RELEVANT STRESS INDICES IN AQUATIC ECOTOXICOLOGY

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ABSTRACT

Stress indices in different biological organization levels are analyzed as tools in ecotoxicological studies. Individual stress indices of high organization levels are proposed to obtain a confident knowledge of the damages or disadvantages caused by pollutants on individual organisms, under the assumption that any disadvantage (caused by stress agents) on individuals provide a confident evidence of a detrimental condition of the population. Particularly, relevant individual animal responses related with changes of the use of environmental resources, alterations of avoidance responses, shifts of biotic preferences, and decrease on functional capacity and performance are proposed as useful indices in ecotoxicological studies.

Palabras clave: ecotoxicología, contaminantes, estrés, índices de estrés, toxicología

RESUMEN

Se analiza el manejo de índices de estrés en estudios ecotoxicológicos y se propone el uso de índices de mayores niveles de organización biológica para obtener una aproximación confiable de los daños causados por los contaminantes en los organismos. Lo anterior se fundamenta en la premisa de que las desventajas que se manifiestan en los organismos como resultado de la exposición a condiciones de estrés son indicadores confiables del deterioro de la población. Particularmente, se proponen como índices algunas respuestas relacionadas con el cambio en el uso de recursos ambientales, alteraciones en las respuestas de escape a ciertos niveles de factores ambientales, cambios en las preferencias abióticas y bióticas y disminución de la capacidad funcional en medios cambiantes. Se señalan de manera particular algunos indicadores útiles en ecotoxicología.

INTRODUCTION

The earliest goal of toxicology was to identify the environmental pollutants and then describe their toxic mechanisms and physiological effects on individual animals and their components. These studies were made on chemical, biochemical, and cellular areas, being biased toward the effects on humans (Moriarty 1988). However, the increasing rise of chemical discharges into the environment, and the deterioration that it caused, demanded the study of the consequences of this pollution source on natural systems, and the establishment of legislation to control emissions. In a short time, the impact of pollutants on the environment changed the objective of toxicological research. The study of the effects of pollutants on individuals, their components and their organic structure, turned on to the investigation of the alterations caused by chemicals on the environment itself. The interest of scientists in describing, understanding, and predicting consequences of chemical discharges on the environment established the beginning of ecotoxicology (Truhaut 1977).

This discipline investigates the cumulative consequences caused by pollutants on individual organisms, populations, communities, and/or ecosystems. Its approach is partly based on the effect of pollutants on the structural unity of ecosystems (individual organisms) in order to identify the alterations able to induce changes in their role and function in the environment (Brouwer *et al.* 1990). Ecotoxicology is historically derived from toxicology, thus toxicology and ecotoxicology are a continuous spectrum on the study of the effect of pollutants on the biological systems, with the individual organism as a unit of study shared by both disciplines. Some researchers regard both disciplines as similar, however on the basis of their dissimilar goals the tools and methods used to study individual organisms should differ. This paper comprises an extensive analysis of literature assessing the use of stress indices. Relevant individual animal responses useful as stress indices in ecotoxicological studies are proposed according to the author perspective.

Stress at different levels of biological organization

The impact of environmental chemicals on the whole animal is the basic approach of the ecotoxicological studies, which has been traditionally accessed by the use of stress indices. Initially, Selye (1950) defined stress as "the sum of all the physiological responses by which an animal tries to maintain or restablish its normal metabolism in the face of physical or chemical force". In this earliest definition, stress is limited to changes performed at functional level. Subsequently, several definitions of stress have been used, all of them considering a stimulus induced by environmental forces on biological systems and their subsequent response (Pickering 1981, Wedemeyer *et al.* 1990). According to the definitions, "stress " can be described in three different categories: a) deviation of "normal" biological processes; b) alterations of homeostatic processes, mainly identified by the loss of the physiological steady-state or by the inability to control routine responses; c) compensation responses, exhibited according to the functional requirements to cope with natural fluctuation or environmental challenge. The stress indices in these categories involve a wide extent of biological organization, from the molecular level to individual behavioral expressions (Pickering 1981, Adams 1990).

