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Clinical indicators of stress-induced changes in aquatic organisms

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Introduction

The effects of chemical and physical alterations of the environment have traditionally been monitored by observing changes at the population or community levels of biotic organization (GAUFIN 1973, LUGO 1978, WARD et al. 1978). This type of monitoring is limited because adverse effects in the environment have already occurred. While responses to stressors are observed at the ecosystem, community, and population levels or organization, the effects are manifested at the organismal level by causing death or impairing organismal function. More specifically, chemical toxicants exert their effects by interacting with biomolecule receptors in organisms. For predictive purposes, the tolerance of organisms to toxicants has been reported as acute lethality or effects on reproduction or behavior. More recently, as the discipline of aquatic toxicology has matured and environmental regulation has become more complex, responses to toxicants at the sub-organismal levels of organization, such as physiological, histological and biochemical, which are generally referred to as "clinical" measures, have been considered as viable monitors of responses of organisms to stressors (WEDEMEYER & YASUTAKE 1977, CAIRNS et al. 1984, BARRETT & ROSENBERG 1981, PICKERING 1981, GOLDSTEIN 1975).

Discussion

Stressors

The term stress is often used in relation to the effects of toxicants on individuals, populations or ecosystems. Unfortunately, this term has been used in so many different toxicological contexts that it has lost any critical connotation. Stressors can be defined as any force that pushes the functioning of a critical subsystem beyond its ability to restore homeostasis through ordinary, nonemergency adjustment processes (LUGO 1978). In my discussion I distinguish between stressors and stress in the manner of SELYE (1956) and FITCH & JOHNSON (1977). A stressor is the causative agent, condition or situation that causes strain or deviation from the normal homeostatic range and results in an organism mobilizing its resources and increasing its energy expenditure. Stress is the organism's response to the stressor.

Tolerance to a stressor is an organism's ability to operate normally within its homeostatic range without resisting the stressor in a demonstrable way, whereas resistance is an adaptive response to the stressor where energy is used to do work to resist the effects of the stressor. This could be the synthesis of replacement or alternative functional or structural proteins or substrates.

General adaptation syndrome

SELYE (1976) described the response of an individual vertebrate organism to stressors in terms of a succession of physiological and biochemical reactions. These responses were observed to occur in a variety of organisms exposed to a variety of stressors, thus SELYE referred to the responses as the "general adaptation syndrome" (GAS).

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The GAS is divided into three main parts: the "alarm stage", the "resistance stage" and the "exhaustion stage". The alarm stage is characterized by the "flight or fight" response, during which the stressor elicits a response to mobilize the organism's defenses. In vertebrates, epinephrine, which is released into the blood system from the adrenal glands, increases the activity of the sympathetic portion of the autonomic nervous system, which results in a wide range of physiological and biochemical changes (PICKERING 1981, CAIRNS et al. 1984). The second phase of the GAS has been described as the resistance stage and offers general long-term protection against stressors. The third and final stage of the GAS is refered to as exhaustion and occurs after long-term, continuous exposure to a stressor. In the exhaustion phase, energy stores become depleted and an organism's ability to continue to resist the stressor is reduced and death of the stressed organism follows.

Based on the above discussion, a model of an individual's response to a stressor can be constructed. An undisturbed individual operates within a normal homeostatic range. Also, there is a wider range of conditions for any given parameter within which an organism can continue to function normally for some finite period of time. Organisms can adapt to even greater deviations from the normal range by alteration of function or activity. Such alterations will, in time, affect other systems. The dimensions of homeostatic ranges vary within- and among-species both spatially and temporally. The response parameter can be either an enzyme activity or substrate concentration and can increase or decrease in response to a stressor. The utility of clinical measures of toxicant-induced stress is to relate short-term changes in biochemical and physiological parameters to imminent adverse effects on individuals and thus population and communities.

The concept of a general monitor of stressor-induced responses in organisms is important because one limitation of clinical indicators is their specificity. That is, advanced knowledge of the potential stressor is necessary before an appropriate clinical measure can be devised. Thus, while individual clinical indicators may be sensitive and measurable antecedent to population-level effects, the probability of choosing the appropriate system to monitor may be small. It is for this reason that non-specific indicators of stress have been advocated.

