the realized performance capacity.

a fish relies almost solely on stored forms of energy, in part because feeding behavior is disrupted (eg, reduced feeding, digestion, assimilation, etc.) and blood flow to certain organs is diverted for use by those needed in fight or flight. Also, glycolysis generates adenosine triphosphate more rapidly than oxidative phosphorylation. Death resulting from acute stress would likely be due to cardiovascular failure and happens during the time the fish is exposed to the stressor or soon thereafter (delayed mortality). If death happens within a week or more following a stressor, then it is likely due to secondary disease caused by replicative pathogens (those that can complete their entire life cycle in a host) with which the fish are infected because of the immunosuppressive effects of cortisol. However, there are situations where the cause of mortality starting about a week after an acute stressor cannot be determined (see Section 1.1.3).

p0265 In terms of total energetic costs we propose that chronic stress would impose a significantly greater allostatic load than could be experienced during acute stress, even those that are quite severe but not injurious; that is because of the prolonged energy demand under chronic conditions. Under severe chronic stress, from which the fish could not either fully compensate or recover, energetic costs would be sufficiently large enough that stored energy and food acquisition would be insufficient for growth, reproductive development, and ontogenetic processes. They would be negatively impacted or completely turned off. At the highest levels of allostatic load there would be insufficient energy for general activities, and death due to other challenges becomes more probable; for example, they cannot mount a response to a random encounter with some threat such as a predator or an infection because of immune failure. In this event, even replicative and nonreplicative parasites (those requiring another host to complete their life cycles) could cause death. These paradigms are supported by the syntheses proposed by Schreck (2010).

s0035 1.2. Dynamics of the Stress Response and Effects on Performance

p0270 The response of the primary, secondary, and tertiary stress response factors to stressors are dependent on the severity and the duration of the stressor. They are also contingent on the genetic heritage of the fish, the environment in which the fish are located, their prior experiences, and the ontogenetic stage they are in when exposed to a stressor. These variables also shape the impact of the stressor on each individual (Schreck, 1981; Schreck and Li, 1991). Fig. 1.3 depicts conceptual responses of the primary, secondary, and tertiary stress response factors to stressors of different types, severities, and exposures. Because of the inherent variability associated with the dynamics of the stress responses and because of differences in



f0015 **Figure 1.3.** A conceptualization of how the final state (well-being in terms of stress response) of a fish would differ when exposed to stressors of differing severity for various periods of time and under differing potential starting physiological conditions of the fish using a landscape perspective. Envision balls (representing the physiologic state of the fish) of differing mass (depicted as size) falling onto a downward sloping landscape of uneven terrain; where the balls would end up at the bottom would be different. How far down the hill a particular ball lands would represent the duration of a stressor, with a shorter duration being closer to the end (downhill). The shape of the landscape is affected by the initial state of the fish: the better the initial condition, the better the final state would be at the bottom of the slope. If the ball were to take a trajectory ending near the far left (near the left side of the double-headed arrow), the fish would experience eustress. A mild stressor of short duration would create a landscape where the state of the fish would be somewhat similar to what it was prior to the stressor (somewhere toward the left but not as far as for eustress along the double-headed arrow). Increased stressor severity, durations of stress, and decreased quality of initial fish condition would result in a trajectory down landscapes leading to various degrees of poor final state of the fish and at extreme stress exhaustion (toward the right end of the double-headed arrow). The double-pointed arrow represents a continuum ranging from maximum eustress on the far left to AU:7 extremely poor health and condition on the far right)…. This conceptualization is depicted somewhat similar to Waddington's (1977) epigenetic landscape.

experimental animals and research designs and procedures, studies on stress in fish can differ in major ways from each other relative to the dynamics of the stress responses. This variability between studies is a function of differing initial states of the fish (genetics, developmental stage, history, general health, etc.), nature of the stressors involved, duration of the stressful events, and sampling periodicities. Thus, interpretation of results using a single sampling time are particularly problematic. This confounds

comparisons from which generalizations can be precisely drawn regarding descriptions of the temporal dynamics of the responses involved with stress. While we can describe the shapes of responses, we cannot overlay these with accurate times or magnitudes.

p0275 There is a strong genetic component to the dynamics of the stress response (Fevolden et al., 2002; Øverli et al., 2005), with large differences between taxa. Thus, the heritability must be quite high. Although not quite as large, individuals within a population differ considerably in their stress responses (Ellis et al., 2012). There can also be sexual dimorphisms in how fish respond to stressors ranging from gene activation (eg, see Momoda et al., 2007) to the magnitude of responses by GAS factors. Therefore, resting values for stress response factors, magnitudes of the response, and temporal dynamics of the responses can all vary considerably with genotype. These differences are reflected in how well different taxa and individuals within taxa can tolerate different stressors; some have small scopes of tolerance, others have quite broad ranges of tolerance. In addition, there is lack of concordance of how different taxa and different individuals of a population respond to different stressors. We speculate that variability exists between different ontogenetic stages of fish within a taxon, but there is an extreme paucity of data in this area. Winberg et al. (see chapter: Variation in the Neuroendocrine Stress Response) and Vijayan (see chapter: The Molecular Stress Response) review variation the genetic aspects of the stress response in detail.

