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**Regulation of Life History Strategies Within
Individuals in Predictable and Unpredictable
Environments**

by

Jerry Dale Jacobs

A dissertation submitted in partial fulfillment
of the requirements for the degree of

Doctor of Philosophy

University of Washington

1996

Approved by

John C. Wingfield

(Chairperson Of Supervisory Committee)

[Signature]

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Program Authorized
to Offer Degree

Zoology

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University of Washington

Abstract

Regulation of Life History Strategies Within Individuals in Predictable and Unpredictable Environments

by Jerry D. Jacobs

Chairperson of the Supervisory Committee: Professor John C. Wingfield
Department of Zoology

Animals must adjust their physiology and life histories to conform with changes in the environment. These changes can be fairly predictable, such as the changes in the season, or they can be unpredictable, such as an attack by a predator or an unusual storm. A finite state machine model was developed to understand individual responses to changing environments. The model was used to investigate life history diversities, and changes in animals associated with unpredictable environmental events. Several conclusions were drawn from the model with regards to unpredictable events: Animals in very predictable environments, very unpredictable environments, and animals in constrained environments would not show the normal adrenocortical responses to unpredictable events. The predictions were tested with experiments, and follow the general trend of the data present.

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Chapter I:

Using a Finite State Machine Model to Understand Organismal Responses to Changes in the Environment.

Introduction

No free-living organism lives in a truly constant environment. All species are exposed to fluctuations in environmental conditions, some of which may be extreme. For example, in arctic environments, temperature, food availability, and the background color of the environment vary radically in a yearly seasonal cycle. In some tropical regions, the dry and rainy periods occur at specific times of year. Even in places that at first seem exceedingly stable, the environment fluctuates. In caves, where the yearly temperature may only fluctuate on the order of 2 C°, seasonal flooding may occur, providing changes in the nutrient input from non-cave sources (Poulson, 1964). Some cave animals get their food from nematodes within bat feces, or on the fungus which on the guano. Thus there may be seasonal variations in food availability within the cave due to seasonal changes in insect populations (food for the bats) outside, and changes in the activity level of the bats (Chapman, 1993).

Unless the environmental fluctuations are very small, an organism will have to modify its physiology to survive as conditions change (e.g. Prosser, 1986). In this dissertation, the term physiology is used in its broadest sense to include all the

biological properties of the organism, including morphology and behavior (Eckert et al., 1988). Physiology can be adjusted several ways to maximize survival in a fluctuating environment. Some invertebrates use a developmental strategy to adapt to long range, predictable changes. The phenotype of the adult is determined by changes in the development pathway, leading to adults with appropriate physiology (e.g., Tauber *et al.*, 1985 and Mustafa and Hodgson, 1984), or individuals that will enter diapause in order to overwinter (Hairston, and Olds, 1986). In others, the presence of predators triggers developmental switches inducing defensive phenotypes. Developmental changes are not reversible within an individual, and are often determined before fertilization. Developmental switching of phenotypes is very successful for some animals such as rotifers (Gilbert, 1980), *Daphnia* (e.g. Sibor, 1992), and bryozoans (Harvell, 1992). These animals have life cycles much less than a year, and can respond to changes in the environment by adjusting the physiology of its progeny, or permanently changing their own morphology. In these short lived organisms, the temporal scale of environmental changes is *greater* than the generation time of the organism. Other organisms, particularly long lived ones, have generation times and life spans that may be many times greater than the temporal scale of environmental change. They show dramatic changes in physiology within the individual, and these must be reversible, or cyclic so as to acclimate to repeated environmental fluctuations (Prosser, 1986). These latter responses will be the focus here.

Phenotypic Expression and Life History Stages

Richard Levins (1968) described several strategies of adaptation in a changing environment. If a fitness curve for a phenotype is plotted against a gradient of some limiting environmental variable, a more or less bell shaped curve results (Figure 1.1a). This curve need not be symmetrical (and more often than not will not be), but for simplicity we will use symmetrical curves in this discussion. If an organism were not limited in any way, an infinitely tall and infinitely wide curve would result so as to maximize fitness under all situations (unconstrained phenotype, Figure 1.1b). Clearly, this is not possible, as the physical and biological composition of an organism limits it, so that an animal (or plant) can not be perfectly adapted to all niches. An ant, for example, no matter how well adapted to living in an *Acacia* bush, would probably not do well in a swamp, and certainly could not penetrate the temperatures, pressures, and aquatic nature of the habitat at deep sea hydrothermal vents. Thus organisms have constrained phenotypes. The height and width of the fitness curve is limited by the biological and physical structures of the animal's genotype. For any given phenotype, the fitness curve will have an optimum for that environmental variable, and regions where the fitness is near or equal to zero. (Figure 1.1a)

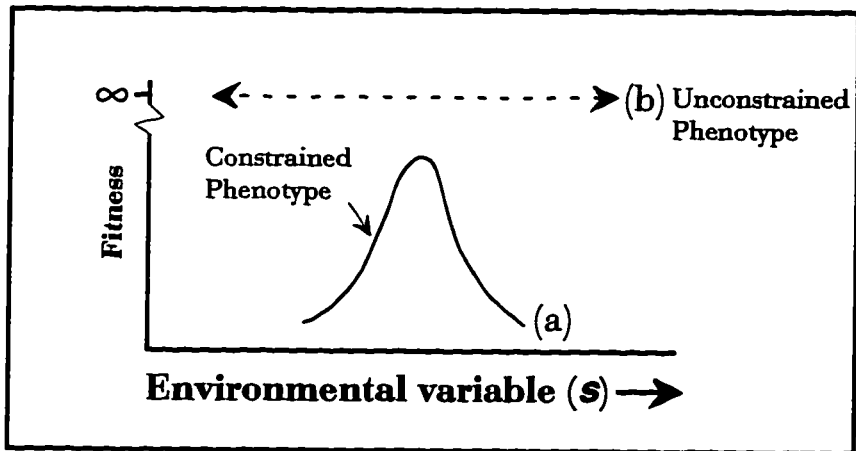


Figure 1.1 - Fitness curves (W) for a phenotype, showing a phenotype which is constrained by its physiology/environment (solid line) and one which is neither constrained by its physiology nor by the environment (dotted line).

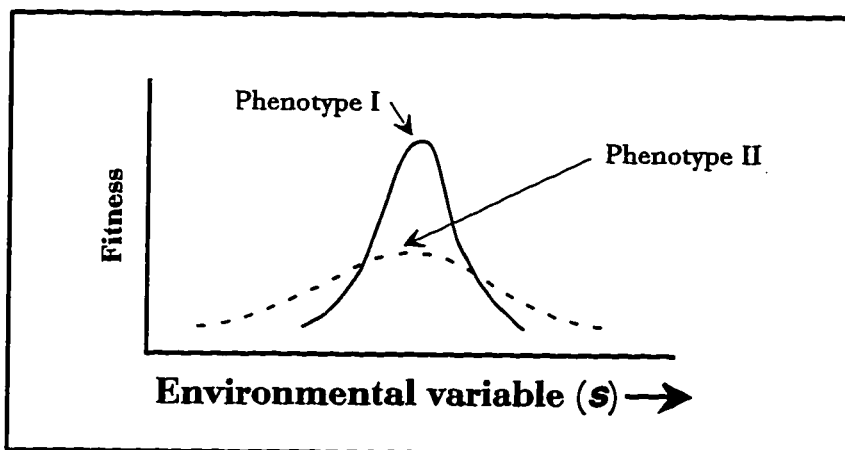


Figure 1.2 - Fitness of a phenotype, showing the effects of the principle of allocation. Since the area under the curve is limited by the genotype, if the curve is broadened (adaptable to a broader range of the environmental variable) then the maximal fitness is then reduced.

Levins (1968) asserted the principle of allocation, which basically states that an organism has limited resources, and that these resources must be allocated between different demands on the organism such that:

$$\int W(s)ds = C$$

where C is a constant, s is some environmental variable (such as temperature, salinity, hydrostatic pressure....), and W is the fitness curve for the phenotype. Because the shape of the curve is determined by the intrinsic limitations of the species, the dotted curve which is broader (phenotype II, Figure 1.2), must have a lower peak than the narrower curve (phenotype I, Figure 1.2) and thus reduced maximum fitness. The out come of this principle is that if a phenotype is adapted to a broader range of the environmental variable s , the maximal fitness for the phenotype will be less, because the area under the fitness curve is assumed constant (See figure 1.2). Both the dotted and solid curves have the same area under them. In biological terms, we interpret this as follows: a phenotype adapted to a broader range of s , will either require a larger number of enzymes and receptors, etc. that must be expressed simultaneously even though they may only be used intermittently, or enzymes which are functional over a broader range of s , but have lesser maximal rates (Somero, 1978 and 1995). These clearly would incur greater energetic costs, or reduced maximal efficiency compared to a phenotype adapted to a narrower range of s and thus only expresses enzymes and receptors specific for that narrow range. Enzymes specialized for a narrow range

of an environmental variable usually have higher maximal rates than those adapted for broader rates, though this is not always the case (Hochachka and Somero, 1973).

Organisms can “cheat” the allocation principle by being able to express multiple phenotypes within the same genotype during successive generations (see Stearns, 1989). These phenotypes are optimized to particular niches within the environment that the genotype experiences over time and space (*e.g.* Gilbert, 1980). Such phenotypic responses can be a limited number of polyphenisms which are designed to cope with specific problems in the environment such as predators and overwintering, or they may be a continuum of phenotypes which develop in direct response to the environment (norms of reaction - Schmalhausen, 1949; Stearns and Koella, 1986). Let’s examine a simplified environment which varies along a single environmental variable S , which could be temperature, salinity, etc. Assuming that the organisms have some means of anticipating changes in the environmental variable, switching between the 2 different phenotypes from one generation to the next will provided a higher fitness for the genotype. This fitness gain hinges on a number of factors. The generation time for the organism must be less than the time between the extremes, as if the generation time is longer than the period of the environmental variation, the organism can not produce progeny which match the environment, as they reproduce too slowly. The cost of switching phenotypes combined with the cost of maintaining the

genetic components necessary for the switching must be less than the gain in fitness from the switching. If the fitness cost of the phenotypic switch is too high, it will exceed the fitness gained by the switch. The changes in the environment must have a predictable component, because if the environment were totally chaotic, it would be impossible to time the correct progeny with the proper environment, and thus a generalist (mixed) phenotype would be superior. The genotype must be able to survive the periods where the fitness is very low. If a genotype has two phenotypes optimized for the extremes of its environment, and environmental variation is large, there would be a region of the environmental gradient where the fitness is very low (Figure 1.3c). If this region of reduced fitness represents a period where conditions are unfavorable for reproduction, and is of short duration, then this low fitness region poses no problem. If the reduced fitness is due to impaired survivorship, then the genotype will die off during the "zero-fitness" period as the environment switches between the extremes.

For an environment with only a very small variation in S (Figure 1.3a), two alternate phenotypes would be very similar, and switching between them would probably not provide any advantage over expressing a generalist phenotype with only slightly reduced maximal fitness (dotted line, Figure 1.3a) due to costs inherent in switching phenotypes. As the variation in S becomes larger, the difference between the maximal fitness of the generalist phenotype (dotted line, Figure 1.3b) and phenotypes I and II (solid lines, Figure 1.3b) becomes larger and begins to offset the switching cost. If the variation in S is moderate to large

(Figures 1.3 c,d), and switches predictably between the extremes in S , the genotype may not be able to persist when the environment is in the middle range for the variable (i.e. the region of the variable where if the organism is forced to complete a generation, the fitness is very low or zero [see Figure 1.3d]). Generalist phenotypes now have greatly reduced maximal fitness. In such a situation, a third phenotype (or even more) would be required to fill the gap (Figure 1.4a). If the cost of switching phenotypes is small and the generation time is small, the organism can smooth out the overall fitness curve for the genotype, by having a larger number of similar phenotypes (Figure 1.4c). Note again that the generalist phenotype would have greatly reduced maximal fitness (dotted line, Figure 1.4a,b,c). Expression of many different phenotypes from a single genotype (as in Figure 1.4c) would probably result in higher costs than advantages gained in fitness. This example would be advantageous only if the organism lived in both an extremely predictable environment, so that the timing of the expression of appropriate phenotypes could be precise, and that the organism had a very short life cycle in order to map many phenotypes with corresponding changes in the environment. In an unpredictable environment, an organism can take the "shot-gun" approach to producing young adapted to the environment, described by Kaplan and Cooper (1984). In this model, a variety of different phenotypes are