Advantages of clinical measures

Clinical indicators of the effects of chemical and physical stressors are attractive alternatives to more traditional measures for several reasons. Under field conditions, organisms are exposed to a multiplicity of both chemical and physical stressors, against a backdrop of naturally occurring seasonal fluctuations and ontogenetic processes, which in and of themselves are potentially stressful to the organism. One goal of biochemical ecotoxicology is to investigate general integrative measures of stress, which will allow one to evaluate the subsequent strain elicited in individuals. Clinical indicators have the potential to act as integrative measures of the sub-organismal level to indicate adverse conditions antecedent to population-level effects. In addition, since the toxicological response to a chemical is caused by the interaction between the toxicant and a biochemical receptor, biochemical responses would be expected to be the most immediate. That is, these responses would occur before responses at higher levels or organization. Therefore, clinical indicators should be more sensitive to toxicants. They should change at smaller

concentrations of toxicant than would be required to elicit organism-level responses and they should respond more rapidly than the whole organism.

The specificity of clinical indicators can also be a useful characteristic. While nonspecific clinical indicators of adverse effects have been developed, most clinical indicators of toxic effects are more specific than measures at more complex levels of organization. In many cases the primary receptor or locus of toxic action can be identified. Examples include inhibition of acetylcholinesterase activity in brain tissue by organophosphate compounds (Coppage et al. 1975) and α-aminolevulinic acid dehydratase activity by lead (Hodson et al. 1984).

Clinical measures of toxicant effects can be useful under both laboratory and field conditions. Previously, I noted that clinical indicators can be useful under field conditions because the responses are manifested at the biochemical level of organization before population level effects occur. This is also an important attribute in laboratory testing schemes because of the costs of conducting bioassays. If shorter-term end points, rather than growth or reduced fecundity, can be developed, screening tests could be conducted for shorter periods of time and be less costly.

Limitations of clinical indicators

Within a single species a number of parameters such as diet, genetic strain, sex, age, reproductive state, water temperature and sampling techniques can affect the "normal" range of biochemical indicators such as enzyme activities and substrate concentrations (Barnhart 1969). Also, activities of enzymes can vary greatly among tissues within the same organism (Boucr 1980) and among species. Therefore, I suggest that representative species be selected for intensive study in different habitats. For instance, because of the large volume of information presently available, rainbow trout are one such species.

Mayer (1983) and Mehrle & Mayer (1980) have discussed the paradox of clinical tests in aquatic toxicology. Understanding the biochemical toxicology of aquatic organism is impeded by the lack of knowledge of the basic biochemistry of aquatic organisms, including background "normal" physiological ranges (Mehrle & Mayer 1980). This paradox greatly limits the applicability of many clinical tests for aquatic organisms. Much is known about the physiology and biochemistry of humans and standard surrogate species so that changes in enzyme activities or substrate concentrations can be evaluated, relative to an established normal range for individuals of a given geographic region or genetic stock. Developing a strong background data base for specific organisms is essential. However, not only does one need to establish the "normal" range of a particular indicator, but one also must establish the statistical confidence in determining that the value for a parameter is out of the normal range. Confidence, expressed as statistical power, is dependent on the variance of the parameter values, as well as the sample size. I advocate determining the standard deviation of the parameter values and power analysis to establish the required number of samples to demonstrate a statistically significant effect at a given power (Type I and Type II error) (Giesy & Allred 1985, Wedemeyer & Yasutake 1977, Bayne et al. 1981). Few studies provide information on the range of values for a parameter, let alone allow one to calculate a standard deviation. However, Wedemeyer & Nelson (1975) provide the basis for calculating both gaussian (parametric) and nonparametric tolerance intervals to estimate "normal" ranges for physiological and biochemical parameters in aquatic organisms.

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It is extremely important that the response of clinical indicators be related to ecologically relevant responses of individuals and populations (MEHRLE & MAYER 1980). Clinical measures of pathological effects are successful in humans because a large body of correlative information exists. Because this type of information is lacking for aquatic organisms, biochemical indicators will never be as useful in assessing toxicant-induced effects in aquatic organisms as they are for mammalian species.

Measurable endpoints

While a number of different end points have been used as measures of the effects of stressors, they can be placed, for purposes of discussion, into six major categories: 1) Hormones; 2) Energetics; 3) Enzyme activities; 4) Osmoregulatory electrolytes; 5) RNA/DNA and protein content; and 6) other substrates. Because they have been frequently used as clinical indicators and are the chemical communication system of the body and known to respond to stress and responsible for initiating changes to allow homeostatic regulation, I will briefly discuss the use of hormones assess the health of aquatic organisms, as an example of "clinical" measures of stress.