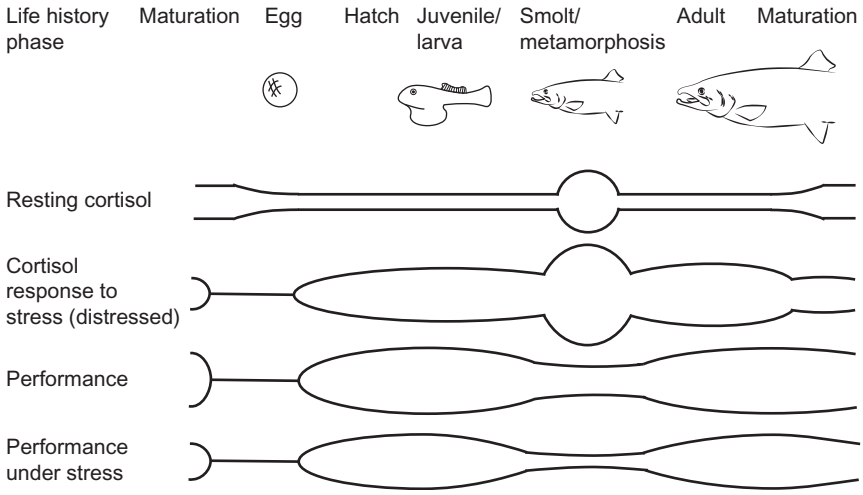
p0280 The ambient environment in which fish experience a stressor influences the physiological responses, the tolerance levels, and their rates. Fish live in a variable environment and therefore potentially stressful (Schulte, 2014), and environmental variables can affect the stress response of fish differently because they operate via different mechanisms. Some examples include (1) temperature can affect the dynamics of the stress response elements by affecting the rate of reactions because of the Q_{10} phenomenon; (2) oxygen concentrations can affect metabolic capacity, thereby influencing the magnitude and duration of the allostatic load; (3) the social environment can cause fish hierarchies that respond differently to a common stressor; (4) state of digestion (full vs empty stomach) can affect stress tolerance; and (5) pathogen infection can alter the responses and tolerances compared with healthy individuals. Chapter 2, Variation in the Neuroendocrine Stress Response, Chapter 5, Stress and Growth, Chapter 7, The Stress and Stress Mitigation Effects of Exercise: Cardiovascular, Metabolic, and Skeletal Muscle Adjustments, Chapter 10, Stress and Disease Resistance: Immune System and Immunoendocrine Interactions, and Chapter 12, Stress Management and Welfare all consider effects of the environment, including fish welfare, in some fashion.

p0285 A fish's prior experience, especially the environments in which they have been residing, can greatly affect how they respond to and during stress. An easy way to visualize this is to consider differences between starved and well-fed fish. Also, prior experiences with stress can potentially either debilitate stress responsiveness and/or tolerance, or could physically and psychologically "harden" the fish to better handle another stressor. Chapter 2, Variation in the Neuroendocrine Stress Response, Chapter 7, The Stress and Stress Mitigation Effects of Exercise: Cardiovascular, Metabolic, and Skeletal Muscle Adjustments, and Chapter 9, Cognition, Learning, and Behavior discuss the importance of fish condition further.

p0290 An emerging area is how the experiences of its parents and even perhaps grandparents can influence the response to a stressor though an epigenetic effect. There is considerable literature on mammals concerning epigenetics as related to stress (Fish et al., 2004), including environmental stressors (Feil and Fraga, 2012). Some of the epigenetic effects of stress appear to be mediated by the corticosteroids (Lee et al., 2010). While there is a paucity of literature regarding epigenetics in fishes, stress must also have profound epigenetic consequences. For example, Mommer and Bell (2014) and McGhee and Bell (2014) found maternal effects of stress in fish on their progeny, as in mammals, and the latter demonstrated that the effect appears to operate via a DNA and histone methylation processes.

p0295 Different developmental stages of a fish's life cycle have differing responses to and tolerances of stressors. It appears that when fish are going through some ontogenetic transition in their lives they are more sensitive to stressors. Thus, the magnitude of the stress response is greater and their tolerance to stressors are lower than at times when they are not undergoing such a transition. Transitional stages when the fish are less resistant to stressors include the embryo before the eye pigments are formed, hatching, the time of onset of per os feeding, metamorphosis (or smolting), and near the end of reproduction (Fig. 1.4). The ability to respond to stressors appears quite early in a fish's development. The embryo is capable of producing cortisol starting around the time when the eye pigmentation becomes apparent. Elevated crowding of embryos at this stage impaired cortisol secretion and feedback (Ghaedi et al., 2013, 2014).