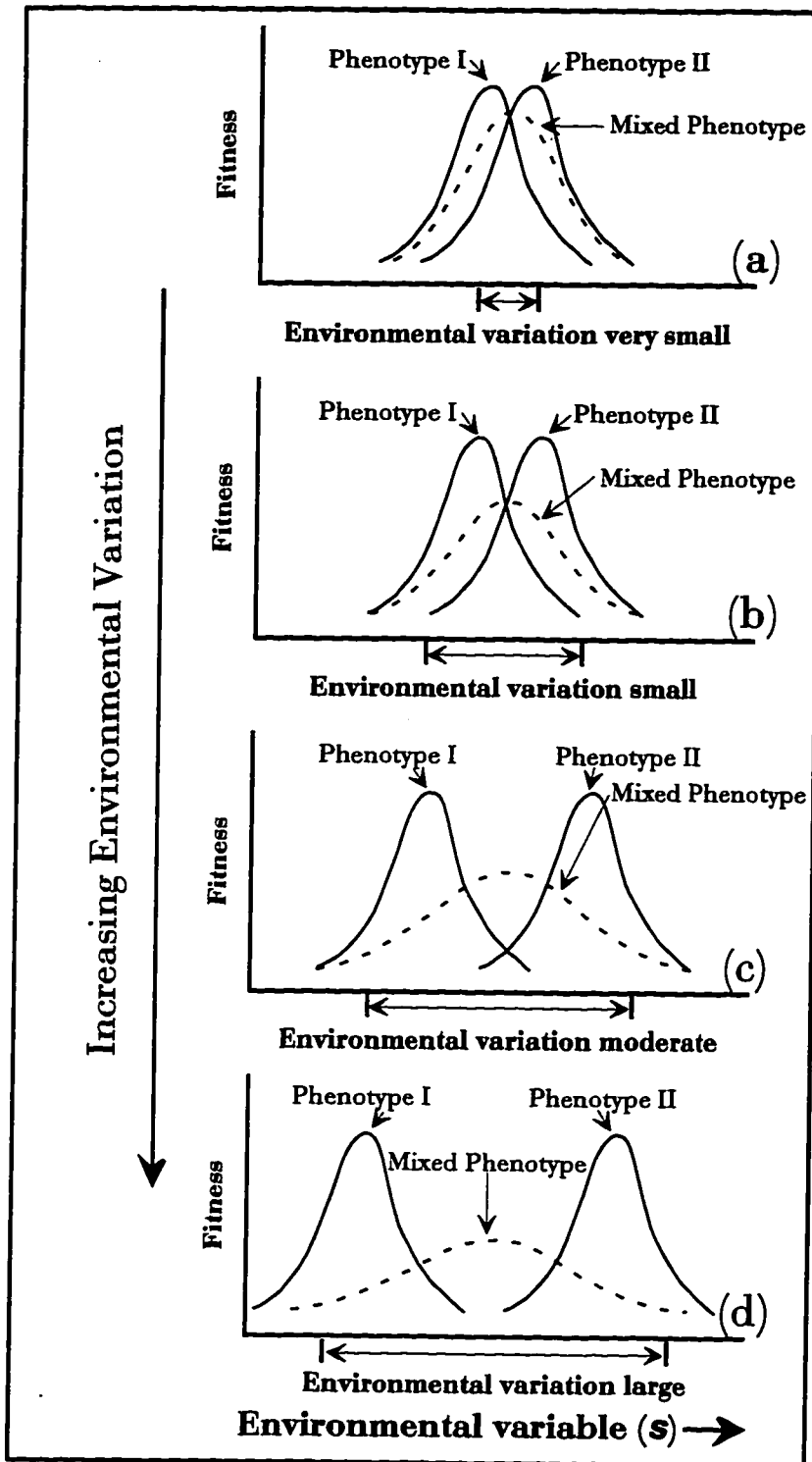


Figure 1.3 - Fitness as a function of the environment of 2 phenotypes from the same genotype. The dotted curve represents the fitness of a mixture of the two phenotypes (a generalist phenotype). If the environmental variations is small, the mixed phenotype will have a higher fitness over all fluctuations in the environment.

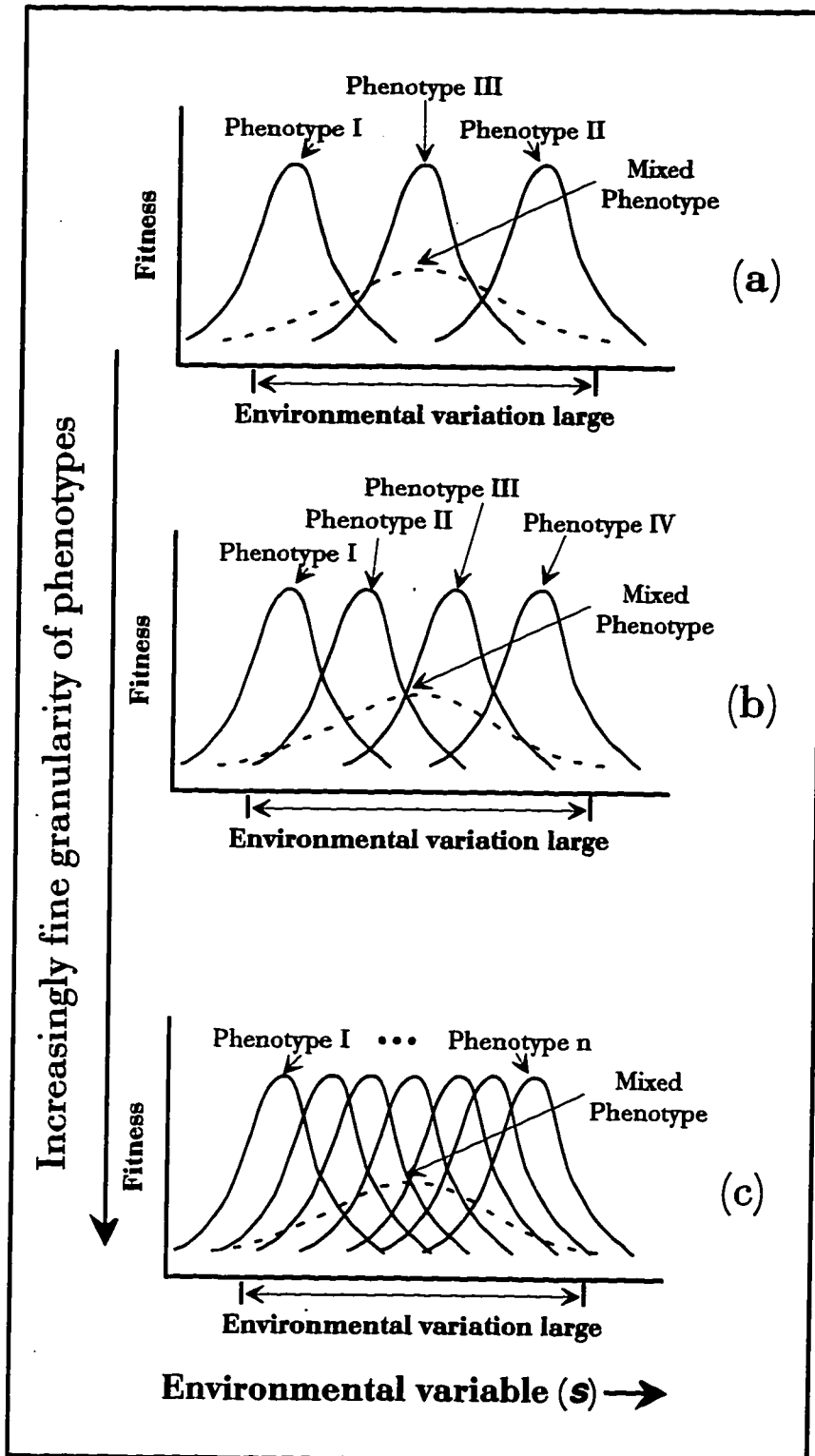


Figure 1.4 - Fitness as a function granularity of phenotypes. The dotted curve represents the fitness of a mixed phenotype (a generalist phenotype). As the granularity of the phenotypes becomes finer, the overall fitness for an animal.

produced such that the probability of some offspring surviving in the changing environment is increased.

Life History Stages Within Individuals

Most vertebrates live at least one year. Within the period of a single life cycle, environmental conditions will probably fluctuate (cycle) many times. This makes the strategy of “hard-wired” phenotype switching between generations untenable and the polyphenism type of phenotypic plasticity common in invertebrates is not found in vertebrates. This chapter is not intended as a review of phenotypic plasticity within populations (well developed by Stearns, 1989, and West-Eberhard, 1989), but rather to develop a model for physiological changes *within* an individual during its life cycle.

An individual must be able to survive a wide variety of environmental fluctuations. It does this by regulating gene expression to adjust morphology, physiology, and behavior to track or anticipate environmental changes (Prosser, 1986). The organism will often (particularly in temperate or arctic species) pass through a series of *phenotypic stages*, each appropriate for a part of the year, and the functions required at that time of year. *Phenotypic stages* are similar to the phenotypic changes described above, but they occur *within* an individual. For example, many animals undergo changes in coloration to adapt to seasonal changes in the color of the habitat or for thermoregulation. In the snowshoe hare, *Lepus americanus*, in the winter, the fur is white to match the snow cover and is

brown in summer to match the exposed forest floor. In spring and fall, as the hares molt between winter and summer colorations, a mottled brown and white pelage results which matches the scattered snow cover during these seasons (Ingles, 1947). Another striking example is the willow ptarmigan, *Lagopus lagopus*, which undergoes similar changes in plumage coloration on the arctic tundra (Pielou, 1994) and these will be addressed later (See Figure 1.6). Other examples of phenotypic stages include adaptations to different temperature ranges as in the goldfish (*Carassius auratus*; Fry *et al.*, 1942; Guderley, 1990) and the goldfinch (*Carduelis tristis*; Buttemer, 1985), or physiological changes associated with life history processes (*i.e.*, reproduction and molting). The temporal progression of phenotypic stages is driven by time and often in relation to changing seasons. During a complete life cycle, an animal will pass through a number of discrete stages which are species specific. These stages represent major events in the life cycle, such as development, breeding, migration, and molting. They can overlap, but usually are temporally separated and sometimes are mutually exclusive.

For further discussions, a few terms need to be defined. *Phenotype* (as used in this discussion), when used to refer to vertebrates, means the sum expression of all genes and stages within a given individual. A number of *phenotypic stages* corresponding to the different suites of extended phenotypes in the various life history stages exist within each *phenotype*. These *phenotypic stages* can represent transient conditions such as breeding and migration in birds, or they may be more

permanent changes, such as sex reversal in sequentially hermaphroditic fishes. Again, the progression of these *phenotypic stages* is analogous to the switching of phenotypes described above, only the “switch” occurs within an individual.

This leads to the logical extension of Levins’ (1968) phenotypic models to *phenotypic stages* (Figure 1.5). In this extension, the *phenotypic stages* take the place of the phenotypes, and because we are now referring to individuals, rather than a population, we will be plotting a *fitness probability*, rather than fitness (since an individual can only have one fitness, at least as represented by progeny in the next generation). The *fitness probability* reflects both the animal’s potential for producing offspring, and its potential survivorship. If the cost of transition between *phenotypic stages* is relatively small, the overall fitness probability for the individual can be increased by expressing a greater number of phenotypic stages. Again, as in the polyphenism, the transition of *phenotypic stages* requires a predictable component in the environment so that individuals can anticipate change and activate the appropriate *phenotypic stage*. It would be impossible match a *phenotypic stage* to the environment, if the environment were changing in a completely random manner, and any change in the *phenotypic stage* would more than likely be inappropriate. For a population, the “shot-gun” approach to producing offspring to match a changing environment (Kaplan and Cooper, 1984) can be used, but this is not an option for an individual.

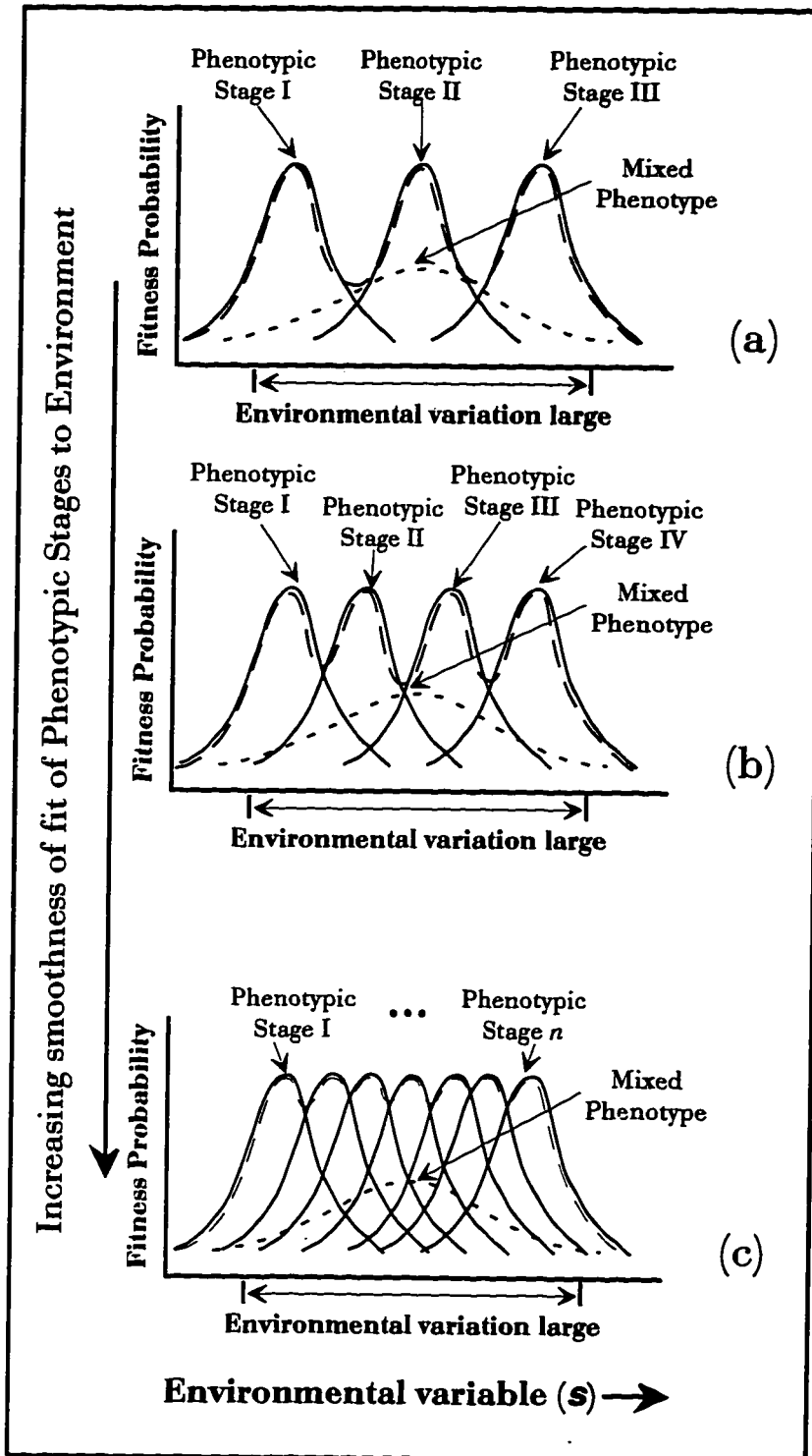


Figure 1.5 - Fitness probability as a function of granularity of phenotypic stages. The dotted curve represents the fitness probability of a mixed phenotype (a generalist phenotype) which is attempting to cover the entire environmental range. As the granularity of the phenotypic stages becomes finer, the overall fitness for an organism increases dramatically.