Hormones

To maintain homeostasis, aquatic organisms must compensate for the physiological and biochemical alterations incurred by exposure to a stressor. Coordination of the physiological and biochemical processes necessary to regain homeostasis involves the coordination of neuronal and hormonal elements. Thus, an assessment of the hormonal status of an organism can yield information on the intensity of stress impinging on an organism. This approach has been used extensively with a few well-understood hormones in fish. However, little research on the effects of stressors on the hormonal systems of invertebrates has been performed due to the much smaller body of information on invertebrate endocrinology.

Endocrine responses to stress may be: 1) primary response, aimed to increase the survival of the organism, 2) a secondary or tertiary response, an attempt to maintain biochemical homeostasis within the organism, 3) an effect on plasma clearance of toxicant due to metabolism and tissue uptake, or 4) a toxic effect on the endocrine organ that causes altered hormone synthesis or release.

Fish

Hypothalamus – pituitary interrenal (HPI) axis

The HPI axis is stimulated due to a wide variety of stressors, including toxicants or physical stressors such as confinement and handling (DONALDSON 1981, WEDEMEYER & YASUTAKE 1977). The HPI axis is rapidly stimulated by exposures to physical stressors, which leads to increased concentrations of cortisol in plasma.

Exposure of fish to toxicants causes alterations in plasma corticosteroids and interrenal ascorbic acid concentrations. The magnitude and duration of these effects are dependent on the type of toxicant, toxicant concentration, species, and acclimation temperature (WEDEMEYER 1971, SWIFT 1981, THOMAS et al. 1981a, SWIFT 1982). In general, plasma cortisol concentrations in fish increase rapidly following onset of exposure and return to pre-exposure concentrations within 6 t0 12 h (THOMAS et al. 1981b). In addition

to stressors, numerous biotic variables affect the activity of the HPI axis in fish. These factors must be considered in utilizing an HPI axis assay in the laboratory and will make field studies on plasma corticosteroid concentrations difficult to interpret.

Quantification of the HPI axis and chromaffin tissue activity are useful indicators of stress in fish, however, at present it has not been demonstrated that toxicant exposures cause increased catecholamine concentrations in fish plasma. Indices of HPI axis and chromaffin cell activity are not expected to be especially useful long-term indicators of stress because: 1) concentrations of corticosteroids and catecholamines in plasma occur for only a short duration after the initial stress; 2) stressor-mediated effects on the HPI axis and chromaffin cell activity have not been directly related to ecologically relevant effects on growth, reproduction or mortality. Thus, I feel their use in establishing safe levels of stress are currently limited and should be used in experiments in which blood is sampled shortly after the stress such as in handling, transportation and stocking of fish. In fact, these acute stressors will probably mask any chronic toxicant-induced effects. I do not recommend these techniques be utilized to assess the effects of long-term, toxicant-induced effects in fish.

Catecholamines

A variety of physical stressors and hypoxia alter concentrations of catecholamines in both plasma and chromaffin tissue. Unfortunately, the effects of chemical stressors on these hormones have not been investigated. Much of the work which has been conducted on the effects of stress on catecholamines in fish has been revised by Mazeaud & Mazeaud (1981). In teleosts subjected to a variety of stressors, concentrations of both adrenaline and noradrenaline in plasma increase. Forced swimming, hypoxia, cannulation, and handling all result in rapid and usually transient increases in plasma catecholamine concentrations (Nakano & Tomlinson 1967, Butler et al. 1978, Wahlqvist & Nilsson 1980, Mazeaud & Mazeaud 1981). During physical stress, the time course of the increase of catecholamine concentrations in plasma and the specific hormone exhibiting the greatest increase is species-specific. There is little information on the effects of biotic factors on plasma catecholamine concentrations in fish.

Other hormones

The effects of stressors on concentrations of hormones other than the corticosteroids and catecholamines in plasma have not been well studied. However, effects would be expected since hormones are involved in maintaining homeostasis and chemical stressors are known to perturb this homeostasis. Thyroid hormones are important in regulating intermediary metabolism in fish (Plisitskaya et al. 1983) and appear to play a role in the primary stress response; however, additional research into the function of and effects of biotic factors on these hormones is needed.