p0300 Stress can negatively affect the reproductive fitness of adult females (Schreck et al., 2001), by either lowering fecundity (number of eggs ovulated) or lowering the quality of the eggs that are ovulated. We conceptualize that the reproductive responses to stress as a series of tradeoffs. Females under stress must partition available energy between recovery from, or tolerance of a stressor and energy investment into maturing eggs to an acceptable level of quality before ovulation.



f0020 **Figure 1.4.** Conceptual model depicting circulating resting and distressed fish cortisol levels and performance capacity in the rested (performance) and stressed (performance under stress) fish over the life history of a fish. The space between the lines represents the magnitude of either the plasma cortisol concentration or the performance capacity (ability to perform work). This conceptualization can be likened to the dimensions of the *n*-dimensional niche hypervolume concept of Hutchinson (1958).

The ultimate tradeoff is between probabilities of female survival versus net lifetime reproductive success (number and quality of offspring). However, a stressed female may not invest in maturing eggs and spawning, delay spawning and potentially lose any investment already made in egg production, mature as many eggs as possible that are nutritionally deficient and result in progeny with lower fitness but in higher numbers, or allow some eggs to become atretic and maximize reproductive investment into a smaller number of eggs that have higher nutritional content and fitness. Given that energy availability is a common currency for these various tradeoffs, nutritional stress is obviously of fundamental importance in setting a female off along one of these trajectories. Examples of all of these tactics for coping with a stressor during egg maturation can be found among fishes. Maternal transfer of biologically active substances associated with the stress response such as cortisol can occur in eggs prior to ovulation. We know very little regarding how stress affects the male's stress response or its consequences. Chapter 2, Variation in the Neuroendocrine Stress Response, Chapter 8, Reproduction and Development, and Chapter 12, Stress Management and Welfare review various roles of ontogeny and the stress response in more detail.

s0040 1.2.1. The Response of the Physiological Systems

p0305 Perception of a stressor initiates an essentially immediate chemically mediated cascade (the primary stress response) that results in qualitatively more or less similar secondary and tertiary stress responses. Neuronal signaling, within a fraction of a second, triggers chromaffin tissue to produce and secrete catecholamines, which control the secondary and tertiary responses. Other neuropeptides such as dopamine and GABA are also affected. The rapidity of catecholamine release into the bloodstream (within seconds and peaking after about 30 min; reviewed by Milligan 1991) makes it difficult to study in fishes, in part also because of the difficulty of obtaining a blood sample from free-swimming fish without further stress (blood vessel cannulation techniques are required). The other hormonal release is slightly slower and more prolonged (within minutes and lasting hours) and is initiated by the production of corticotropic hormone releasing hormone (corticotropin releasing hormone (CRH), also referred to as corticotropic releasing factor) in hypothalamic cells of the brain. The CRH travels to the adrenocorticotropic hormone (ACTH) producing cells in the anterior pituitary, which are specialized to produce and secrete ACTH into the circulation. ACTH targets interrenal cells in the head kidney, causing synthesis and secretion of cortisol in the circulation. Both CRH and ACTH can directly affect secondary stress responses, with ACTH operating centrally directly on the brain (eg, see Clements et al., 2001) and both CRH and ACTH acting peripherally as on the immune system (eg, see Schreck and Maule, 2001). In fact, there is considerable cross-talk between chemical mediators of the endocrine and immune systems related to stress (for a review of psychoneuroimmunology, see Ader, 2007).

p0310 There is considerable variation between species in how long it takes before significant elevations in circulating cortisol levels can be seen following onset of a stressor, with most fish studied requiring several minutes. Chapter 2, Variation in the Neuroendocrine Stress Response reviews the variation seen in the dynamics of various stress response factors and species and Chapter 3, The Endocrinology of the Stress Response in Fish covers the endocrinology involved.

p0315 The main role of the stress response is to make energy available for processes involved in fight, flight, or coping. Stored forms of energy are metabolized and liberated from organs such as the liver into the circulation in forms more readily utilized by tissues. For example, both the catecholamines and cortisol cause hepatic glycogenolysis, making glucose readily available for use by skeletal muscles. In addition, cortisol can downregulate glyconeogenesis systems, thereby keeping the glucose available to the tissues. Free fatty acid and protein concentrations in blood may