The willow ptarmigan (*Lagopus lagopus*) is a good example of the relation between progression of discrete phenotypic stages, and a generalist phenotypic stage that tries to adapt to the entire suite of environments simultaneously. Normally, the willow ptarmigan goes through a number of phenotypic stages including discrete molt patterns (Pielou, 1994; Figure 1.6a). During the winter, the bird is entirely white, to match the complete snow cover at that time. In spring, the head region is molted to match the patchy dark areas where the snow is melting through to the ground below. In summer, the ptarmigan completes the molt, and with the wings tucked in, the mottled pattern blends in well with its background. In fall, the molting from the mottled summer phase, into the white winter phase, yields a 'peppered' phase which blends well with the streaky pattern of the first snows in the fall. If the ptarmigan were to adopt a generalist phenotypic stage intermediate to the four normal phenotypic color stages (trying to cover the entire range of ground colors/patterns, Figure 1.6b), the resulting generalist molt would be inappropriate for almost all conditions the animal was exposed to. A ptarmigan expressing this generalist phenotypic stage would probably be quickly removed from the gene pool by predators. The ptarmigan generation time is too long to produce new individuals for each environmental phase. Furthermore, breeding is only possible during one part of the year, further limiting the possibility of generational responses to seasonal fluctuations in the environment.

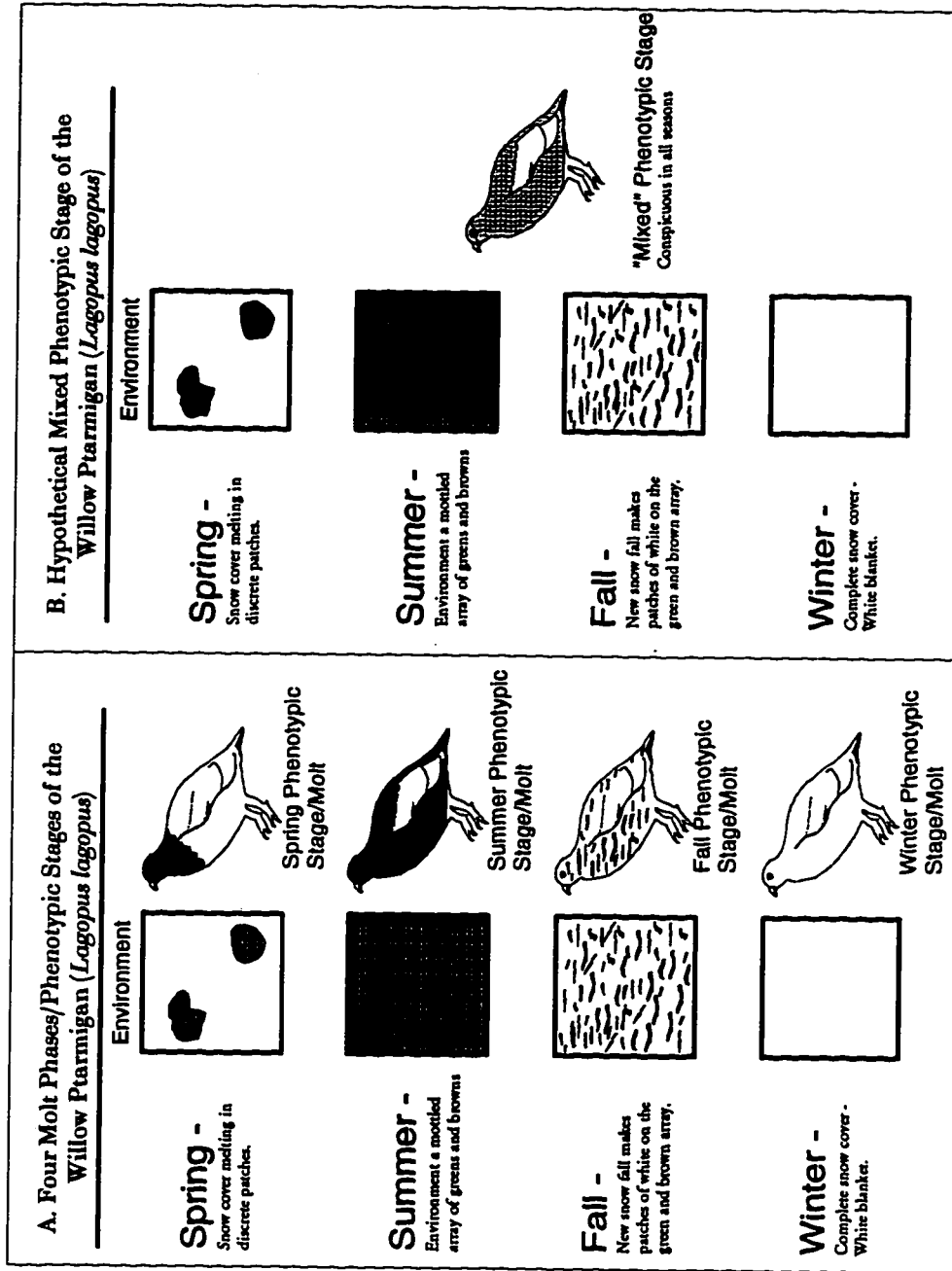


Figure 1.6 - Seasonal phenotypic stages in the molt cycle of the willow ptarmigan, *Lagopus lagopus*, and the ground cover for the corresponding season. (A) Phenotypic stages found in wild willow ptarmigans. (B) Hypothetical 'mixed' phenotypic stage which attempts to take a 'middle-of-the-road' approach to adapting to seasonal changes in the ground cover.

The discussion thus far has assumed that the changes in environmental conditions are more or less predictable. Transition either between phenotypes in a population, or among *phenotypic stages* within individuals must be timed so as to coincide with appropriate environmental changes. In either case, information from the environment (environmental cues; Levins, 1968) permits organisms to anticipate the change. The importance of these cues is the topic of the next section.

Environmental Cues

In an environment with predictable components, each *phenotypic stage* shows a progression of events, driven by cues from the environment. These cues comes in five basic classes: developmental, initial predictive, supplementary, synchronizing and integrating, and modifying factors (see also Wingfield and Kenagy, 1991).

- 1) Developmental cues come in several different types: growth factors/morphogens, tissue interactions, intrinsic factors, and external factors. They most often come from the internal environment of the embryo, but some cues come directly from the external environment (i.e., extrinsic factors). Growth factors/morphogens (such as fibroblast growth factor [FGF] and Retinoic acid) trigger/regulate the differentiation of tissues and structures (limb development, differentiation of muscle cells...). Both the presence and absence of growth factors can act to stimulate differentiation. When FGF is removed

from cultures of dividing myoblasts, they differentiate and begin to fuse into myotubes (Linkhart, Clegg, and Hauschka, 1980). During vertebrate limb development, a gradient of retinoic acid is set up which regulates differentiation and pattern formation of the limb (Eichele, 1989). Tissue interactions can act as signals for differentiation. Development of the optic vesicle stimulates the overlying ectoderm to differentiate into a lens (Jacobsen and Sater, 1988). Intrinsic factors, such as the nuclear/cytoplasmic ratio can signal differentiation events. In developing *Drosophila melanogaster* embryos, the formation of the cellular blastoderm is stimulated by a critical nuclear to cytoplasmic ratio (Edgar, Kiehle, and Schubiger, 1986), and in embryos of the African clawed frog, *Xenopus laevis*, the mid-blastula stage transition (where nuclear genes first become activated) is also regulated by a critical nuclear-cytoplasmic ratio (Newport and Kirshner, 1982a, b). External factors, such as temperature in reptiles (Janzen and Paukstis, 1991) and fish (Conover and Fleisher, 1987) and pH in fish (Schaefer and Rubin, 1985) can shunt a developing animal into a different path of development. In some freshwater teleosts, the sex of the fry can be all male if the eggs are developed in low pH water, and all female if developed in high pH water (Schaefer and Rubin, 1985). In some reptiles, such as alligators (Ferguson and Joanen (1983) and some turtles (Lockwood *et al.*, 1991), the sex of the embryo is determined by the temperature at critical periods during development (*e.g.* Crews, *et al.*, 1994).

- 2) Initial predictive cues are environmental signals that allow the organism to make long term predictions about the environment. Photoperiod is a good example of a cue which provides information on future events in diverse environments. Increasing day length is an excellent predictor of the approach of spring and summer, and triggers gonadal development in anticipation of the breeding season in many species (Follett, 1984; Gwinner, 1986).
- 3) Supplementary cues (such as food availability) provide fine tuning for responses to initial predictive cues, and also provide short term predictive information about the environment (Marshall, 1959; Wingfield and Kenagy, 1991). As the initial predictive cues only provide a general idea of what the environment might be, supplementary cues are important to ensure the organism makes the correct responses at the correct time. In other words, they can accelerate or inhibit the responses to initial predictive cues.
- 4) Synchronizing and integrating cues are social stimuli provided by other members of the population (Hinde, 1965; Lehrman, 1965). They ensure that the animal's responses to the external environment are appropriate to the condition of the other conspecifics. For example, the sea star *Acanthaster planci* releases its eggs and sperm into the ocean where they are fertilized. If males and females spawn at the same time, over 70% of the eggs are fertilized up to 8 meters downstream of a single spawning male and greater than 5% even 100 meters downstream (Babcock et al., 1994). Fertilization would be

exceedingly unlikely if spawning was not synchronized as the sperm and eggs would become diluted in the vast volume of sea water, and the shed eggs and sperm would thus be wasted.

- 5) The final class of cues/factors are the modifying factors or transient perturbation factors (TPF or "stressors"). Unpredictable events in the environment (e.g. inclement weather, frost, flood, capture) provide the basis for these factors (Wingfield, 1988). In addition, for an event to be a TPF, the organism must be either qualitatively, or quantitatively unprepared for the event. For example, if a gold fish (*Carassius*) is acclimated to summer temperatures of around 27°C, it cannot survive temperatures of 7°C, but if it is acclimated to 17°C, it can survive at 7°C, and even down to near freezing (Fry, *et al.*, 1942). A temperature of 20°C may be fine for a temperate fish, or even to a tropical fish, as this temperature is within their normal range. However, even such a moderate temperature is fatal to the Antarctic icefish *Trematomus* (Somero and DeVries, 1967), which is adapted to much colder temperatures (average temperature of -1.1°C), and normally experiences only very minor changes in temperature (yearly variation of around 0.1°C). TPFs stimulate the expression of a *transitory emergency state* (TES or stress response) which has many characteristics which are similar regardless of the TPF. The TES and TPFs will be discussed in great detail in Chapter III.

Transitions Between Phenotypic Stages

A transitional phenotypic stage may be induced by some environmental cue, with no outward expression of this transitional phase. For instance in example II, a bird can become photorefractory, even while still tending young (Wingfield and Farner, 1979). Development of the stage usually requires stimulation of the cell cycle, leading to cellular growth and differentiation, such as in the recrudescence of gonads in seasonal breeding birds. This stimulation probably involves the action of hormones acting either via genomic receptors (such as those of the steroids and thyroid hormones) or by slow acting membrane receptors which lead to cascades of messenger resulting in stimulation of the cell cycle.

