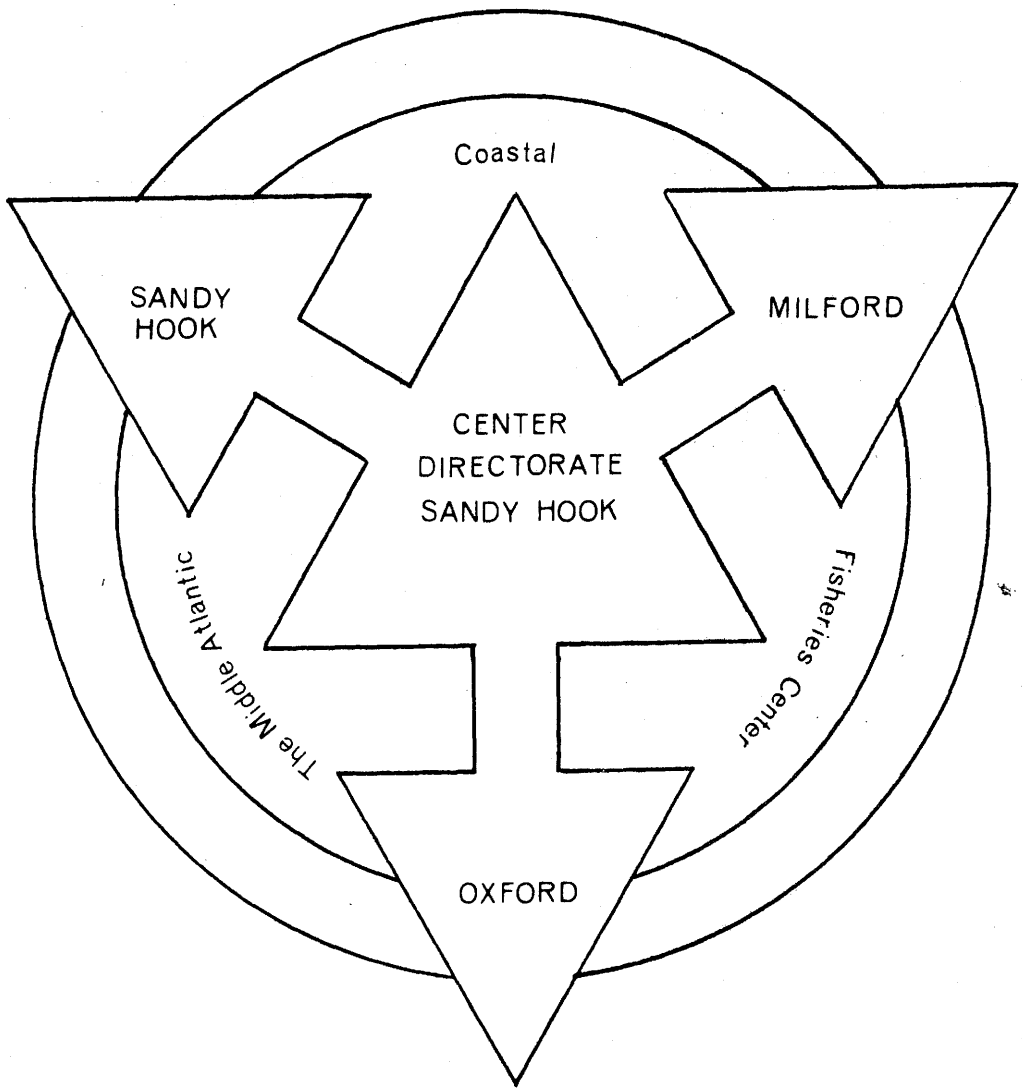
BEHAVIORAL MEASURES OF ENVIRONMENTAL STRESS: MARINE FISHES AND INVERTEBRATES. ENERGY RESEARCH AND DEVELOPMENT PROPOSAL (RENEWAL) CONTRACT NO. AT(49-7)3045, 1977

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Region

MIDDLE ATLANTIC COASTAL FISHERIES CENTER



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ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION PROPOSAL

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INTRODUCTION

The basic aim of our research is to observe and describe patterns of behavior in marine organisms as related to life habits and environmental requirements. Once established, the various strategies possessed by the organism to deal with the total range of selected environmental factors, can be identified, including those levels which may be stressful.