also be affected, as explained in Chapter 5, Stress and Growth. The catecholamines trigger cardiorespiratory responses (recruit gill lamellae; increase cardiac output, blood pressure, and gill ventilation) that help provide more oxygen to tissues; Chapter 7, The Stress and Stress Mitigation Effects of Exercise: Cardiovascular, Metabolic, and Skeletal Muscle Adjustments reviews this aspect of the stress response. However, an increase in functional gill surface area enhances water as well as oxygen flow, termed the osmo-respiratory compromise. Hence, stressed fish in freshwater take on water down its osmotic gradient, while those in seawater lose water; there can thus be weight gain in the former due to stress and weight loss in fish in the latter (Stevens, 1972). Blood ion fluxes are also evident during osmo-respiratory compromise, and any resulting change in ionic concentrations must be restored. Concentrations of differing electrolytes may not follow similar response patterns during and after exposure to a stressor; this is likely explainable by differing cellular processes relative to each electrolyte (Stewart et al., in press). One of the main roles of cortisol is the activation of pumps involved in transporting ions into and out of the fish at the gill, gut, and kidney. Restoration of osmotic and ionic equilibrium must be one of the main roles of cortisol following the hydromineral disequilibrium consequent to stress. Chapter 6, Homeostatic Responses to Osmotic Stress reviews the effects of stress on hydromineral balance in fish. The time-course for measurable hydromineral disturbances is extremely variable.

p0320 Stress affects the immune system. The initial phase of an immune response is the activation of certain nonspecific responses, increasing processes such as apoptosis. However, even very acute stressors can suppress the acquired arm of the immune system, which is highly specific. Cortisol suppresses the capacity of lymphocytes to synthesize antibodies and the processes leading to leukocyte production and mobilization. An inevitable consequence of this suppression is that exposure to a stressor makes fish more susceptible to infection and disease until the immune status of the fish oscillates back to prestress immune competence.

p0325 Stress also can affect biological processes by shifting the normal timing of such processes. Schreck et al. (2001) and Schreck (2010) discussed this phenomenon relative to reproduction. It appears highly likely that other "biological clocks" can be interfered with when fish are under stress (Sánchez et al., 2009).

p0330 There are also other physiological responses to stressors that are somewhat general but that do not fit neatly into the GAS paradigm. For example, heat shock proteins (HSPs) are part of the cellular stress response. These proteins, also referred to as stress proteins, can be classified into several families of proteins based on their molecular weights. Each family

can have somewhat different functions. Basically, these proteins are protective. One of their main functions is to serve as chaperone proteins, helping preserve the tertiary structure of other proteins by preventing their deformation by stressors. These proteins are produced by cells in response to a variety of stressors including heat, hypoxia, oxidative stressors, and pathogens, and they are found in most teleosts (Iwama et al., 1998; Basua et al., 2002; Kayhan and Duman, 2010; Kayhan et al., 2010; Roberts et al., 2010; Currie, 2011; LeBlanc et al., 2012; Templeman et al., 2014; Stitt et al., 2014). They appear to have similar functions in elasmobranchs (Renshawa et al., 2012).

p0335 While we know that many processes at the level of the whole organism such as growth, disease resistance, reproduction, and development are impaired to some degree by stress, the nature of the pattern of this impairment or the duration of the impairment are not well studied. Of course, under severe stress, there would be no recovery and death would result.

s0045 ### 1.2.2. Acute, Chronic, and Multiple Stressors

p0340 The magnitude and duration of the individual components of the physiological stress response are highly dependent on both duration and severity of a stressor such that the magnitude of the response to an acute, severe stressor could be as great as that due to a prolonged, mild stressor. Even so, the dynamics and magnitude of the response to the alarm phase tend to be more stereotypical irrespective of the magnitude or duration of the stressor, unlike the resistance and compensation/recovery phases (Schreck, 2000). The physiological responses to acute stressors and the subsequent effect on the capacity of fish to perform necessary life functions (e.g., grow, develop, reproduce, and resist pathogens) can vary with exposure to acute stressors (Fig. 1.5) and to chronic stressors (Fig. 1.6).