Timing of development of the stage, as well as the onset of mature capability (Figure 1.7) are modulated by supplementary, integrating and synchronizing cues. These cues can either act as accelerators, or inhibitors, speeding up, or slowing down the progression of the phenotypic substages, as well controlling the "path" among physiological states within each substage. Onset of processes characteristic of a phenotypic stage (i.e. phenotypic substages; see below), are regulated by environmental cues in the same categories, but presumably would act throughout different mechanisms. These processes are probably regulated by messengers acting via fast acting membrane receptors to regulate the function of cells, rather than the slow acting effects via stimulation the

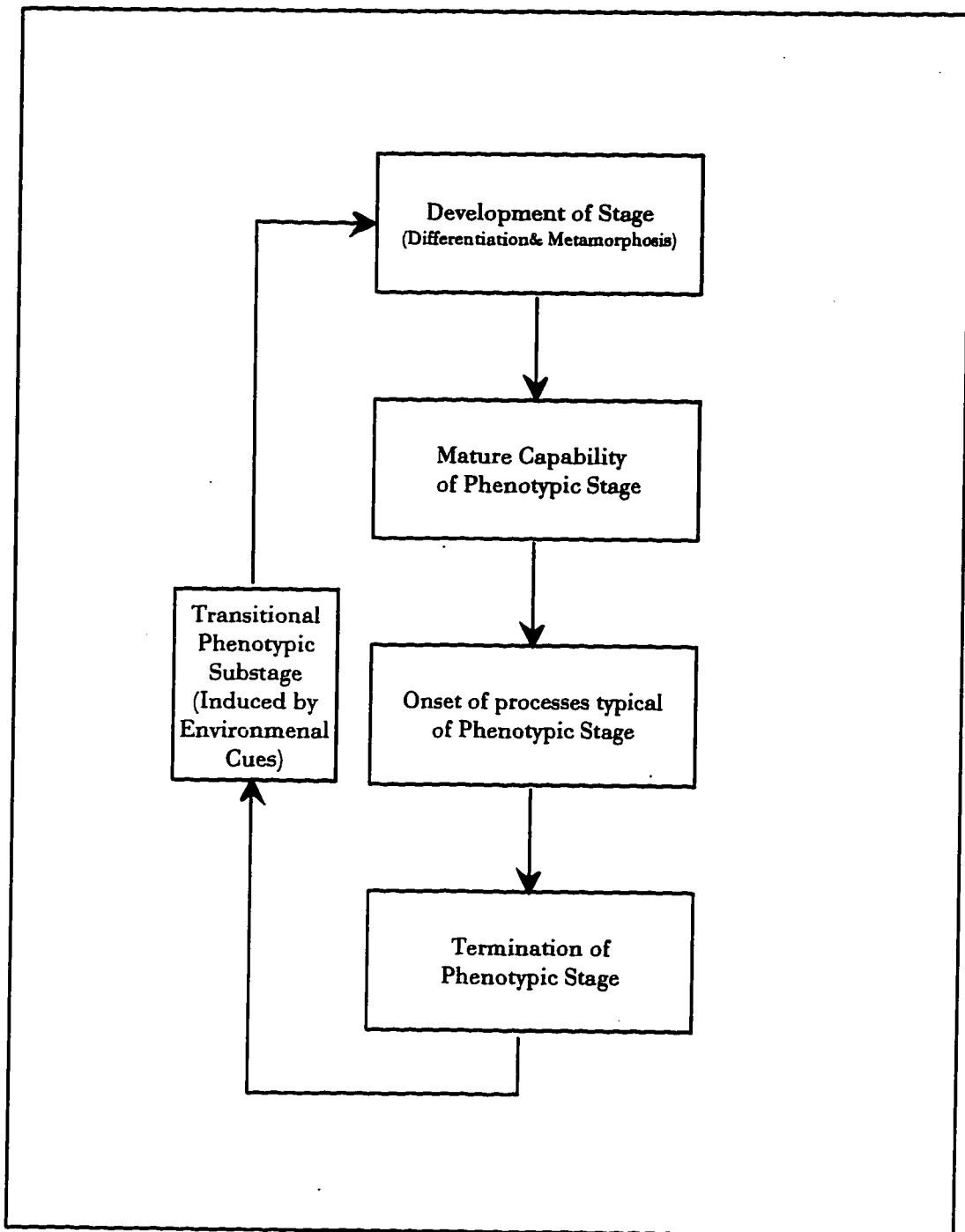


Figure 1.7 - Cyclic nature of the development and expression of phenotypic stages. Environmental cues (often initial predictive cues) trigger a transitional phenotypic substage that is committed to the next phenotypic stage. Development of the characteristics of the phenotypic stage may occur some considerable time from the transitional phenotypic substage. Development of the structures involved in the phenotypic stage often occurs before the behaviors associated with the phenotypic stage.

cell cycle. Termination of a phenotypic stage may be initiated by any of the cues, including the unpredictable modifying factors.

A number of potential *physiological states exist* within each phenotypic stage (as well as within phenotypic substages characteristic of the stage). These physiological states represent the actual condition of the organism, including factors often included in the so-called extended phenotype (Dawkins, 1982), such as territory quality, and mate. The physiological state will change with time, associated with changes in the “extended phenotypic factors,” as well as adjustments in the physiology of the organism associated with fluctuations in its environment. Note, these changes are triggered by environmental cues in a manner different from the transition between *phenotypic stages*.

If we look at the potential regulation of single genes, and the states of all cells within the individual, one sees an apparent infinite number of physiological states for an organism. But for the most part, a vast majority of these “micro-physiological states” will be functionally and selectively identical (or nearly so) and can be grouped into a finite number of *physiological states*. Although all the individuals within a physiological state will not be completely identical, their ability to interact with the environment will be indistinguishable.

Finite State Machine Model

If we step back and view an animal's life cycle at a coarse level, the life cycle appears as a series of discrete stages (i.e., reproduction, migration, molting...).

These *phenotypic stages* occur in a more or less cyclical progression, proceeding from one stage to the next in response to a wide number of environmental cues or stimuli. Within a given phenotypic stage, the organism can exist in a finite number of functionally different *physiological states*. The finite nature of these *phenotypic stages* and *physiological states* allows us to view the animal's life cycle as a finite state machine. These physiological states are similar to the concept of states as used by Mangel and Clark (1986) and Houston and McNamara (1992).

Finite state machines (FSM) are commonly used in computer programming literature to describe the decision making process of a program (see Gill, 1962). Three different type of variables are involved in a FSM: (1) excitation variables provide the stimulus for the machine, (2) response variables are the behavior of the system of interest to the investigator, and (3) intermediate variables, which are neither excitation or response variables, and are often difficult to measure as they occur within the machine. A FSM can be represented as a black box (figure 1.8). The "box" has a finite number of inputs (excitation variables), and finite number of outputs (response variables). The intermediate variables are inside the black box, and though they are important in determining the state of the machine, they are not easily determined, and can be ignored for dealing with transitions between stages. While intermediate variables can be ignored when dealing with state transitions, they are none the less quite interesting. In biological system, they encapsulate the physiological mechanisms within the organism and cells which

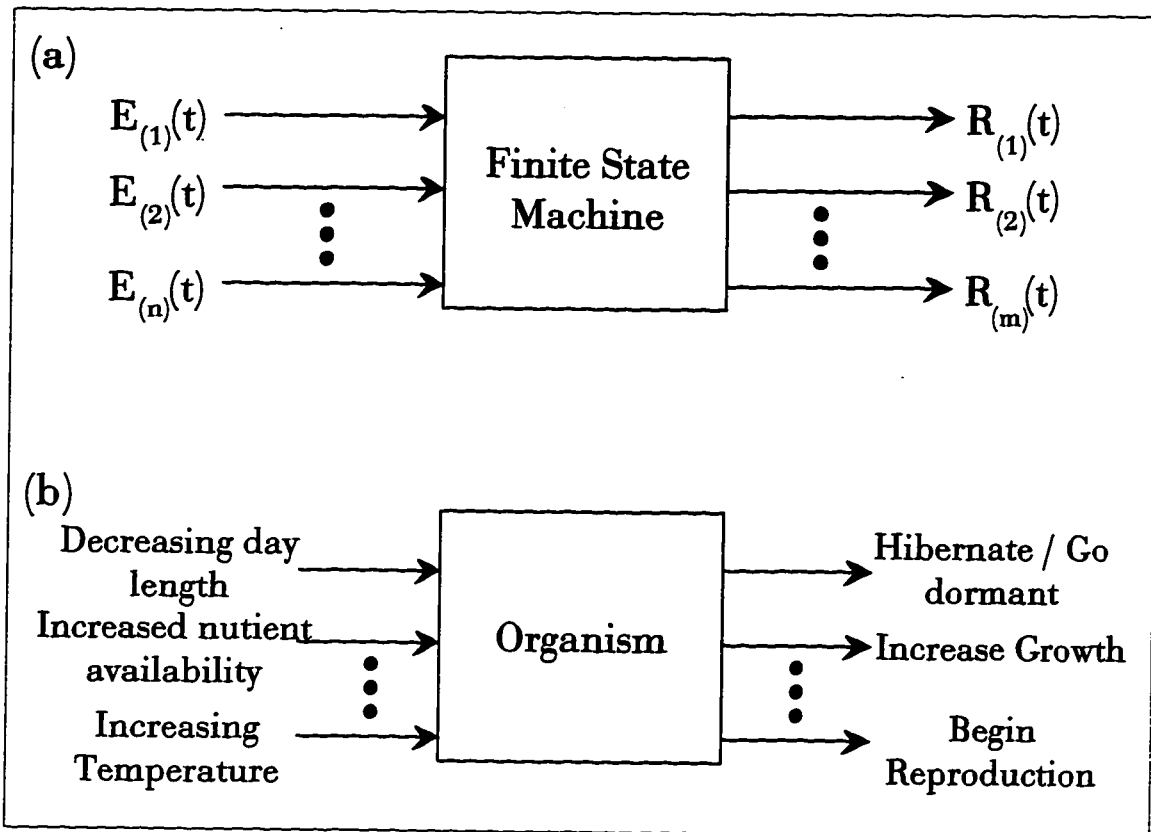


Figure 1.8 - Black box representation of a finite state machine. [Figure 1.1a redrawn from Gill, 1962.] (a) Inputs enter the black box from the left, and responses leave on the right of the black box. Excitation variables are represented as E , and response variables are represented as R , where t is the sampling time, and m and n are the number of response and excitation variables respectively. (b) Biological 'Black Box'. A vast number of excitations (stimuli) impinge on the organism. The animal has a finite number of responses to these excitations. Ellipsis (...) indicate that there are many different stimuli and responses for an organism, though only 3 are shown.

determine the actual state of the organism, and are thus very important, but their exact nature need not be understood to use the finite state model. However, a finite state model may indicate lines of investigation to determine mechanisms.

For a biological system, the excitation variables are the environmental cues described above (e.g. increasing day length, decreasing temperature, increasing food availability), and the response variables can be transitions between phenotypic stages and substages (e.g. begin gonadal recrudescence, initiate hibernation) or they may be changes in physiological state within a phenotypic stage or substage (e.g. increase growth, increase fat stores, abandon nest, and shiver to increase body heat) (Figure 1.8b).

Finite-state machines conform to several basic assumptions. The machine must be controlled by an independent *synchronizing source*. The system variables are not measured constantly, but rather at discrete intervals when certain specific events occur, called *synchronizing signals*. These discrete time instances are called the *sampling times*. An additional assumption of the system is that the behavior of the system at sampling time t_v (where v is an integer indicating the time interval) is independent of the interval between t_v and the previous interval t_{v-1} . Thus the system is not dependent on time, but rather on an ordinal number associated with the sampling times. This does not preclude events occurring at regular intervals, but such regularity is not required for the system. Environmental information can come from internal biological clocks as well. This independence from the interval

between samplings is important in biological systems, as they are responding to their environment, and not to the ticking of a simple clock. Time may be critically important, but only in that the cues from the environment will occur at more or less predictable times and the responses must coincide with appropriate conditions. Although biological clocks have been documented in a large number of biological systems (*e.g.* Aschoff, 1980, Gwinner, 1986, and Turek, 1985), these clocks are responsive to the environment, and the timing in the absence of environmental cues is usually different from timing in their presence. Thus, if a biological clock does not receive synchronizing signals from the environment, it loses its entrainment (Aschoff, 1980; Gwinner, 1986; Hastings *et al.*, 1989 and Turek, 1985).

Another basic assumption of the FSM is that the number of both the excitation and response variables is finite, and that the machine can only exist in one of a finite number of states during a given time interval. The current set of response variables (output) is uniquely determined by the present state of the machine, and the excitation variables at that time. The previous history of the machine is only important in that it uniquely determines the present state of the machine. The state at the next sampling time is solely determined by the present state, and the excitation variables. Thus, the machine can be characterized by a state function and a response function:

$$\begin{aligned}
 response_i &= f_{response}(excitation_i, state_i) \\
 state_{i+1} &= f_{state}(excitation_i, state_i)
 \end{aligned}$$

where $state_i$ is the state of the machine at time t_i ($i = 1, 2, 3, \dots$), $excitation_i$ is the set of excitation variables received during interval i , $state_i$ is the state of the machine at interval i , $state_{i+1}$ is the state of the machine in the next time interval after i , and $response_i$ is the response of the machine at interval i . Both the response function ($f_{response}$) and state function (f_{state}) have the current state of the machine ($state_i$) as a dependent variable, thus the response of the machine to a given excitation cannot be predicted, even if the response and state functions are known, unless the state to which the excitations are applied ($state_i$) is known, or the machine always has the same response to the excitation. Many excitations do not cause the transition to another state, but act to maintain the current state. For example, an excitation inhibits the transition to another state.

A simple example may better illustrate this state dependence. A coin toss game provides a very simple finite state machine (Figure 1.9). The coin has two sides, heads and tails. Points are only awarded for three heads in succession, without any intervening tails. In this game, the excitation variable are either heads or tails. The response variables are with score, or no score. There are three states: no heads in a row, one heads and two heads. The machine always responds to "tails" in the same way (no score \rightarrow no heads), and thus is always predictable, regardless of whether the state of the machine is known or not. Both

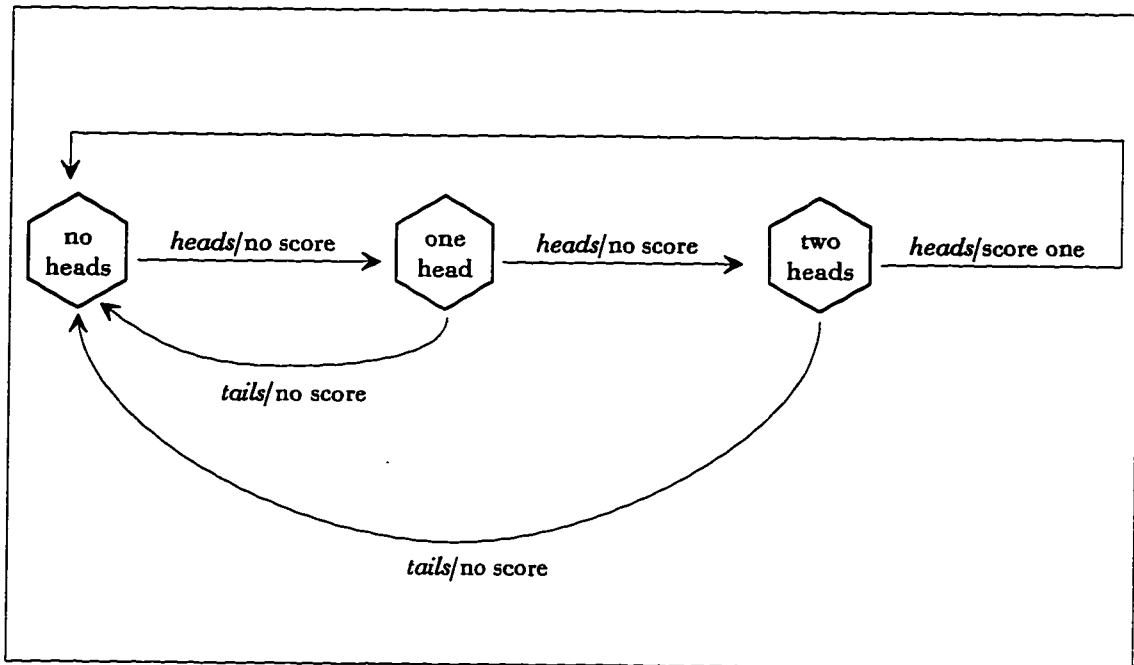


Figure 1.9 - Transition diagram of a simple coin toss game. In this game points are only awarded for three consecutive coin tosses resulting in heads. States are enclosed in hexagons, and excitations are in italics followed by the response (*excitation/reponse*).

the response, and the resulting state are ambiguous for “heads”, unless you know the state of the machine. If you were to enter the room while the game was in progress, and the player flipped a “heads”, you could not know whether the point was scored, without finding out from the players. You could not know whether the previous streak of heads was one, two, or zero, and thus could not predict the outcome (response) even though you knew the excitation (heads).