Such understanding of behavior under natural conditions may be used to design experiments in the laboratory, with the formulation of questions and interpretation of results derived from and related to the field observations of natural behaviors. From these findings we may predict the effect of man-induced perturbations, with the highest probability of predictive success on those which approximate natural stresses.

Our research efforts concentrated, at first, on measuring the direct effects of perturbations (primarily thermal) on specific patterns of behavior, particularly activity, with the effect observed within a normal range of predictability, i.e.,the possible responses being: no change, a decrease, or increase. For example, as temperature departed from acclimation levels, in pelagic species, activity increased reflecting avoidance potential and the ability of these animals to move in response to changing tem-

perature (Olla and Studholme 1971; Olla et al. 1975b). When temperature returned to acclimation levels, or to that range which the animal did not avoid, activity returned to prestress levels.

As our research effort expanded we began to consider the behavior of marine fish within their natural environment. Arising from these studies predictions on the effects of temperature perturbations were made that could not have been made without this knowledge. For example, young tautog, in contrast with adults, were shelter-dependent throughout the year (Olla et al. 1974). Because of this dependence, we hypothesized that these fish would have a reduced capability (as compared with pelagic species) for avoiding thermal perturbations, and in fact, would seek shelter when subjected to environmental perturbations. Laboratory experiments designed from knowledge of these animals under natural conditions, confirmed the hypothesis of shelter-seeking when under stress.

If investigations on young tautog had gone no further, we would have considered that shelter-seeking was a generalized response of young fish subjected to stressful changes in the environment. However, again, further knowledge of the animal under natural conditions indicated that this may not be the case. Preliminary results from field studies still in progress showed that when young tautog are subjected to shelter which is not appropriate the animal responds by moving from the area. (We are current-

ly attempting to define the essential physical and biological components of shelter; see Report and Proposal). Once it has left, the behavior in searching for appropriate shelter and associated resources is much more complex than simply entering cover. Predictions must now take into account the increase in complexity and probabilities of survival arising from such complexity. It is apparent that the hierarchy or ordering of the environmental requirements of the animal relates closely to the nature of the perturbing event.

Based on our findings to date, our research will center on defining comprehensively the natural flexibility of behavioral patterns expressed under various environmental regimes.

Where the stressing event is one that may be part of the evolutionary history of the organism, predictive confidence may be high. The serious problem arises when the stress is a novel event, i.e., one not part of that history. The predictions in this case will depend upon the ability of the animal to 1) detect the event; 2) distinguish the novel event as inimical, and 3) behave appropriately to mitigate damage. Thus, the environmental event itself must be considered and compared with others with which the animal previously has been biologically equipped to deal. It is our opinion that such considerations should be an integral part of almost any behavioral measure of environmen-

tal perturbation, as they will be in our own research.

Our research effort will be divided into laboratory and field programs. As in the past, laboratory studies will concentrate on 1) reproductive behavior in adult tautog, *Tautoga onitis*, and the effects of temperature on both behavior patterns and normal embryological development; 2) effect of low temperature on activity and feeding of juvenile bluefish, *Pomatomus saltatrix*; 3) chemosensory behavior of the hake, *Urophycis chuss*, and the effect of temperature on the behavior; 4) effect of temperature on the behavior of blue crabs, *Callinectes sapidus*, with a related study on chemosensory mechanisms in this species.

The field program will continue to focus on juvenile tautog and cunner, *Tautogolabrus adspersus*, and their environmental requirements for recruitment from the pelagic stage through the first year, relating the findings to the capabilities of dealing with environmental perturbations.

A. Laboratory Studies

1. The Effect of Temperature on Normal Reproductive Behavior of Adult Tautog, *Tautoga onitis*.

Two separate groups of adult tautog (two males and one female comprising each group) spawned in our 121-kl aquarium enabling us to describe reproductive behavior in detail (see current Progress Report for research results). Characteristic behavioral patterns associated with the progression of events leading to daily spawning were consistent from study to study, making it possible to establish clearly quantifiable components of the behavioral repertoire.

The reproductive behavioral repertoire is currently being utilized as a baseline to detect the effects of thermal stress on the second group of tautog.