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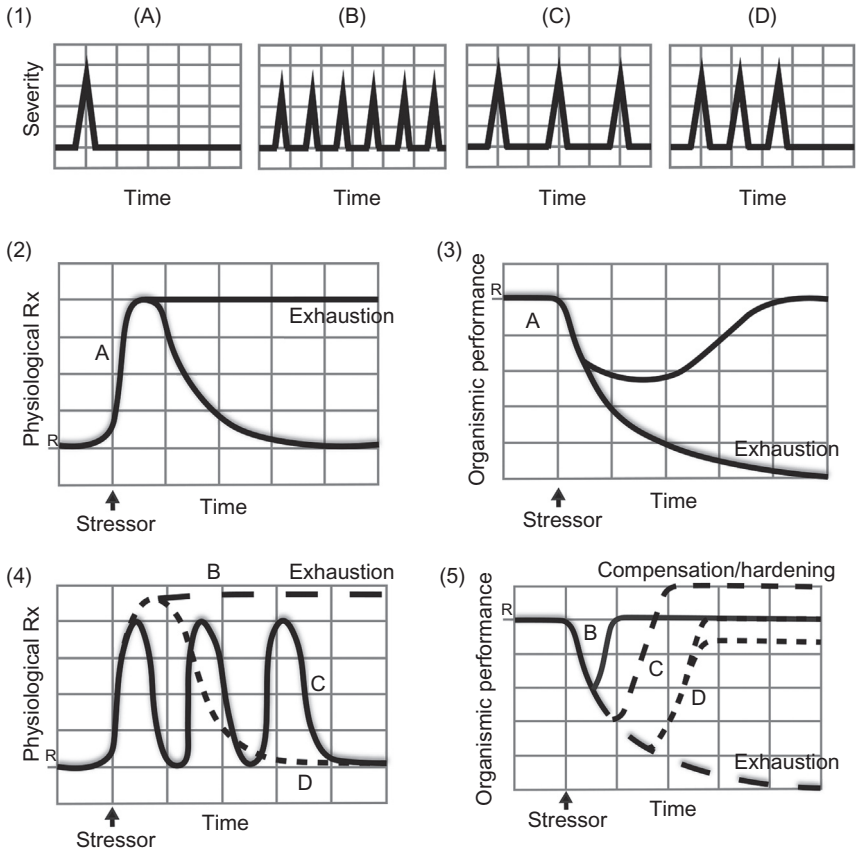
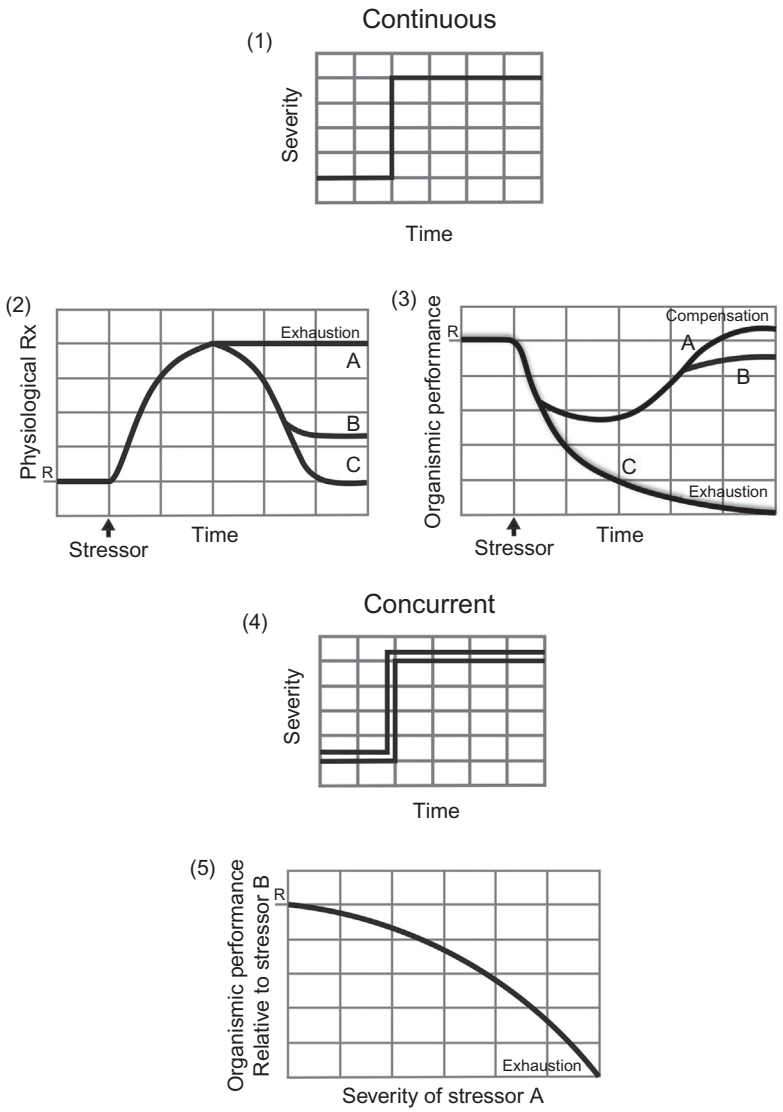
p0345 Regarding chronic stressors, Boonstra (2013) argues that chronic stressors in nature do not lead to pathology in wild animals, basing his views on the functionality of the hypothalamic–pituitary–adrenal axis (hypothalamic–pituitary–interrenal (HPI) axis in fishes). We view this slightly differently and suggest that other coping mechanisms may maintain life and even a functional HPI axis in the face of a common stressor (eg, Patino et al., 1986). That is not to say that under chronic stress the stress response is not adaptive, it is. However, there are bioenergetic costs associated with physiological resisting and coping mechanisms that can lead to poorer capacity to perform other of life's functions (eg, resist pathogens) (see Section 1.1.4). How we define stress also can affect how we interpret effects of chronic stressors. The question really is, does chronic stress lower an animal's fitness? It does not need to be lowered to zero (as implied by Boonstra, 2013), in fact not even very much, to have the potential for major