Groups of states can be associated into a submachine which is in essence a “superstate” for the machine. Submachines can be composed of any number of layers, and can be used to encapsulate various layers of complexity (particularly when drawing transition diagrams.) When considering the transitions between submachines, transition between the states within the submachine can be considered as actions of intermediate variables.

Let’s now examine a simple biological example of a finite state machine. Consider a stream animal, like *Hydra*, which is attached to the bottom, waiting for prey to be brought to it by the current. The prey are at a low density, so the *Hydra* is always hungry. For this example we will impose the limitation that while the *Hydra* is eating a food item, it cannot capture another prey item. As with all biological systems, the synchronizing source is the environment, and in this example the stream is the synchronizing source (as it moves the food to the animal). The environment changes through time, and this environmental variation provides the synchronizing signal. For our *Hydra* example the flowing

water is the synchronizing source. Environmental variation provides the excitation variables to the machine (the animal or plant), in the form of environmental cues (synchronizing signals). The machine integrates these cues, and responds appropriately to the state of the machine. For an animal, these states correspond to physiological/behavioral states.

In our simple *Hydra* feeding FSM, the excitation variables (see Table 1.1) are all supplementary cues: the presence or absence of a prey item and the completion of eating the food item. The Hydra has three responses: to wait, capture the prey, or to eat the prey. The "machine" has 2 states: eating, and waiting. During the eating state, the response set is: continue eating and wait. During the waiting state, the response set is: continue waiting, and capture prey. Even with only three different excitations in this model system, the reaction of the machine (response and transition to the next state) can not be determined, unless the state of the machine at the time of the excitation is known (see Table 1.2). This factor cannot be over emphasized, since it is important to all experimental manipulations of plants and animals: you can not predict the response of the system, unless you know both the physiological state of the organism, as well as the whole suite of excitations being experienced by the organism. A close examination of the transition diagram (figure 1.10) for this system shows this quite clearly. The response to the presence of prey is very different, depending on the current state of the *Hydra*.

Table 1.1 - States, Excitation Variables, and Response Variables for a Simple Biological Finite State Machine. A hypothetical freshwater stream predator remains attached to the bottom and captures prey from the stream as water flows past.

| States | Excitations | Responses |
|---------|---------------|-----------------|
| waiting | prey | wait |
| eating | no prey | capture prey |
| | prey devoured | continue eating |

Table 1.2 - Transition Table for the Same simple Biological Finite State Machine as in Table 1.1. N.A. means that the excitation is not available to the current state of the machine.

| state _v \ excitation _v | response _v | | | state _{v+1} | | |
|--|-----------------------|----------|----------|----------------------|--------|---------|
| | prey devoured | prey | no prey | prey devoured | prey | no prey |
| waiting | N.A. | capture | wait | N.A. | eating | waiting |
| eating | wait | eat prey | eat prey | waiting | eating | eating |

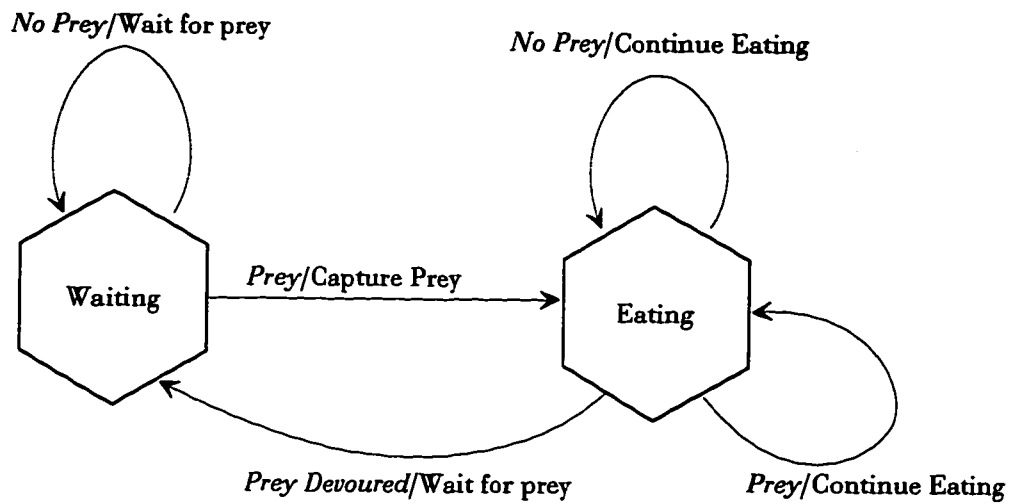


Figure 1.10 - Transition Diagram for a Simple Biological Finite State Machine. A hypothetical freshwater stream predator remains attached to a surface and captures prey from the stream as water flows past. The 2 states are represented by the hexagons, and excitations are in italics. Responses of the machine in response to the excitation are to the right of the slash-marks for the corresponding excitation.

Application of Finite State Machine Models to Biological Systems

Example I. Modeling Sex Change in a Sequentially Hermaphroditic Fish

The bluestreak cleaner wrasse, *Labroides dimidiatus*, is a widely distributed coral-reef fish found from the Red Sea, down through the Great Barrier Reef of Australia (Meyers, 1991). It is a protogynous species, but unlike some other species of wrasses, all individuals are born as females (*i.e.*, no primary males). A terminal phase male (resulting from the sex change of a large female) guards a territory, and maintains a harem of 3-6 mature females, and several juvenile females (Robertson, 1972). When breeding, the male mates with each mature female once a day. A large number of aggressive interactions within the harem resulting in a dominance hierarchy within the harem. The male sits at the top of the hierarchy, keeping the dominant female (who is usually the largest) from sex reversing with aggressive threats and displays.

Death (or removal) of the territorial male initiates sex reversal in the dominant female within 1.5 hours. The reversal is first expressed as male-like aggressive and territorial behaviors, then male courtship and spawning behaviors develop in 2-4 days. By 14-18 days, the transformation is complete, and the terminal phase male is able to release sperm.

Sex reversal of females in this species appears to be inhibited by aggression of more dominant animals. Within the harem, more dominant females inhibit the reversal of lower status females by aggression. Aggression from the male inhibits

the reversal of the dominant female. Reversal is also dependent on the presence of a less dominant female, as an isolated female will not undergo sex reversal, probably because a lone female can join a harem and immediately begin breeding, but a lone male must find and establish its own territory, as well as attract females in order to breed.

In this system, the signals maintaining social status and sex come in the form of integrating cues from other members of the group (or the lack of those signals). Three phenotypic stages occur within the life history of this wrasse: (1) immature female, (2) mature female and (3) terminal phase male. The mature female phenotypic stage has three phenotypic substages: low-status female, unattached female, and dominant female. An individual may not go through all stages or substages, depending on their environment, and life span. Unlike many biological systems, the phenotypic stage transitions are not governed by initial predictive cues. The excitation set has 7 members, all but one of which are integrating cues: (1) reaching sexual maturity, (2) aggression from more dominant female, (3) no aggression from a dominant female + smaller females in harem, (4) no aggression from a dominant female + no smaller females in harem, (5) male aggression, (6) joining a harem with a larger female, and (7) joining a harem with only smaller females. These excitations do not come at specific times or at specific intervals. The response set is: (1) become low status female, (2) remain submissive, (3) become unattached female, (4) become dominant female, (5) remain dominant

female, (6) sex change to terminal phase male. These data can be used to create a transition diagram (Figure 1.11). The first thing that is noticeable from this diagram is that not all of the excitations are applicable to all stages, and that the response set for each state is more limited than the response set for the entire system (e.g. the terminal phase male cannot become a low status female). This property is characteristic of biological systems, because the present state of the system puts major constraints on the possible responses of the system, as well as the transition from one state to the next. The excitation set is composed of elements which represent a suite of stimuli (e.g. the combined effect of *No male aggression* + *No smaller females* leads to a different outcome from *No male aggression* + *Smaller females*). In fact, in biological systems the summation of cues often is critical to both the timing and the initialization of *phenotypic stage* transitions.

Example II. Modeling Phenotypic Stage Transitions of the Gambel's White Crown Sparrow

If we examine the life history of the migratory, temperate Gambel's White Crown Sparrow, *Zonotrichia leucophrys gambelii*, we see that the animal progresses between a number of discrete functional phases, such as migration, breeding, molting, and overwintering (Figure 1.12). These can be viewed as *phenotypic stages*, as they involve very different physiological expressions (in the broad sense as described above), and are hence phenotypically different with respect to

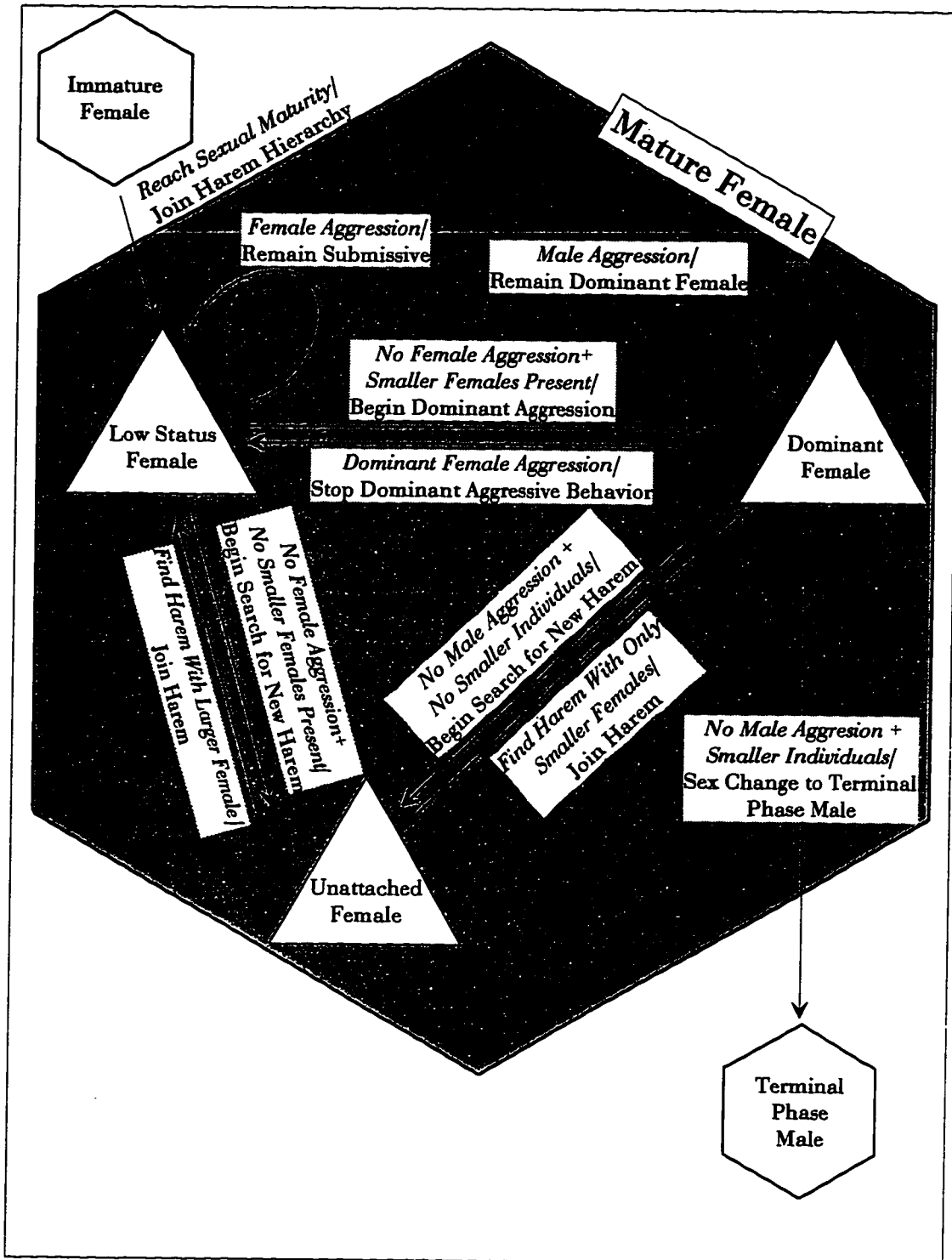


Figure 1.11 - Transition diagram for a sequentially hermaphroditic female Cleaner Wrasse *Labroides dimidiatus*. The diagram shows the transition between *phenotypic stages* in the life of a tropical reef wrasse. Phenotypic stages are within hexagons, and phenotypic substages are within triangles. The large mature female phenotypic stage includes three phenotypic substages.