Prolactin is produced and released by the pituitary and is believed to be under inhibitory control by a hypothalamic hormone. It is generally believed that prolactin affects osmoregulation in fish and has been shown to affect branchial water permeability and ion flux at the gill and kidney (Folmar & Dickhoff 1979, Flick et al. 1984, Wendelaar Bonga et al. 1984). Insulin is produced in the pancreas and secreted to promote cellular glucose uptake and utilization. Insulin causes hypoglycemia, decreased hepatic glycogen,

FOLMAR, L. C. & DICKHOFF, W. W., 1979: Plasma thyroxine and gill Na^+-K^+ ATPase changes during seawater acclimation of coho salmon, *Oncorhynchus kisutch*. — *Comp. Biochem. Physiol.* **63A**: 329—332.

FONTAINE, Y. A., 1975: Hormones in fishes. — In: *Biochemical and biophysical perspectives in marine biology.* **2**: 139—212. D. C. MALINS & J. R. SARGENT (eds.). Academic Press, New York.

GAUFIN, A. R., 1973: Use of aquatic invertebrates in the assessment of water quality. — In: *Biological methods for the assessment of water quality*: 96—116, J. CAIRNS, Jr. & K. L. DICKSON (eds.). ASTTM STP 528.

GIESY, J. P. & ALLRED, P. M., 1985: Replicability of aquatic multispecies test systems. — In: *Multispecies toxicity testing*: 187—247, J. CAIRNS (ed.). Pergamon Press, New York.

GOLDSTEIN, A., 1975: Biochemical indicators of environmental pollution. — In: *Indicators of environmental quality*: 109—131, W. A. THOMAS (ed.). Plenum Press, New York.

HODSON, P. V., BLUNT, B. R. & WHITTLE, D. M., 1984: Monitoring lead exposure of fish. — In: *Contaminant effects on fisheries*: 87—98, V. W. CAIRNS, P. V. HODSON & J. P. NRIAGU (eds.). John Wiley and Sons, New York.

LUGO, A. E., 1978: Stress and ecosystems. — In: *Energy and environmental stress in aquatic systems*: 62—101, J. H. THORP & J. W. GIBBONS (eds.). Techn. Information Center, U. S. Dept. of Energy.

MAYER, F. L., 1983: Clinical tests in aquatic toxicology: A paradox. — *Environ. Toxicol. Chem.* **2**: 139—140.

MAZEAUD, M. M. & MAZEAUD, F., 1981: Adrenergic responses to stress in fish. — In: *Stress and fish*: 49—76, A. D. PICKERING (ed.). Academic Press, London.

MEHRLE, P. M. & MAYER, F. L., 1980: Clinical tests in aquatic toxicology: State of the art. — *Environ. Health Perspect.* **34**: 139—143.

NAKANO, T. & TOMLINSON, N., 1967: Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri)* in relation to physical disturbance. — *J. Fish Res. Board Can.* **24**: 1701—1705.

PICKERING, A. D. (ed.), 1981: *Stress and fish.* — Academic Press, New York, 267 pp.

PLISETSKAYA, E., WOO, N. Y. S. & MURAT, J. C., 1983: Thyroid hormones in cyclostomes and fish and their role in regulation of intermediate metabolism. — *Comp. Biochem. Physiol.* **74A**: 179—187.

SANGALANG, G. B. & FREEMAN, H. C., 1974: Effects of sublethal cadmium on maturation and testosterone and 11-hetotestosterone production in-vivo in brook trout. — *Biol. Reprod.* **11**: 429—435.

SELYE, H., 1956: *The stress of life.* — McGraw-Hill Book Co., New York.

— 1976: *Stress in health and disease.* — Butterworth Publ., Boston, MA.

SWIFT, D. J., 1981: Changes in selected blood component values of rainbow trout, *Salmo gairdneri* RICHARDSON, exposed to hypozia or sublethal concentrations of phenol or ammonia. — *J. Fish. Biol.* **19**: 45—61.

— 1982: Changes in selected blood component values of rainbow trout, *Salmo gairdneri* RICHARDSON, following blocking of the cortisol stress response with betamethasone and subsequent exposure to phenol or hypozia. — *J. Eish. Biol.* **21**: 269—277.