f0025 **Figure 1.5.** Four temporal patterns of a stressor (1): (A) a single acute stressor, (B) numerous sequential stressors with relatively short time intervals in between each, (C) sequential stressors with relatively longer time intervals in between each, and (D) sequential acute stressors with relatively short time interval between each. Temporal dynamics of primary or secondary stress factors (2) and performance (ie, ability to perform work) (3) when exposed to a stress pattern A. Stressor effects can be mild, resulting in recovery to the resting state, or severe, resulting in exhaustion (ie, death). Temporal dynamics of primary or secondary stress factors (4) and performance (5) when exposed to stress patterns B, C, and D. R represents the resting state. Single or sequential stress patterns (A, B, C, D) are initiated starting at the Stressor arrow. Stressor effects range from those having no long-term performance consequences to those that are mild, resulting in compensation/hardening (ie, eustress), or those that are severe, resulting in exhaustion (ie, death).

evolutionary consequences. It has been suggested that acute stressors are much more common than chronic stressors in nature, at least for terrestrial animals (Boonstra, 2013). This may not be the case for fishes, particularly those in freshwater environments that can experience prolonged elevations



f0030 **Figure 1.6.** Temporal pattern of a chronic, continuous stressor of various severity (1). Temporal dynamics of primary or secondary stress factors over time (2) and performance (ability to perform work) (3) when exposed to a continuous stressor initiated at the time of Stressor arrow. A mild stressor leading to compensation and eustress (A), severe stressor leading to distress (B), and an extremely severe stressor leading to exhaustion and death (C) are represented. R represents the resting state. Stressors may also occur concurrently and continuously (4). The effects of increasing severity of stressor A on the ability of the fish to perform or tolerate stressor B to which it is exposed concurrently (5).

in temperature, reduced flows, or contaminant exposure. Even marine environments can experience prolonged cooling or warming, ocean acidification, and hypoxia that at times can push some species of fish beyond their tolerance limits. The role of social status related to chronic stress and its effects is important (see Sapolsky (2005) for an interesting analysis of this in primates). In any case, there is an extreme paucity of information on the physiology of fish in nature, and much of what we think is inferred from studies conducted in laboratory or hatchery environments.

p0350 Fish can often be exposed to the same acute stressor in some sequential order, such as in mark-recapture experiments. We know that such situations can lead to cumulative effects. However, how stress response factors respond temporally and in magnitude to multiple stressors is very poorly understood. We do know that acute stressors experienced a few hours apart lead to nearly additive effects on stress response elements, for at least three exposures, but we do not know how many more could continue this trend. We also do not know the minimum amount of time between stressors before two or more stressors are perceived as one event rather than multiple events. Similarly, we do not know how much time needs to elapse between sequential stressors before the presence of a former stressor is no longer apparent (Schreck, 2000) (Fig. 1.5).

p0355 When two different stressors are encountered simultaneously (eg, crowding in low oxygen environments), the presence of one stressor can lower the tolerance to another stressor (Schreck, 2000) (Fig. 1.6). However, a gradual downregulation of the stress response in a fish that is coping and slowly compensating for a chronic stressor should not be confused with an apparent exhaustion, attributable perhaps to lack of capacity to physiologically respond to more stressors. The diminished capacity to physiologically respond to other stressful encounters (Huntingford et al., 2006; Pankhurst, 2011; Zuberi et al., 2014) during chronic stress is covered by Sneddon et al. (see chapter: Stress Management and Welfare). During compensation for stress, fish purposely downregulate the stress response, while a diminished stress response can also occur because of an overload of stressors and a response cannot be mounted.

p0360 Care is also needed to distinguish when fish condition themselves to stressors. After prior experiences to other mild, sequential (perhaps daily) stressors for a period of time, the magnitude and duration of the stress response can become attenuated. This is likely due to the physical conditioning that occurred during the earlier exposures to the earlier stressors. A mental component can play a role in the enhanced performance of fish that have had prior experiences with mild stressors. Habituation to repeated stressors is a good example. For example, positive conditioning

where food (a reward) was given to fish following brief and mild daily stressful experiences improved performance better to subsequent severe challenges compared with no reward. The positively conditioned fish had lower physiological responses to subsequent stressful events and were also better at tolerating other challenges such as lower dissolved oxygen, increased salinity, and disease (Schreck et al., 1995). Schreck (2010) referred to this as stress hardening. Chapter 2, Variation in the Neuroendocrine Stress Response discusses variation in how fish respond to and handle stressful situations.