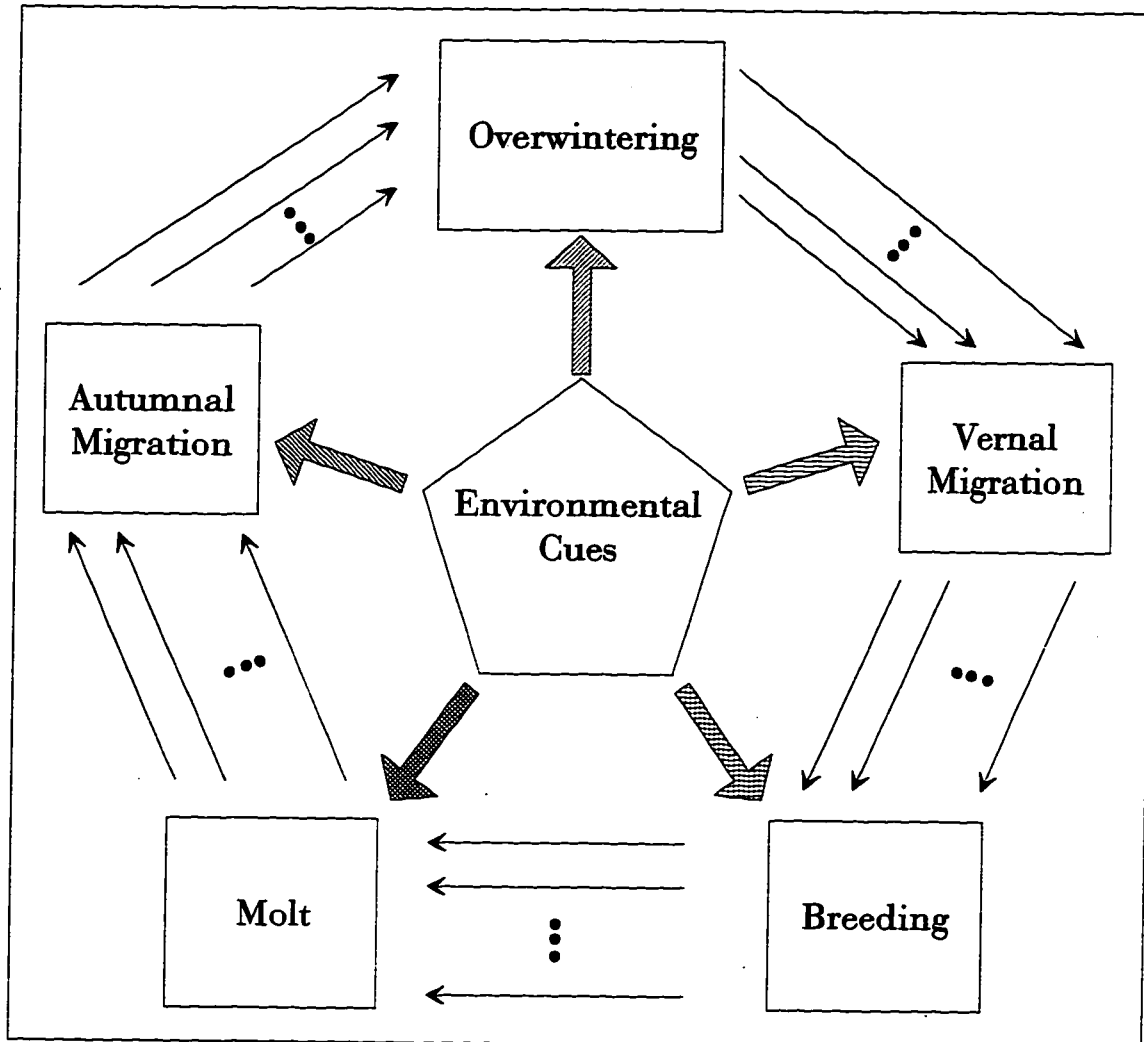


Figure 1.12 - The Seasonal Life Cycle of a migratory bird as diagrammed as a series of finite state submachines. Each submachine (drawn as boxes) receives a number of stimuli from the environment in the form of environmental cues (shown as large arrows). These stimuli are usually different for each submachine (as indicated by the different hatching of the arrows). The stimuli trigger a number of internal responses in the sub-machines (intermediate response variables), and also trigger the transition from one sub-machine to the next (indicated by simple arrows). The elipses (...) between the simple arrows indicate that there may be many ways to transition between submachines.

selection on the animal during the particular season. The transitions between the stages are controlled by cues from the environment, and these cues will obviously vary throughout the year (Figure 1.13). The transition between phenotypic stages can follow several different paths, and yield several different physiological stages, depending on both the integrated cues, and the initial physiological stage before the transition (Figure 1.14). Each of these *phenotypic stages* represents a finite state submachine, as each contains a distinct set of *phenotypic substages* (or super-states) designed for specific tasks. Within each of the *phenotypic substages* are a suite of *physiological states* that are determined by constraints imposed by both the environment and the genome. The exact nature of these *physiological states* will depend on such things as energy stores within the animal, quality of the territory, presence or absence of mate, and a whole set of physiological considerations as determined by the expression of the genome. Again, the excitation cues are important in determining the timing of the transition between *phenotypic stages* (submachines), but the state of the “machine”/individual is critical to the timing and direction of the transition. For a seasonally breeding bird, the transition from one *phenotypic stage* to the next is unidirectional, due the seasonal nature of the excitation cues (Figure 1.13). A bird that has migrated to the Arctic and begun breeding cannot then begin the vernal migration, because the state of the animal does not allow transitions to the vernal migration submachine (in part because the bird is already at the end point of migration), and the cues which normally trigger

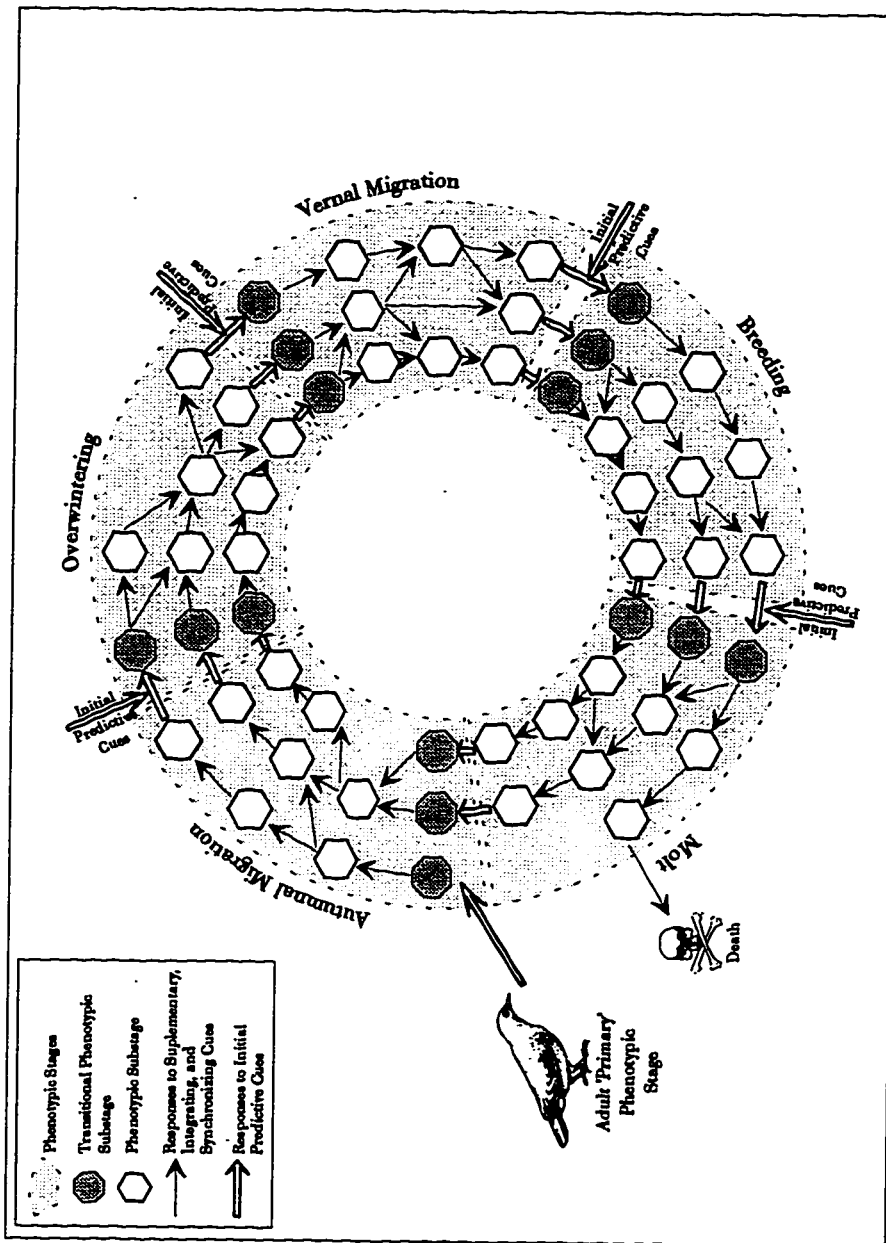


Figure 1.13 - Representing the adult life cycle of a bird as a finite state machine. The cycle is divided into a several phenotypic stages (breeding, vernal migration, autumnal migration, overwintering, etc.) which are finite state submachines acting to complete a specific task. Within each submachines there are a number of superstates, or phenotypic substages (see figure 1.14), dealing with a particular aspect of the phase. Each of the phenotypic stages typically has a transitional phenotypic substage which is usually triggered by initial predictive cues. Death, the terminal state for the life cycle, can occur at any time during the cycle, and is the only state which can be reached from all other states.

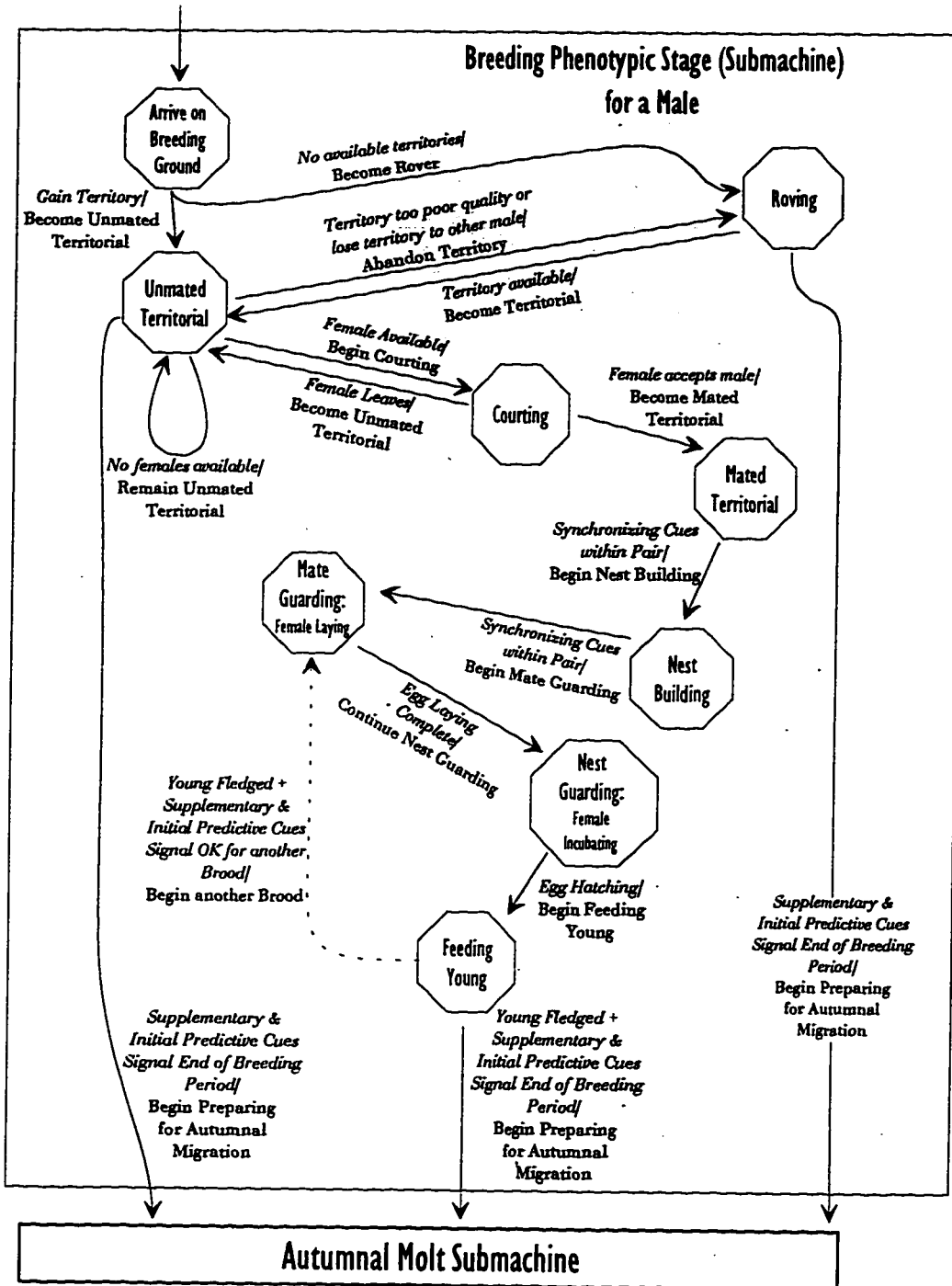


Figure 1.14 - Transition Diagram of the Breeding Phenotypic Stage for a male white crowned sparrow *Zonotrichia leucophrys*. Shows transitions between phenotypic substages (Finite State Submachines of the Breeding Submachine/*phenotypic stage*). This diagram depicts only the major transitions between substages, and does not attempt to show all transitions between *phenotypic substages*.

the vernal migration are not present during the late summer and early autumn, and also not present in the Arctic. As all transitions depend on the physiological state of the bird, there are several ways to transition between *phenotypic stages*, yielding different *physiological states* within the next *phenotypic stage*. The resulting physiological state depends on the initial state, and the combination of cues (excitations) leading to the phenotypic stage transition. Cycling of phenotypic substages can occur within a phenotypic stage, such as in the case of multiple broods (Figure 1.14), but after the bird has entered the next phenotypic stage, it must pass through the rest of the *phenotypic stages* before returning to the *phenotypic stage* it just completed (Figure 1.13). In cases where cycling of substages is possible (such as in the case of multiple brooded birds), the bird will integrate initial predictive and more proximal cues at the cyclic branch point to determine if another cycle is feasible. If, for example, the day length (an initial predictive cue) indicated that the breeding season was nearly over, the bird would not initiate another brood, even though the food supplies (a supplementary cue) might be very plentiful. The same would be true if the food supply was insufficient for another brood, the bird would not reneest unless the initial predictive cues were correct. This cycling is most important in the breeding period.