THOMAS, P., WOFFORD, H. W. & NEFF, J. M., 1981 a: Biochemical stress responses of striped mullet (*Mugil cephalus* L.) to fluorene analogs. — *Aquat. Toxicol.* **1**: 329—342.

THOMAS, P., CARR, R. S. & NEFF, J. M., 1981 b: Biochemical stresss responses of mullett *Mullet cephalus* and polychaete worms *Neanthes virens* to pentachlorophenol. — In: *Biological monitoring of marine pollutants*: 73—103, J. VERNBERG, A. CALABRESE, F. P. THURBERG & W. B. VERNBERG (eds.). Academic Press, New York.

THORPE, A. & INCE, B. W., 1974: The effects of pancreatic hormones, catecholamines, and glucose loading on blood metabolites in Northern pike (*Esox lucius* L.). — *Gen. Comp. Endocrinol.* **23**: 29—44.

VERNBERG, W. B., CALABRESE, A., THURBERG, F. P. & VERNBERG, F. J., 1982: *Physiological mechanisms of marine pollutant toxicity.* — Academic Press, New York, 564 pp.

WAHLQVIST, I. & NILSSON, S., 1980: Adrenergic control of the cardiovascular system of the Atlantic cod, *Gadus morhua*, during "stress". — *J. Comp. Physiol.* **137**: 245—250.

WARD, J. V., CANTON, S. P. & GRAY, L. J., 1978: The stream environment and macroinvertebrate communities: Contrasting effects of mining in Colorado and the Eastern United States. — In: *Energy and environmental stress in aquatic systems*: 176—187, J. H. THORP & J. W. GIBBONS (eds.). U. S. Techn. Information Center, U. S. Dept. of Energy Symposium Series 48 (CONF-771114).

WEDELAAR-BONGA, S. E., VAN DER MEIJ, J. C. A. & FLICK, G., 1984: Prolactin and acid stress in the teleost *Oreochromis* (formerly *Sarotherodon*) *mossambicus*. — *Gen. Comp. Endocrinol.* **55**: 323—332.

WEDEMEYER, G., 1971: The stress of formalin treatments in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). — *J. Fish Res. Board Can.* **28**: 1899—1904.

WEDEMEYER, G. A. & NELSON, N. C., 1975: Statistical methods for estimating normal blood chemistry ranges and variance in rainbow trout (*Salmo gairdneri*), shasta strain. — *J. Fish Res. Board Can.* **32**: 551—554.

WEDEMEYER, G. A. & YASUTAKE, W. T., 1977: *Clinical methods or the assessment of the effects of environmental stress on fish health.* — Techn. Paper No. 89, U. S. Fish and Wildlife Service, Dept. Interior, Washington, DC, 18 pp.

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decreased serum fatty acids, and decreased plasma amino acids (THORPE & INCE 1974, FONTAINE 1975, WENDELAAR BONGA et al. 1984). Plasma insulin concentrations may prove useful in understanding the response of plasma glucose and hyperglycemic hormones during stress in fish.

Thyroid hormones, prolactin, insulin, and other hormones may be excellent biochemical tools for assessing a fish's response to stress. However, at present insufficient information on the function of these hormones and their response to stress is available. To date no research has been performed to relate ecologically relevant effects on growth, reproduction or survival to changes in hormones. We recommend further research be conducted on the effects of stressors on these hormones and that quantification in blood be considered for future use to better understand the mode of toxic action of persistent environmental contaminants.

In contrast to the effects of stressors on other hormones, concentrations of circulating sex-related steroids, such as the androgens and estrogens are altered directly by toxicant effects on hormone synthesis or steroid metabolism. Altered concentrations of the sex steroids in plasma are believed to cause in reproductive impairment. Determination of sex-steroid concentrations in plasma and metabolism of these hormones may be of use in assessing the effects of stressors on reproduction in fish. Unfortunately few studies have linked effects on steroid production, metabolism, and concentrations in blood with altered reproduction (SANGALANG & FREEMAN 1974). Thus, a determination of the effect of stressors on reproduction has not been possible. We recommend research be conducted to link effects of stressors on the fertilization process, gamete production and embryo survival with sex hormone dynamics in fish. At that time it will be possible to determine how stressor-induced effects on reproductive hormones should be interpreted. Sex hormone dynamics may prove useful for assessing reproduction effects of stressors, however, their general use as indicators of stress is not recommended nor biologically supportable.