p0365 Conceptually, we view the response dynamics of both physiological stress factors and animal performances to stressful situations as a multidimensional family of curves that cannot simply be described or displayed. The nature of the response curve due to a stressor is contingent upon multiple factors including those endogenous to the fish such as the fish's genetics, developmental stage, and prior experiences by the fish (environments and stressors experienced in the past). They are also contingent upon external factors such as severity and duration of the stressor, developmental stage of the fish, and the present environment of the fish. All of these factors operate in concert to affect the way fish respond physiologically to stressors. They also affect the performance capacity of the fish; that is, they affect how well the fish can perform all other life functions (Schreck and Li, 1991). Fig. 1.3 illustrates conceptually the pattern and duration of responses to acute and chronic stressors in a much simplified manner.

s0050 1.3. Contemporary View of the GAS: Eustress versus Distress

p0370 We need to revisit the concepts of eustress and distress to properly acknowledge that the responses of fish to various stressors or durations are not necessarily unimodal. Bimodal responses are common, such as a low severity of a stressor has one effect, while a higher level of severity has the opposite effect. This concept, hormesis, was discussed more fully by Schreck (2010). It can be considered an adaptive response that induces compensatory biological processes following an initial disruption of homeostasis. How the immune system of fish responds to various severities of stress is a classic example of this phenomenon. Low levels of stress result in just slightly elevated levels of cortisol that have a positive effect on the immune capacity by upregulating leukocyte redeployment, innate immunity, effector cell function, and cell-mediated immunity. The net effect of this is enhanced resistance to infections and cancers. This positive level of stress is referred to as eustress (Dhabhar, 2008; Dhabhar and McEwen, 2001; Schreck, 2010).

p0375 As the severity of a stressor increases and hormones such as cortisol become somewhat more elevated over eustress states, the immune system teeters in a state where the positive effects on the immune system start to disappear and those that are all negative appear. This condition, resilience, is followed as the severity of the stressor increases yet more by a suppression of all the immune factors mentioned earlier. The net effect of this is suppressed resistance to infections and cancers. This state of stress where effects are negative is referred to as distress.

s0055 ## 1.4. Sensory Systems and Perception

p0380 As mentioned earlier, a classic stress response is only initiated consequent to the perception of a threat. Most of the literature concerning the perception of stressors in fish follows the paradigm that stressors are sensed through activation of the classic senses (vision, hearing, touch, smell, and likely taste in fishes). These activate the neuroendocrine response in the hypothalamus of fish. Schreck (1981) suggested that from an anthropogenic perspective that those events in a fish's life that can cause fright, discomfort, or pain result in a stress response. However, other sensory systems also appear to play a role in stress perception and a subsequent stress response. For example, exposure to environmentally relevant levels of acidity (Barton et al., 1985), hypoxia (O'Connor et al., 2011), hypercapnia (Schreck, 1972; Sandblom et al., 2013), osmostress (see chapter: Homeostatic Responses to Osmotic Stress), and pathogens/parasites (Suzumoto et al., 1977) can result in elevated cortisol. These may affect the stress response system via neural routes not part of the classical senses. Stressors affecting mucosal surfaces, such as gills, stomach-intestine, or skin, can generate responses at these sites that first develop as a local response that subsequently leads to a neuroendocrine or systemic response. This is the case with stressors such as unbalanced or deficient diets that affect the gut and may become chronic stressors (Montero et al., 2001). Similarly, contaminants or pollutants at subacute levels can affect gills locally. Clearly pathogens that act at as the portals of entry such that gill, skin, or gut can generate local responses that involve cytokine expression and production and consequent activation of the HPI axis. It is difficult to precisely determine when the HPI axis will be activated after the entry of a pathogen or a parasite as it contingent upon the virulence of the pathogen and infection intensity. Once the HPI axis is activated, their messenger elements perform their actions in the target tissues through their specific receptors. Chapter 3, The Endocrinology of the Stress Response in Fish and Chapter 10, Stress and Disease Resistance: Immune System and Immunoendocrine Interactions discuss the roles of the stress hormones and their receptors within this context.

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s0060 ## 1.5. Adaptation versus Nonadaptation Aspects of the Stress Response

p0385 Physiological adaptation is the sum of mechanisms that allow the organism to overcome (adjust to or recover from) the challenge posed by the stressor. These mechanisms could involve compensatory responses and/or habituation. This involves all physiological response mechanisms, including energetics, immune responses, and also learning and coping strategy.

p0390 Nonadaptation is the result of not meeting the resources to overcome the stressor, and/or the uncontrollability of the reaction. In fact, fishes, like other vertebrates, have a number of abilities and mechanisms to cope with stressor stimuli and overcome the consequences of such stressors. As mentioned earlier, the stress reaction depends on the type of stressor, its intensity, and its eventual repetition. However, two factors, uncontrollability and unpredictability, will always make very difficult the coping possibilities, since in such conditions the homeostatic will increase, and consequently, the allostatic load (Korte et al., 2007; Schreck, 2010).