This study presents a rare opportunity to measure sublethal effects on reproductive behavior of a marine fish species. Since we have comprehensive measures of the normal and stress repertoire of the species (see current report) we will be able to make insightful interpretations that otherwise would not be possible.

The major aim of this study will be to determine quantitatively the effects of a prolonged exposure to elevated, subleth-

al temperature on the reproductive behaviors of adult tautog. Concurrently, the effects of this stress on the development of the eggs and embryos will be investigated.

Procedure: Maintain three adult tautog (two males and one female) approximately 40-60 cm in the 121-kl aquarium under naturally changing photoperiod at an acclimation temperature of 18-20°C. Previous tests have shown that because of the normal territorial requirements and limitations of the facility, this is the optimum number of fish to be used. As in the two preceding tests, provide three terra-cotta tiles to serve as shelter. Supply live mussels as needed to serve as a natural food source and allow *ad libitum* feeding. Prior to spawning, using previously developed procedures (see current Progress Report on adult tautog), make measurements 15 min each hour (0800-1500) on activity (time swimming in water column or moving about on the sand). At the same time record sequentially for each fish for 50 counts the following: zone location, number of feeding ingestions and aggressive interactions to establish baseline measures. Observe in 4-day sets each week.

As spawning season begins, record continually for 1 h prior to each spawning the characteristic behaviors associated with daily gamete release. Behaviors observed will include frequency

and intensity of aggressions, frequency of prespawning rushes toward the female, description of progressive shading changes in the female and the final courtship activities culminating in gamete release as correlated with specific components of the courtship repertoire (see Progress Report).

Following each spawning, collect eggs and transfer to aerated containers maintained at the same temperature as the aquarium water. At 1, 12, 18, 24, 36 and 48 h, examine eggs and/or embryos using a binocular microscope fitted with an ocular micrometer and grid, measuring mean diameter of egg capsule and yolk, standard and total length of embryo, and head width. For all samples, calculate percent of development to each stage and viability. Preserve all samples in a buffered 5% formalin solution.

After reproductive activity stabilizes and egg development proves to be normal, gradually raise temperature (0.04°C/h) until changes in reproductive behavior and/or development of eggs and embryos indicate possible temperature effects. If subsequent acclimation of adults and/or resumption of normal embryological development is apparent, again raise temperature (0.04°C/h) until effects are evident(comparing behavior with previous experiments; current Progress Report). Continue daily and prespawning observations on adults as well as sampling and staging of eggs and em-

bryos to determine if development is normal. Depending on findings, decrease temperature (0.04°C/h) to determine if normal behavior and embryological development is reestablished.

2. The Effect of Low Temperature on Activity and Feeding of Juvenile Bluefish, *Pomatomus saltatrix*.

The past year we conducted two preliminary experiments on the effects of low temperature on juvenile bluefish, the results of which were encouraging enough to plan a series of 12 experiments to be performed this coming summer and fall. The number of experiments to be completed will depend on the degree of effort required to complete the study on tautog reproduction as well as the field program. Besides the comparative value of the results in gaining an understanding of behavioral response capabilities, the results of these studies may be directly applicable to problems encountered during power-plant shut-downs in winter when significant mortalities of juvenile bluefish were observed. These results will be compared with previous studies on adults (Olla and Studholme 1971) and on juveniles to elevated temperature (Olla et al. 1975b).

Procedure: For each test, establish a group of seven juvenile bluefish, approximately 18-25 cm, in a 1.5-kl experimental testing system (for description of system see Olla and Studholme 1975; an additional testing system will be constructed to allow replicate testing). Hold fish for 3-4 weeks under a constant 12-h photoperiod at an acclimation temperature of 20°C. During this period

begin feeding test fish at 2-day intervals, measuring normal chemosensory response to a standardized freeze-dried clam extract, including latency to detection, excitation and localization. Three minutes after extract introduction, introduce live prey, Fundulus heteroclitus (5.0-7.0 cm) using 1 prey/test fish/introduction, measuring for each introduction latency for ingestion by the first fish, duration of feeding, total number of prey captured, and motivation to feed based on swimming speeds. Introduction of food will cease when capture rates fall to 20-30%.