Although pollutants affect a wide range of levels of biological organization, the concept of stress should be flexible accordingly to the attributes of the particular level focused on. The stability and generalized function of lower organization levels, including molecular, biochemical, and cellular systems allow definitions calling stress as any deviation from a "normal condition" (Hochachka and Somero 1980, Genoni 1997). Meanwhile, the wide environmental pressures, the diversity of strategies to cope with the environment, and the plasticity of individual responses (functional, morphological, and behavioral) drive the organisms into a dynamic equilibrium with respect to the environment. In this vital equilibrium, the homeostatic processes endure and "normality" might be a confused conception (Pickering 1981). The concept of stress depends on the aims, self-experience and on the perspectives of the researchers. Discrepancies about the concept of stress are common in the literature, however the meaning and specific use of "stress" is important in ecotoxicology. This does not mean that "stress" must be restricted to certain theoretical or practical boundaries, but that it must be clearly defined in each ecotoxicological study.

By the other hand, compensatory responses involve a functional sequence of routine processes performed to withstand with the natural fluctuation of the environment. Selye (1950) and Mazeaud *et al.* (1977) point out that the quantitative approach of stress caused by pollutants is sustained by functional sequence of responses, which compress the integration of different levels of biological organization (cells to systemic organs). In this scheme, the "primary changes" involve neuroendocrine responses associated with the release of catecholamines and corticosteroids. These hormones induce "secondary changes" related to biochemical adjustments (including changes in enzyme activity, metabolite production, etc.) that finally are expressed as physiological and behavioral responses (including changes in respiratory rate, ionic regulation, etc.). Each of these changes over different levels of the spectrum of the biological organization have also been recognized as stress indices (Sprague 1971, Adams 1990), and can be identified into the three categories cited before. However, the overall meaning of the responses on the individual performance varies according to the hierarchical biological organization approached (Heath 1987). The components of larger biological systems are less capable of coping with the environmental challenges than the system they comprise. For example, in freshwater cyprinids, 1.6 mg of nitrite is enough to cause ultrastructural alterations in the respiratory epithelia, meanwhile the respiration is not modified by the exposure to a concentration 3 times higher (Alcaraz and Espina 1997). Most of the changes observed at lower organization levels, like cells and tissues, are partial compensation responses of individuals or effects that are not an evident sign of a deleterious condition or evidence of decreased performance capabilities of organisms. These changes do not have ecological significance since they do not reflect the performance of the individuals (Schreck 1990); instead, some of them have evolved and they operate as partial homeostatic mechanisms to allow the animal to cope with usual changes of the environment (Pickering 1981). On the other hand, at higher organization levels (individual organism) compensation responses are completed, accomplished by communication channels and regulatory pathways of their biological components, determining the overall capability and efficiency of coping with the environment (Hochachka and Somero 1980). In this sense, since whole animal responses show the entire physical condition of the animal, they are confident indices of the physical fitness of the individuals.

An important goal of ecotoxicology is to estimate the changes caused by toxicants on individuals, but also to estimate and predict the effects and consequences that these changes may cause to the environment (populations, communities, etc.). Specific efforts have been made to establish a reliable relationship between suborganismal responses, whole animal effects and ecological consequences, however a few reliable relationships have been established (Clemens and Kiffney 1994). The use of stress indices of lower biological levels may have insufficient utility as a tool to understand and predict alterations that may modify the environment, when no direct link between low level responses and individual performance is established. In ecotoxicology, the use of stress indices of low levels of organization requires strong evidences of linking between them and individual performance and fitness. The last does not mean that the approach on one level is more important that others. On the contrary, it means that it is important to determine the appropriate organization level according to the goals. The study and understanding of the changes induced by pollutants on the functional components of individual organisms (cells to organs) is very important, but if the aim is to study the effect of pollutants on the environment, higher biological levels are more appropriate (Heath 1987, Alcaraz 1999).