p0395 Fish in aquaculture are subjected to a number of constrictions, in particular the movement restriction and the regular environment. This means that the learning features and hardening will be different from wild fish.

p0400 For an adequate fish stress management under captivity conditions, it is very relevant to know the main behavioral responses, the capacity of coping, and therefore a certain predictability of the fish responses. In fact, most of the work done in the area of fish welfare over recent years has investigated the behavioral traits that determine the capacity of coping with stressors and the limits of welfare. Model fish should provide a more complete framework (see chapter: Stress in Fish as Model Organisms).

s0065 ## 1.6. Key Unknowns

p0405 Contemporary research approaches have provided major new insights into the stress physiology of fish and have considerably broadened the paradigms that describe how fish respond to stressors (Table 1.1). However, there are numerous unknowns that must be resolved for us to have a more comprehensive understanding of the stress in fish. The following describe some of these key unknowns and questions.

p0410 Our ability to predict when a fish is stressed is relatively good. For example, stress hormone levels that are above resting concentrations allow reasonable inference that fish are stressed. Similarly, elevated concentrations of HSPs also allow fairly robust speculation that fish are stressed to near the point of cellular damage, which needs to be managed. However, there is little basis upon which to predict when fish have recovered from exposure to either acute or chronic stressors. In other words, circulating concentrations

t0005 **Table 1.1**
Classical and contemporary approaches and thinking regarding stress characteristics in fish

Subject	Classical	Contemporary
Behavioral response	Species-specific	Species-specific Coping strategy
Biological hierarchy	Organismic	Organismic Cellular Molecular
Endocrine stress response	Hierarchical (eg, hypothalamus, pituitary, interrenal)	Nonhierarchical (multiple directions)
Learning and adaptation	Stereotyped	Learning and adaptation
Physiological regulation	Nervous system Endocrine system	Nervous system Endocrine system Immune system
Regulation	Homeostasis	Homeostasis Allostasis
Response dynamics	Unimodal	Unimodal Bimodal
Response variables	Physiological Metabolic	Physiological Metabolic Energetics/resource allocation
Sensing	Central (nervous system)	Multiple (central and peripheral)
Signaling	Hormones	Hormones Cytokines
Stress effects	Organismic Physiological	Organismic Physiological Pain Psychological
Stressor effects	Distress	Distress Eustress
Stressor traits	Nature of stressor Intensity of stressor Duration of stressor	Nature of stressor Intensity of stressor Duration of stressor Control of stressor Predictability of stressor
Variation	Interspecific	Interspecific Individual

of stress hormones or other stress factors do not necessarily mean that the fish have recovered fully; they just indicate that either the fish have recovered or they have been able to lower these concentrations.

p0415 Information is needed to allow conclusions regarding the magnitude of stress response factors over resting levels. For example, slight elevations in

magnitude of response may be adaptive if they lead to a eustress condition while slightly higher magnitudes of response may be maladaptive in either the short or long term. We do not know where this tipping point is. Further, we do not know if higher magnitudes of a stress response indicate that a fish is more stressed than at lower levels of response once magnitudes are above those associated with eustress; similarly, we do not know if the magnitude of a response is the same or differs over a range of severities of the same or different stressors. Another dilemma is that we do not know if stressors perceived through different sensory systems lead to similar or dissimilar stress responses. Information is needed to inform the question: Is the response to a stressor operating under a graded or a threshold control system? For example, do increasingly elevated concentrations of stress hormones cause continuously increasing levels of responses, or are there minimum concentrations needed for a response with higher concentrations having no additional effect, until potentially some other set point is reached?

p0420 Information on how fish in the wild respond to stress is extremely rare. It is important to validate that what is learned about stress from fish in captivity can be extended to their wild counterparts. It would also be exceptionally useful to determine how to study the initial phases of stress in wild fish that avoid potential error introduced due to stressors associated with the fish and sample collection process.

p0425 A large number of studies in fish have provided data that should allow for the establishment of normal, nonstress ranges in values for stress factors and for their functions. However, this is actually quite problematic because the numerous factors affecting resting and response levels and their interactions coupled with intra- and interspecific differences basically defy the proposals of generalities. Because of this, interpretation of data concerning the GAS is not simple. Further confounding interpretation of data is the likelihood that some GAS factors operate more via threshold rather than continuous control, and there is no hard data on this phenomenon that would allow for establishment of globally applicable thresholds. The bottom line is that it would be exceptionally useful if a validated "cookbook" could be developed that allowed for interpretation of stress measurements.

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NON-PRINT ITEM

Keywords
Adaptation; allostasis; definitions; distress; eustress; stress response