Invertebrates

Information on invertebrate endocrinology is available and some studies have been conducted on the effects of chemical stressors on the endocrine system of aquatic invertebrates (VERNBERG et al. 1982). However, the number of studies is much fewer than that conducted on the effects of chemical stressors on fishes. This fact, along with the limited understanding of the regulatory processes of these systems, severely limits the use of changes in the hormonal milieu of invertebrates as in in-situ indicator of toxicant-induced stress. In addition, sensitive analytical techniques for measuring specific hormones on a routine basis generally is not available. Also, as with many of the fish hormones, the sensitivity of the response to physical (handling) and normal chemical stresses, such as salinity changes, may impede one's ability to identify chemical-induced effects. Given that many of these limitations can be overcome by basic research on invertebrate endocrinology and the development of a strong data base on natural variations in hormone titres, endocrine alterations may, in the future, have applicability as a monitoring tool.

Conclusions and recommendations

I am of the opinion that environmental regulation is a very complex science and that to be effective ecotoxicologists must make use of all available tools. Ultimately, the

ecosystem and all of its components is the level of organization that we want to protect. Therefore, ecosystem-level monitoring will continue to be important, as will population and community-level studies. Biochemical indicators will not replace measures of toxicant effects on individuals, populations, communities or ecosystems but rather will add to and enrich our understanding of toxicant-induced effects. Specifically, an understanding of biochemical responses will elucidate the modes of action of toxicants, which will provide greater generality. This increased understanding will subsequently serve as the basis of improved predictability of toxic effects among toxicants and species and allow better extrapolation to different environmental conditions. Furthermore, understanding of biochemical toxicology will allow one to better predict the interactions of toxic mixtures.

Few clinical measures have been sufficiently well developed to be used in routine monitoring programs. We presently lack the knowledge to implement many of the clinical measures. Therefore, I endorse the continued development of these sensitive assays for use under field conditions.

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References

BARNHARDT, R. A., 1969: Effects of certain variables on hematological characteristics of rainbow trout. — *Trans. Amer. Fish. Soc.* 3: 411—448.

BARRETT, G. W. & ROSENBERG, R., 1981: *Stress effects on natural ecosystems.* — John Wiley & Sons, New York, 305 pp.

BAYNE, B. L., CLARKE, K. R. & MOORE, M. N., 1981: Some practical considerations in the measurement of pollution effects on bivalve molluscs, and some possible ecological consequences. — *Aquat. Toxicol.* 1: 159—174.

BOUCK, G. R., 1980: Concentration of leucine aminonaphythylamidase (LAN) and soluble protein in tissues of rainbow trout, *Salmo gairdneri.* — *Can. J. Aquat. Sci.* 37: 116—120.

BUTLER, P. J., TAYLOR, E. W., CAPAR, M. F. & DAVIDSON, W., 1978: The effect of hypoxia on the levels of circulating catecholamines in the dogfish *Schyliohinus canicula.* — *J. Comp. Physiol.* 127: 325—330.

CAIRNS, V. W., HODSON, P. V. & NRIAGU, J. P., 1984: *Contaminant Effects on Fisheries.* — John Wiley and Sons, New York, 333 pp.

COPPAGE, D. L., MATTHEWS, E., COOK, G. H. & KNIGHT, J., 1975: Brain acetycholinesterase inhibition in fish as a diagnosis of environmental poisoning by malathion, 0,0-dimethyl S-(1,2-dicarbethy oxyethyl) phosphodithioute. — *Pest. Biochem. Physiol.* 5: 536—542.

DONALDSON, E. M., 1981: The pituitary-interrenal axis as an indicator of stress in fish. — In: *Stress and fish*: 11—47, A. D. PICKERING (ed.). Academic Press, London.

FITCH, K. & JOHNSON, P., 1977: *Human life science.* — Holt, Rinehart and Winston, Inc., New York.

FLICK, G., WENDELAAR BONGA, S. E. & FENWICK, J. C., 1984: Ca^{2+}-dependent phosphatase and Ca^{2+}-dependent ATPase activities in plasma membranes of eel gill epithelium-III. Stimulation of branchial high-affinity Ca^{2+}-ATPase activity during prolactin-induced hypercalcemia in American eels. — *Comp. Biochem. Physiol.* 79B: 521—524.