Stress indices in ecotoxicology

One important aspect of the Ecotoxicology is to propose and use stress indices based on biological criteria applied through simple and short-term procedures that reveal and identify damages caused by chemical discharges. In the last few decades, acute toxicity tests based on mortality were widely used as indication of toxicity. However, in the environment most of the exposures of organisms to pollutants are at sublethal levels, so these acute toxicity tests have restricted value in practice (Maltby and Naylor 1990). Since the 1960s, researchers interested in environmental studies have used a variety of physiological responses to account for the effect of pollutants on individual organisms, and great efforts have been made to assess stress indices with predictive value. It is well recognized that acute exposures to pollutants that kill few organisms may or may not have ecological impact, whereas chronic exposures that cause delays in development, diseases, reproductive malfunctions, or decrease in probability of survival, might result in ecological consequences (Moriarty 1988). In this sense, some behavioral and physiological integrative responses may be advantageous to account for the health condition of individual organisms, and some of them could provide feasible explanations of higher organization levels. Some relevant stress indices in ecotoxicological studies are listed in **Table I**.

In general, behavioral and physiological responses that give relevant information on the environmental impact of chemicals are those that describe the performance, account for the health of individual organisms, and indicate their chance of survival or long-term opportunity to reproduce (Vaughn *et al.* 1984). Consequently, stress indices in ecotoxicology should be able to recognize functional alterations related to the fitness to their environment. In particular, the changes in the energy handiness and allotment reveal consequences in the fitness of the organisms to the environment through changes in the energy exchange with their environment, within conspecifics, and between organisms of different species. In general, these processes are related to changes in food acquisition, the predatory chance, competitive ability, mating opportunity, environmental tolerance, sensitivity to pathogen agents, etc., which in turn are associated with the performance and fitness of the organisms and thus can be used as sensitive stress indices (**Table I**). In brief some indices related to these processes are:

a. Alteration of processes related to acquisition and use of environmental resources. The energy availability is one of the mayor constrains in natural condition. Thus the efficiency to acquire, transform and use the environmental resources is an important factor in the fitness to the environment. The energy assimilated from food is primary allocated to support maintenance processes. While the remaining energy or “surplus power” (also scope for growth) is allocated mainly to processes such as growth and reproduction (biosynthesis), locomotion and behavioral activities (external work) and expression of secondary sexual traits or ornaments (Sibly and Calow 1987). Assuming that energy is a limited resource, any reduction on the energy acquisition or on the surplus power indicate a decrease in the individual ability to perform one or several of these energy-dependent processes related with the fitness of the organisms. The energy allocated to these processes is variable and depends on the interaction between the genetic control and the environment, including the exposure to pollutants. In literature, many of the individual changes related to the acquisition and use of energetic resources are expressed as alterations related to development, reproduction, and growth of individuals. For example, although a low basal metabolic rate is frequently associated with increased resistance to stress

TABLE I. STRESS INDICES RELATED TO RELEVANT FUNCTIONAL AND BEHAVIORAL PERFORMANCES

Functions and processes	Stress responses	Some references
Acquisition and use of environmental resources	Growth rates	Woltering 1984
	Scope for growth	Rice 1990
	Surplus power	Calow and Sibly 1990
	Times for development	Sibly and Calow 1989
	Reproductive allocation	De Coen and Janssen 1997
	Age at first reproduction	Donaldson 1990
Environmental and biotic preferences	Physicochemical preferences	Alcaraz *et al.* 1993
	Avoidance and escape responses	Lingaraja *et al.* 1979
	Food preferences	Bardach *et al.* 1965
	Mating selection	Parsons 1997
Biological capability and environmental tolerance	Swimming performance	Wedemeyer *et al.* 1990
	Migration ability	Schreck 1990
	Critical thermal maximum (CTM)	Alcaraz *et al. 1997*
	Tolerance to salinity changes	Matthews *et al.* 1986
	Environmental tolerance to hypoxia, stressors, etc.	Wedemeyer and McLeay 1981
Expression of morphological traits	Changes of morphological traits (e.g. ornaments)	Parsons 1995
		Hoffmann and Parsons 1997
	Coloration changes	Hulscher-Emeis 1992
	Fluctuating asymmetry	Nilsson 1994, Markow 1995
Low organization responses with linkage to individual performance	Immune responses	Anderson 1990, Porter *et al.* 1984
	Sensitivity to pathogen infection	Snieszko 1974
	Genetic integrity	Hoffmann and Parsons 1997
	Sensorial perception	Rehnberg and Schreck 1986
	Adenylate energy charge (AEC)	Ivanovici and Wiebe 1981
	Chemical indicators of exposure	Mackay 1982