For a seasonally breeding bird, such as the white crown sparrow, the synchronizing source will be the progression of the seasons as driven by the movement of the earth around the sun. At each step along the life cycle, the bird

will receive excitations in the form of cues from the environment. In some cases the cues will be important individually, but in other cases, the integration of several environmental cues combine to form a single excitation.

Hormones and the Regulation of Phenotypic Stage Transitions

How do animals integrate the signals from the environment, and bring about both stage transitions, as well as changes from substage to substage? Neural integration is important in the initial collation of the sensory inputs, but except for in purely behavioral responses, the signal must be sent to a large number of cells/tissues within the body. One might think that neural innervation of all the tissues would allow for immediate action on the integrated cues, but such a system would have several limitations. First the number of neural connections would be astronomical, and would probably require a massive enlargement of both the brain and spinal cord. Second, it requires that the animal maintain a large network of neurons that have no function for most of the year, as they would only be used during the transition periods, and only a small portion of these regulatory neurons would be needed for any one transition. Clearly these constraints on both the complexity of the system, and energy requirements to maintain such a network would be prohibitive.

The endocrine system may play one of its many roles in reducing the size and complexity of the regulatory systems of an animal. Hormones are carried throughout the body by the blood system and stimulate their target cells at very

minute concentrations. This non-directed transport of the hormones may at first seem a problem: because all cells are exposed to the hormone, how can specific cells and tissues be regulated? As all hormones require receptor proteins in/on their target cells to have their actions, the easy answer to this specificity problem is that the receptors are found on only certain cells, and only at specific times. The distribution of hormone receptors does not really solve our problem, as we then need to know how the distribution of the receptors was determined.

Cells respond to cues from their environment, just as do whole organisms. In many ways, cells within the body also function like finite state machines. First, they respond to excitations from the environment in the form of hormones, growth factors, neurotransmitters, cell-cell interactions, morphogens, and concentrations of nutrients/ions in the extracellular fluid. They also have specific responses to those excitations. The nature of the response will be determined by both the excitation and the state of the cell. A nerve cell that has a strongly hyperpolarized resting potential, will not generate a spike if exposed to a moderate amount of the proper neurotransmitter. If the resting potential of the same cell becomes depolarized to near threshold, an action potential will be generated in response to the same amount of neurotransmitter. If the neuron lacks receptors for a neurotransmitter, then that neurotransmitter ceases to be an excitation for the cell, even if depolarized to near threshold. The same is true for the action of hormones. If a cell lacks receptors for a hormone, the cell will not respond to the hormone.

Part of the state of a cell will be the presence or absence of hormone receptors on/in the cell. Also important to the state of the cells will be the effect of cues from previous time intervals. If the cell become terminally differentiated into a red blood cell or fused myotube, it is certainly not going to respond to any hormone or growth factor by beginning to proliferate. The state of the second messenger systems within the cell will also be critical to the cell's response to a hormone. If the cell normally responds to a hormone by elevating cyclic-AMP levels within the cell, and the cell has elevated phosphodiesterase in responses to other cues, then the hormone may have no effect on the response of the cell.

The actual response to the cell will also depend on the nature of the hormone receptors it has. The expression of hormone receptors will be regulated by the genome of the cell, by cues from the environment, and the state of the cell. During the development of the animal, many of the most important cues will be of the developmental class, as they will determine the location and final function of the cell within the animal. The number of receptors a cell has for a given hormone will vary over time in response to other hormones and concentrations of nutrients and ions within the extracellular fluids of the animal. Many hormones, such as the steroid and thyroid hormones, have permissive actions which set up the state within a cell, such that a second hormone can then have a specific effect that was not possible without the action of the permissive hormone. Also, many hormones have several types of receptors, which have different affinities to the

hormone, and are thus activated at different concentrations of the hormone. Cells with fewer receptors will require higher concentrations of hormones to respond.

This state dependence of cellular responses to hormones is crucial to regulating complex *phenotypic stage* transitions. This allows diverse cell types to respond to the same hormone in very different ways, and allows the same type of cells to respond to the same hormone differently at different times of year, depending on the state of the cell. A metamorphosing frog tadpole would be in serious trouble if all of its cells (rather than just those of the tail) began to die and be reabsorbed in response to the thyroxine signal. Also, this same thyroxine signal has no similar effect in adults.

This state dependent response helps to explain experiments on cellular responses to hormones that have often led to cellular responses that seem to be mutually exclusive, or inconsistent. Again, the response of the cell, even in culture, will be according to its state, so if the cells are in a different state, possibly by growing in media with differing concentrations of permissive hormones, the action of the hormone to be tested may be different. Local specificity of cellular responses to hormones will be determined by the state of the cell, as set-up by the developmental path of the cell, as well as the effect of other hormones. Thus a relatively small suite of hormones can serve to regulate very complex *phenotypic stage* transitions.

Conclusions and Implications

Several ideas develop from using a finite state machine model to understand responses to a changing environment. Because the progression of phenotypic stages is unidirectional, long-lived organisms will cycle through the stages (See Figure 1.12). The tight linkage of phenotypic stages to the environment means that a given phenotypic stage is only appropriate at specific season for the organism. Each of the phenotypic stages will have associated behaviors and other physiological adaptations, specific to that stage.

Within each phenotypic stage will be a number of phenotypic substages carrying out specific functions within the stage (See figure 1.15). A certain amount predictable of cycling between the substages (such as reneating for a second brood), or cycling may be due to unpredictable transient perturbation factors (TPFs), such as reneating after nest predation (TPFs will be covered in chapter III) can occur. The progression of substages follows a limited number of paths through the phenotypic stage and these paths are generally one directional, with a few predictable cyclic branches, and possible unpredictable set backs. In most cases, the phenotypic substages are found only within a single phenotypic stage, and thus restricted to specific parts of the life cycle.

During a phenotypic substage, an organism can exist in a number of physiological states (as defined above). The stage determines the organism's responses to the cues from the environment. From a given state, only a limited

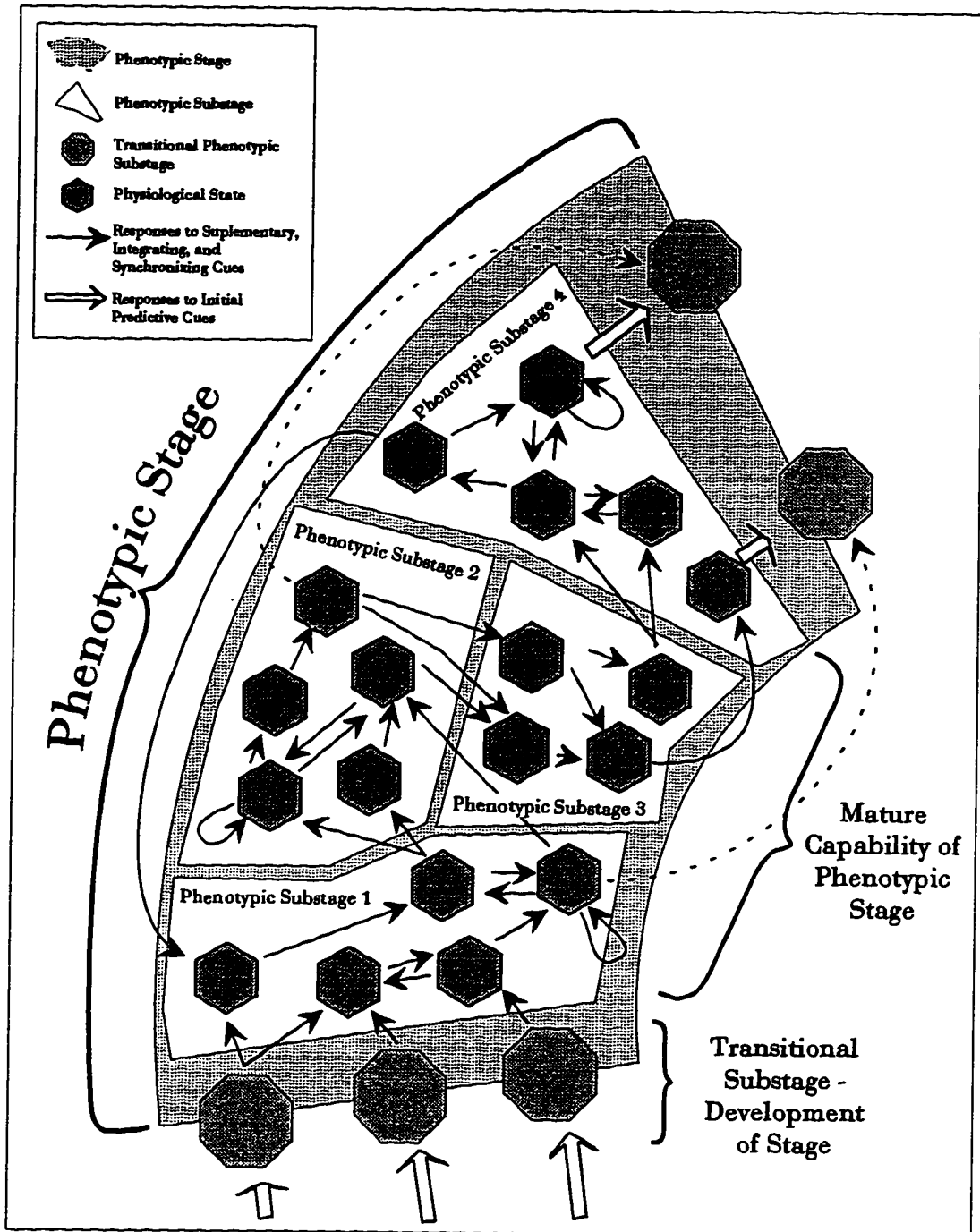


Figure 1.15 - Transition diagram for a phenotypic stage. The stage is triggered by initial predictive cues (though sometimes by supplementary and integrating cues), yielding a transition substage, where cellular processes (including possibly both differentiation and metamorphosis) are stimulated to produce the mature capability of the stage. Transition between substages and phenotypic stages are controlled by supplementary, synchronizing and integrating cues which combine with the initial predictive cues to provide the state specific excitations.

number of other states are obtainable in the next time interval. Such stage transitions are restricted to within a phenotypic stage, or into transitional substages, and are often restricted to within a single substage. Expression of physiological states, though generally progressing through the substage, is not one way, and there are many lateral detours, including those caused by TPFs. Physiological states are generally volatile and transition between states can occur quite rapidly. For example, a thirsty animal can quickly restore its water balance if given access to water. As the environment is changing constantly, so is the physiological state of the organism.

Environmental cues act on phenotypic stage transitions very differently to changes in physiological state. Because phenotypic stages are responding to a somewhat predictable environment, and the stage transitions generally involve stimulation of the cell-cycle, initial predictive cues are often most important for the regulation of these stage transitions. The precise timing of the stages is modulated by supplementary, synchronizing and integrating cues, as these cues can act to either accelerated or inhibit the progression of the stages. Transition between phenotypic states generally does not involve initial predictive cues, except at key points where the organism enters a transitional substage (See figure 1.15).

Hormones provide a possible mechanism for regulating these transitions. Transitions between phenotypic stages could be regulated late responses of hormones, or via genomic receptors, because stage transitions generally involve

activation of the cell cycle, differentiation, and gene expression. Expression of physiological stages is very rapid. While both early and late cell responses to the environment will occur, the transitions will result primarily from early cell responses via membrane receptors, including those for neurotransmitters.

This model provides a number of insights into understanding animals responses, but it also emphasizes a number of cautionary restrictions to experimenters. To make conclusions about the response of an organism to a stimulus (excitation), the experimenter must be able to determine the state of the organism. If overwintering animals are given an injection of testosterone, one can not expect them to respond in the same manner as the same animals during the breeding season. The experimenter also needs to know the normal excitations for the organism. Because excitations result from an integration of multiple cues, isolating a single cue and presenting it to the organism may result in responses not normally expressed in the field.

Thus the model proposed here provides a framework for investigating organismal responses to a changing environment. The model has features which are applicable to both whole organisms, and to cells, and provides insights into such broad questions as to why do animals have hormones.

Chapter II

Diversity Indices of Life History Stages and Their Sub-Stages:

Implications for Endocrine Control Mechanisms.

Introduction

Ecologists have long been interested in describing the diversity of biological communities. It quickly became apparent, however, that simple species lists fail to account for the abundance of individual species. For example, a simple community may have 4 species each with equal abundance whereas another community may have 4 species in which one is common and the other three are rare. Do these communities have the same species diversity? Over three decades ago, information theory provided a basis by which to describe species diversity in terms of number of species and the abundance of each. Many methods have since been derived but one of the most commonly is the Shannon Index (MacArthur and MacArthur, 1961; MacArthur, 1964):

$$H = -\sum_i p_i \cdot \log_e p_i$$

where H = diversity index and p_i is the proportion of all individuals in the community that belong to species i . The Shannon Index will be highest if all species in a community have equal abundance, but will be lower if some species are more abundant than others.