Once feeding has stabilized begin observations to establish normal behavior including activity rhythms, mobility, school orientation and cohesion. For 10 min/each hour, make 5 consecutive readings of swimming speed (time for the fish to swim a fixed distance), the number of fish schooling or grouped, and their positions in the water column. Once these behaviors appear to have stabilized, lower the temperature. Rates of decrease will range from 0.02°C/h to approximately 0.6°C/h with each test concluding when the majority of the fish (4) have died. Analyze data for presence and extent of correlation between rate of temperature decrease and observed behavioral responses.

3. The Effect of Temperature on Chemosensory Behavior of Hake, *Urophycis chuss*.

Up to the present, most of our studies have concentrated on those behaviors which are primarily mediated by visual mechanisms. To gain a fuller understanding of the behavioral response mechanism, we have begun to examine organisms in which chemoreception plays a major role in their life habits.

Preliminary studies performed by us this past year have shown that the hake, *Urophycis chuss*, meets this criterion. In addition, the species is ecologically and environmentally important inasmuch as it resides in areas likely to be impacted by energy-related activities, e.g. established and proposed electrical generating plants and petroleum exploitation and production facilities.

We may divide chemoreception into detection, excitation, localization and motivation for both feeding and reproductive responses. At the outset, we will concentrate our effort on feeding.

Preliminary work this past year has enabled us to standardize a freeze-dried extract for elicitation of feeding responses in *Urophycis*. We intend concentrating on establishing behavioral baselines over the normal range of temperature encountered by *Urophycis in situ*. We will then subject the animal to levels beyond those normally encountered. A future aim (for the next

2 years) is to test the animal to certain chemical contaminants, including petroleum constituents, at varying temperatures.

Procedure: Establish three hake (15-18 cm) in each of a series of 380-l aquaria under a constant 12-h photoperiod and at preferred temperatures. Monitor activity, interfish distance, and aggressive encounters. At various times of the day, introduce trace amounts of freeze-dried extract into each aquarium. Following extract introduction, measure for each fish the latency before detection and excitation, the degree and duration of excitation to establish normal behavioral baselines. Design and develop an experimental maze test system in which to observe and measure normal food searching behaviors which are mediated by chemoreception. Begin studies to determine the effect of sublethal temperatures on normal feeding responses.

4. The Effect of Temperature on the Behavior of Blue Crabs, *Callinectes sapidus*.

Preliminary observations on the relation of temperature and behavior in the blue crab, *Callinectes sapidus*, have shown that the content of the animal's repertoire varies with the temperatures to which the crab is naturally exposed. For example, blue crabs stop feeding below 13°C and are dormant at 4°C (see Progress Report). Because specific temperature levels also appear to influence intensity of aggression (Leffler 1972) and migration (Jaworski 1972) as well as feeding, our aim is to continue observations of the crab's general activities over a range of temperatures in order to discover those temperatures at which certain behaviors wax and wane in the crab's behavioral repertoire. Such an approach is necessary initially not only to facilitate recognition of thermal stress, but also to indicate those thermal conditions under which other pollutants might prove particularly stressful.

Parallel with our proposed efforts for the hake, *Urophycis chuss*, we wish to examine the effect of temperature on the chemoreceptive and subsequent feeding behavior of blue crabs. A solution of freeze-dried clam extract now appears to initiate feeding behavior readily and consistently. Now our aim is to present

this extract to crabs under various thermal regimes and measure the quantitative aspects of the feeding behavior.

a) The Effect of Temperature on Activities and Aggressions.

Procedure: Observe blue crabs in a temperature controlled 1200-l elliptical aquarium already constructed. Maintain juvenile and adult male crabs in water of 12 o/oo salinity and adult females in water of 25 o/oo. Add live mussels, clams and killifish to aquarium as well as adequate sand and shelter.

Mark zones of equal area in the aquarium to aid in measuring inter-animal distances and so to discern any changes in individual spacing or territoriality.

Continue describing the behavioral repertoire and time activities of crabs using a tape recording giving time markers every 4 s. Watch a crab and note its activity in code upon hearing each 4-s mark. A 4-s interval has proved long enough to allow note-taking and short enough not to overestimate behaviors of short duration. Compile the resulting data into a time budget of the crab's activities.