conditions, low rates may decrease the fitness because it can reduce reproductive success (Hoffmann and Parsons 1997). Particularly, exposure to mercury chloride and lindane induce a decrease in the energy budget of females of *Daphnia magna*, which might increase the short-term tolerance to the toxicants; however, the decrease of the metabolic rate also results in a reduction of the broad size (De Coen and Janssen 1997). Additionally, life-history characters such as development period, age at first reproduction, reproductive effort, etc., depend partially on the energy availability and on the resources allocated to these processes, playing an important role in the fitness (Sibly and Calow 1987). The energetic analysis is useful in ecotoxicological studies since it describes the fitness of the organisms to the environment (Widdows and Donkin 1991, Genoni 1997).

b. Alterations on the avoidance response or change of the environmental or biotic preferences. In contrast to sessile organisms, mobile animals have the ability to escape from stress conditions. The organisms respond to stressful conditions by moving to zones where their functions are more efficient. This behavioral compensation is especially important in organisms unable to control its internal environment for a specific physical or chemical factor. However, many pollutants induce retardation of escape responses to extreme environmental stress, or even in some cases fish may show preferences for toxicants, which may reduce their probability of survival or decrease the reproductive success of individuals when facing variable conditions (Kramer *et al.* 1997). Several pollutants impair the sensory capacity of individuals to discriminate the quality of the resources. Particularly, ectotherms perceive and respond to temperature in an adaptive manner, moving to temperatures in which their functional abilities are displayed efficiently, however the exposure to toxicants can modify the thermal preferences (Giattina and Garton 1983). For example, exposure to sublethal concentration of detergents modifies the temperature preference of the grass carp, resulting also in a decreasing growth rate and lower thermal tolerance (Alcaraz *et al.* 1993). Assuming that animals select the habitat conceding the highest fitness, any change on the habitat preference will reduce its performance. In particular, changes in habitat selection induce direct effects modifying the distribution and abundance of the organisms in the environment and indirect consequences via changes on the energy transference between organisms (trophic relationships).

A further important aspect on biotic preferences is the effect induced by pollutants on the mate choice.

Commonly, females receive benefits from choice and mate with males bearing certain traits. Under certain stress conditions, the female discrimination ability may change mainly as consequence of modified sensorial perception, resulting in potential lower reproductive success and changes on the selection and frequency of phenotypes in the environment due to sexual selection (Hoffmann and Parsons 1997). Shifts in biotic preferences induced by pollutants at which the organisms have little or no evolutionary history of exposure may induce severe effects on fitness (Kramer *et al.* 1997).

c. Diminished functional capacity and performance associated with decreases of the ability of the organisms to adjust their physiological rate to the environmental changes. These abilities are particularly important in organisms that inhabit fluctuating environments or extreme conditions. These damages may be identified as a decreased tolerance to fluctuations of physicochemical parameters (such as temperature, salinity, some chemical, etc.) or decreases on the performance ability specially related to some functions, such as swimming. The challenge tests, such as the critical thermal maximum (CTM), are frequently used to assess the performance capability under stressful conditions. These tests must be carefully selected or designed depending on the organisms, on the environmental conditions in which inhabit, and on the functional responses they display in their natural environment. For example, nitrite exposure decreases the resistance to high temperature, measured through the CTM, and the tolerance to hypoxic conditions in the shrimp *Penaeus setiferus*, these changes show a decrease of shrimp ability to face usual environmental stresses in rearing conditions.

d. Changes in the expression of relevant morphological traits. In particular, the changes in color and ornaments commonly result in changes in outcome of the male-male contest or female choice, which consequently results in different frequencies of phenotypes in the environment and therefore shift in the population (Hoffmann and Parsons 1997). Additionally, the alteration of these morphological traits may induce also changes in the relation among species when cryptic coloration or morphology are modified. When these kinds of changes take place, the success of the organisms on their environment decline due to decrease of reproductive success or increase of predatory detection, or due to indirect effect related to both aspects. In this sense, it is expected that changes in the expression of these traits anticipate changes in the relationship within or among species being confident stress indices. Furthermore, the fluctuating asymmetry (FA) of bilateral characters is an important approach in environmental studies. This measure is based in differences between paired traits of the right and left size of the body. FA is high in organisms that have been exposed to stressful condition during their development, providing a confident measure of the physiological condition and previous history of the organism (Markow 1995). The mate attractiveness decreases as the FA increase, showing that female are able to discriminate between males with different physiological condition (mate quality). Although the FA is a relevant stress index it has been occasionally used in ecotoxicological studies (Markow 1995).

The use of relevant physiological and behavioral responses in Ecotoxicology is based on the premise that environmental perturbation may be identified through the biological condition of individuals. Because of individual organisms are the basic units of the ecosystem, the source of population and community deterioration is found at this level. This assumption is based on the linkage between the fitness of individual organisms and the condition of the population.

Additionally, some indices of low biological organization levels (suborganismic functions) show a close linkage with the individual performance of the organisms. Some of these, such as the immune response and the genetic integrity are important aspects related to the fitness of the organisms to their environment. Likewise, some biochemical indices have received special attention, such as the adenylate energy charge (AEC) that quantify the amount of metabolically available energy stored in the adenylate pool (adenine monophosphate, diphosphate, and triphosphate). The proportion of these energetic carriers shows the nutritional condition and energy availability of the organisms to perform certain functions (Ivanovici and Wiebe 1981). These indices of low organization level and other, such as listed in **Table I** represent highly sensitive indicators of the health condition of the organisms.

CONCLUSIONS AND PERSPECTIVES

The goal of ecotoxicological studies is concerned with the effect of pollutants on the environment, however the tools used often cover a wide spectrum of biological levels (Maltby and Naylor 1990). To obtain a close approximation of the damages caused by pollutants on the environment, we should answer questions that approach the problem at the appropriate level. When the research problem is focused on the environment, ecological studies are appropriate. However, the great complexity of the environment requires the measurement and integration of a great quantity of information about biotic and environmental variables. The plasticity of the environment and its dynamic equilibrium (Feder *et al.* 1990) make the understanding of the environmental conditions on studies difficult as they are limited by time and space. Thus, a direct ecological approach requires long-term studies to obtain confident results. Meanwhile, the abrupt environmental changes caused by anthropogenic factors demand that short-term studies be made to evaluate proximate consequences and provide immediate and feasible solutions to the problem. In this sense, a viable alternative is to investigate the causal effect through relevant individual responses (stress indices) capable of constituting a linkage between individual animal responses and the condition of the population and possibly the communities (Seitz and Ratte 1991).

On the other hand, although laboratory studies provide highly accurate data about individual organisms, they might lack ecological realism because of the usual simplicity in which the experimental tests are done (constant and fixed experimental conditions, single toxicant exposure, etc.). Thus, special care must be taken to reproduce more authentic environmental conditions. It is important to consider that individual pollutants rarely occur alone in the environment and that typical single species fail to account for the complexity of the environment. Additionally, no single stress index is appropriate to recognize the condition of the individual or population condition, so multiple indices should be used. Relevant physiological and behavioral processes appropriate to the mode of action of pollutants should be selected to support ecological conclusions, mainly caused by differences on the action mechanisms and target organs (Brouwer *et al.* 1990).

According to Bayne (1985), any disadvantage caused by pollutants on individuals (stress) is enough evidence of a detrimental condition of the population. Nevertheless, three important aspects should be considered in studies based on individual organisms. First, caution must be taken when assuming that any behavioral or physiological change indicate disadvantage or stress. Second, no single index can provide sufficient information about the health and biological capacity of organisms, so multiple stress tests should be made (Heath 1987). Third, appropriate physiological indices should be carefully selected, considering the feature of the organisms that might be studied, the characteristics of the environment, and mode of action of the pollutants.

Finally, it seems unreasonable to attempt the study of the effect of overall environmental pollutants in every organism, so a viable way would be to explore patterns able to bring understanding and generalizations. This goal could be achieved by grouping chemicals with respect to their "behavior" and biological action (Genoni 1997), and studying their effects on ecological groups rather than taxonomic ones. In order to obtain confident conclusions the information should be integrated, to the better understanding of the interaction among the environmental variables, the responses of organisms, and the mode of action of the pollutants. In this sense, the use of relevant stress indices in micro and mesocosm designs that include ecological meaningful processes can be useful, giving resolution to particular problems by determining appropriate linkages between physiological and behavioral responses and population or community conditions.

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