Observe aggression between individuals and measure their frequency, duration and intensity. Perform regression analysis to develop a statistical model of the relation between temperature and the frequency, duration and intensity of aggression.

b) Effect of Temperature on Chemoreception.

Procedure: Blue crabs presented with a freeze-dried hard clam extract readily respond in a way that allows the magnitude of the response to be scored (see Table I). After acclimating crabs to particular temperatures and a controlled diet of hard clam, present a known quantity of clam extract to individual crabs and measure the intensity, latency and threshold of the feeding response. Perform regression analysis to investigate the relation between temperature and the magnitude of the feeding response.

Measure at various temperatures the ability of crabs to find the source of the sapid extract in a Y-maze. Perform regression analysis to indicate how the efficiency of the feeding response is influenced by temperature.

Table I. Tentative Scoring of Blue Crab Feeding Response to Hard Clam Extract.

Score	Criteria
0	No visible response.
1	Increase in antennule flicking and antennule cleaning, Side-to-side motion of third maxillipeds.
2	Dactyl searching
3	Movement. Chelae probing.
4	Object manipulation. Ingestion attempts.

B. Field Studies

1. Environmental Requirements, Shelter-Dependence and Home Range in Young and Juvenile Cunner, *Tautogolabrus adspersus*, and Tautog, *Tautoga onitis*.

The field program, integrated closely with our own laboratory studies, will continue to define home range and environmental requirements of juvenile cunner, *Tautogolabrus adspersus* and tautog, *Tautoga onitis*, beginning at recruitment from the pelagic stage through the fishes' first year. In addition, we will continue to examine the effects of various natural stresses on shelter-dependence in cunner and young tautog.

Procedure: Directly observe and collect juveniles under natural conditions, using SCUBA and varying sampling and collection techniques as employed in previous studies (Olla et al. 1974, 1975a). Determine home range, habitat selection, social behavior and food habits in relation to various physical parameters. Analyze data to determine the extent of correlation between the observed behavior and physical parameters, and determine how certain behaviors are modified as a function of age.

While observing with SCUBA, expose young fish to stresses such as food deprivation, artificial shelters and predation. De-

termine the nature of the behavioral response to the type of stress imposed.

Initiate a tagging-recapture program for young fish. Determine their movements, home range, and selection and utilization of intermittent habitats. Analyze data to determine the presence and extent of correlation between these behaviors and population and seasonal factors.

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MIDDLE ATLANTIC COASTAL FISHERIES CENTER
Highlands, New Jersey

BUDGET

Project: Behavioral measures of environmental stress in marine fishes: Field and laboratory studies.

Project No. : E(49-7) 3045 Mod. No. 8

	FY 76T 7/1/76 - 9/30/76	FY 77 10/1/76 - 9/30/77
Direct Salaries (Includes Benefits)	5.3	12.7
Materials, Services, Supplies: Includes maintaining recirculation system for experimental animals, fish food, miscellaneous operating supplies, travel, etc.	7.1	18.4
Indirect Cost: Services and supplies of a general nature benefiting the project.	2.1	6.2
	14.5 1/	37.3 2/

1/ The costs of the project traditionally run more in the summer months; therefore, this amount is not a straight 1/4 of the cost for a 12 month effort.

2/ This amount inlcudes a 5% cost of living increase.

Middle Atlantic Coastal Fisheries Center
Highlands, New Jersey

BUDGET

Project: Effects of temperature on the behavior of marine invertebrates

Project No.: E(49-7) 3045 Mod. No. 7

	FY 76T 7/1/76 - 9/30/76	FY 77 10/1/76 - 9/30/77
Direct Salaries (includes Benefits)	3.7	18.7
Materials, Services, Supplies: Upkeep and maintenance of experimental tanks, food for experimental animals, operating supplies, travel, etc.	6.5	14.2
Indirect Cost Services and supplies of a general nature benefiting the project	1.5	7.3
	11.7 1/	40.2 2/

1/ The costs of the project traditionally run more in the summer months; therefore, this amount is not a straight 1/4 of the cost for a 12 month effort.

2/ This amount includes a 5% cost of living increase.

May 24, 1976