Community ecologists have used this method and others not only to describe biological communities but also to try and determine what controls diversity. Investigations have related habitat complexity to variation in species diversity (MacArthur and Preer, 1962), as well as latitude (Ricklefs, 1987), competition and predation (Martin, 1988), disturbance (Connell, 1978) etc. These techniques have also been applied to other areas of biology. For example, the Shannon Index has been applied to describe and compare the length of birds' breeding seasons (MacArthur, 1964; Wyndham, 1986). In some avian species the breeding season is constant from year to year (*i.e.* only one or two months in which nesting occurs) whereas in others the breeding season may be highly variable covering 10 months in one year and only two months in another (see also Wingfield *et al.*, 1992, 1993). Thus, simply comparing the number of months that nesting can occur in any one year for each population may be misleading. Wyndham (1986) used the Shannon Index to calculate the number of equally good months (EGM) from the formula above where p_i is now the proportion of nests initiated in the i^{th} month. EGM will vary from 1, when all nest are initiated (eggs laid) in one month in all years to 12, when the number of nests initiated is equal in all months of the year in each year. Wyndham (1986) points out that EGM varies only as a function of the proportion of birds breeding in each month and does not differentiate between single and bimodal breeding seasons. Nevertheless, the calculation of EGM allows a much more precise comparison of breeding

seasons among species in relation to habitat, latitude etc. Application of these calculations to the breeding seasons of an Australasian species, the zebra finch, *Poephila guttata*, showed a highly seasonal component to breeding (Zann *et al.*, 1995). This species has long been considered a pure opportunist capable of breeding in any month in relation to unpredictable rains. However, the analysis suggests that although this species is capable of breeding at any time year there is still an underlying seasonal component. Clearly the control mechanisms for breeding in this species will be different than formerly supposed.

This approach also allows us to choose populations with similar or very different EGM values to then compare endocrine mechanisms experimentally. In this communication we have applied diversity indices (MacArthur, 1964) to life history stages and their sub-stages (see chapter one) within individuals of populations. We then go on to compare diversity of life history stages among populations and in relation latitude, habitat etc. Such comparisons will allow more meaningful assessment of endocrine mechanisms underlying transitions in life history stages and may shed further light on why there is such variation in hormone action among at least vertebrate taxa.

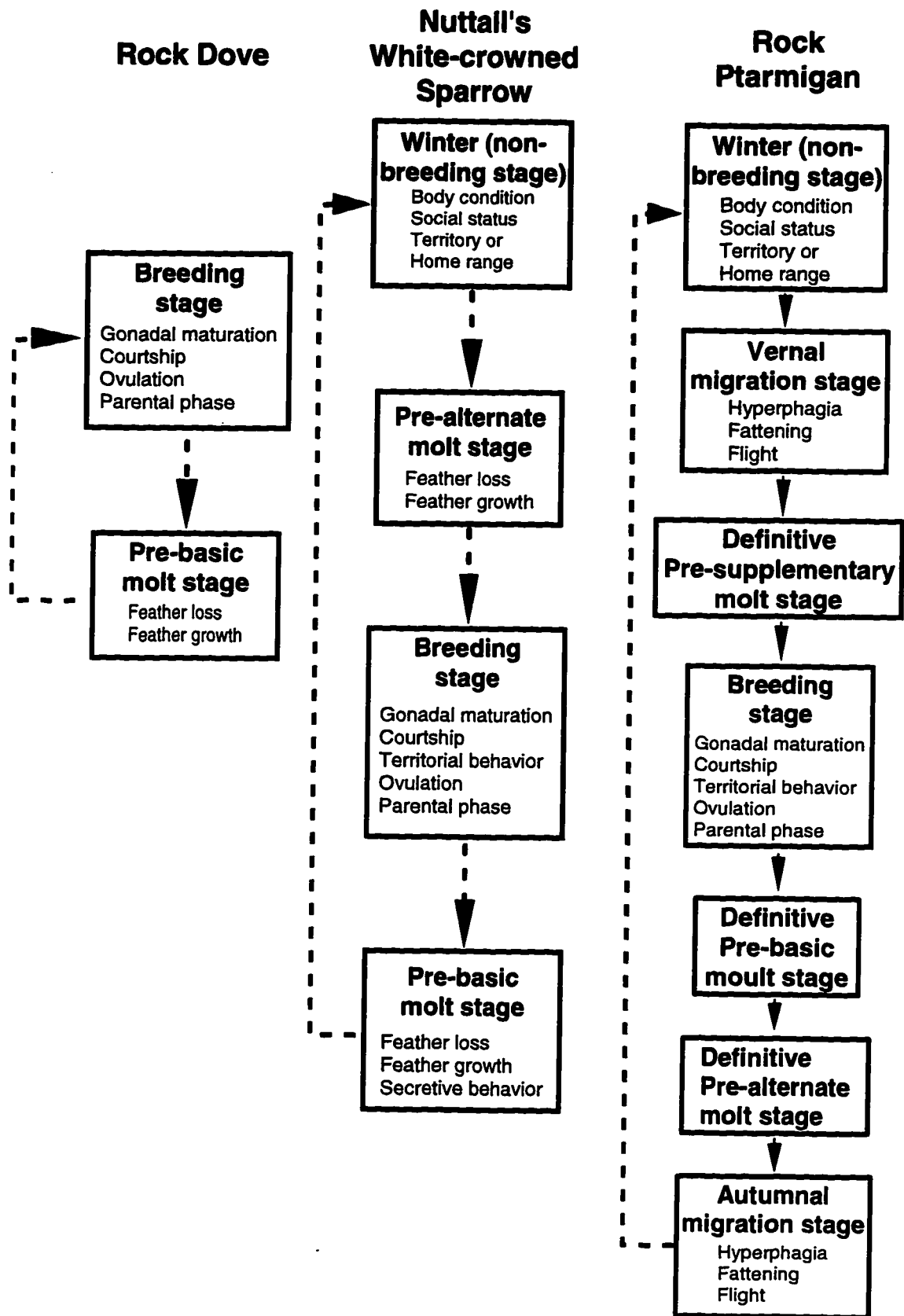
Finite State Machines and Diversity Indices.

A major role of hormones is to regulate transitions in morphology, physiological state and behavior. These transitions usually involve a period of development of a life history stage followed by a 'mature stage' in which a number

of sub-stages can be activated. Hormones may also play a major role in the activation of these sub-stages through mechanisms that are very different from their roles in promoting development (and also termination) of that stage (see chapter one). For each organism, then, we can postulate a finite state machine of several phenotypic (life history) stages that occur in a fixed sequence and often on a schedule determined by, for example, the changing seasons. Each life history stage is independent of the others although some may overlap to varying degrees, and each has a unique set of sub-stages (see chapter one). Some species may have more life history stages than others and even within an individual, some life history stages may have a more complex set of sub-stages than others.

The degree to which variation in life history stages exists is presented for three species of birds in Fig. 2.1. The rock dove (*Columba livia*) has only two obvious life history stages, breeding and molt (from Johnston, 1992), whereas the Nuttall's white-crowned sparrow (*Zonotrichia leucophrys nuttalli*) has four (e.g. Blanchard, 1941; Mewaldt and King, 1977) and the rock ptarmigan (*Lagopus mutus*) has seven (Holder and Montgomerie, 1992). Note also that the number of sub-stages within each stage, and unique to that stage, varies. Comparing these finite state machines, it is clear that a problem remains as to how we compare the complexity of life history stages across taxa. Because these transitions of stage and activation of sub-stages are regulated by hormones, there are also likely to be major differences among species at cell and molecular levels. How do we compare

Figure 2.1 - Finite state machines of life history stages for three avian species. In the rock dove (*Columba livia*) there are two major life history stages, breeding and molt, designated by the boxes. Dashed lines indicate the direction of progression from one stage to another. Note also that the number of sub-stages (finer print within boxes) is greater for the breeding stage than for pre-basic moult. The center column represents four life history stages for the Nuttall's white-crowned sparrow (*Zonotrichia leucophrys nuttallii*). Again, dashed lines and arrows indicate the direction of progression. This temporal sequence cannot be reversed. Finer print within boxes indicates the number of sub-stages. On the right is the most complex finite state machine with seven life history stages in the rock ptarmigan (*Lagopus mutus*). Dashed lines and arrows indicate the direction of progression, and finer print in boxes represents the sub-stages. Sub-stages in the three different moult stages here have been omitted but include feather loss and feather growth in each case. These may be different for each type of moult because different feathers may be moulted, and even if they are in the same feather tracts, the color of the feather that develops is different. So although the sub-stages in these different life history stages may appear to be the same, they may in fact have very different ecological bases and control mechanisms (see Wingfield et al., 1996 for discussion of other examples).



life history stages across taxa, and what additional information does the finite-state machine give us? As with species diversity, simple number of stages (or species) may not be an accurate assessment. We can outline a number of questions that integrate all these possibilities and are derived from those questions posed by ecologists (*e.g.* Pielou, 1969, 1975):

- a). Why are there not more (or fewer) life history stages and will their durations be the same? Another way of stating this would be, do the life history stages differ much in their tolerance ranges for environmental variables (see also chapter one).
- b). Why are some stages widespread and others rare and are their relative proportions (durations) constant?
- c). Are most, or all, of the life history stages within an individual fully adapted to the habitat at that time?

From Fig. 2.1 we see that some organisms have only a few life history stages and others have many. Thus we can apply formulae for ecological diversity (*e.g.* species diversity indices) to finite state machines to give us a measure of finite state diversity. This allows us to go beyond simply counting numbers of life history stages, and indicates the diversity of stages as a function of their duration as well as number.

Before finite state diversity can be measured, the temporal sequence of life history stages within an individual of a given population must be defined and

applied equally to all (see chapter one). This definition must specify the boundaries of the temporal sequence and how each stage is identified and its duration measured. The boundaries of the temporal sequence could be defined at many levels such as day, month, tidal fluctuation, wet season duration, year or longer). Numbers of life history stages must be determined by the same criteria in all populations. In chapter one I defined a life history stage as having a unique set of sub-stages (*i.e.* not shared by other life history stages), and each life history stage occurs in a set temporal sequence that cannot be reversed. For example in the white-crowned sparrow (Fig. 2.1), it is not possible to go back from the breeding stage to pre-alternate molt stage directly. It can only be attained by progressing through pre-basic molt and winter stages. On the other hand, the sub-stages within a life history stage can be activated in any sequence, or repeated many times within a life history stage (see below).

Duration of each life history stage can be measured directly from field observations. In the rock dove (Fig. 2.1), individuals are almost always in breeding condition once adult as indicated by developmental state of the gonads. Pre-basic molt, however, may be restricted to a few months of the year (Johnston, 1992). Thus, the durations of these two life history stages are very different but they also overlap almost completely. In the rock ptarmigan, a larger number of life history stages could mean shorter duration of each stage unless there is considerable overlap. In general, it is likely that some life history stages (such as winter and

molt) are more compatible in terms of overlap whereas others (such as migration and breeding) are much less compatible. The application of diversity indices is one way by which we can describe number, duration and overlap of life history stages across all populations of all taxa.

Finite State Diversity

Bearing in mind the admonition of Pielou (1969; 1975) that we should be careful in how we apply mathematical techniques to biological problems and not take too many liberties in stretching assumptions made in mathematics to highly complex biological processes, we have applied the Shannon diversity index (formula given above) to finite-state machines of life history stages. We use the ideas of MacArthur (1964) to use this formula to calculate the diversity of life history stages as a function of their number, duration and degree of overlap. In this way we are able to formally describe finite state machines of different populations as a frame work to formulating hypotheses about hormonal control. We feel that this objective method will allow us to identify which populations should be compared to give maximum power in determining those mechanisms.

To illustrate these points we present some theoretical examples first as a way of defining our methods. The boundary of the temporal sequence we define here as one year with intervals of months (*i.e.* 12 intervals in Fig. 2.2). The vertical axis is then made up of different life history stages (for example 6 stages in Fig. 2.2). For a given temporal sequence of hypothetical life history stages we can

MSID = 2, TSD = 6

| | | Interval | | | | | | | | | | | |
|-------|--|----------|---|---|---|---|---|---|---|---|----|----|----|
| Stage | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | | | ● | | | | | | | | | | |
| 2 | | | | ● | ● | | | | | | | | |
| 3 | | | | | | ● | ● | | | | | | |
| 4 | | | | | | | | ● | ● | | | | |
| 5 | | | | | | | | | | ● | ● | | |
| 6 | | | | | | | | | | | | ● | ● |

MSID = 4, TSD = 3

| | | Interval | | | | | | | | | | | |
|-------|--|----------|---|---|---|---|---|---|---|---|----|----|----|
| Stage | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | | ● | ● | ● | ● | | | | | | | | |
| 2 | | | | ● | ● | ● | ● | | | | | | |
| 3 | | | | | | ● | ● | ● | ● | | | | |
| 4 | | | | | | | | ● | ● | ● | ● | | |
| 5 | | | | | | | | | | ● | ● | ● | ● |
| 6 | | ● | ● | | | | | | | | | ● | ● |

Figure 2.2 - Schematic representation of finite state diversity. Each column represents an interval in a temporal sequence of hypothetical life history stages. In this example the intervals are months in a year. The rows represent hypothetical life history stages; in this example we have six. The hatched circles indicate in which interval each life history stage is expressed by an organism in a given population. In the top panel, each stage has a duration of 2 months and there is no overlap. In this case, the mean stage interval diversity (MSID) is calculated as 2 and the temporal stage diversity (TSD) is 6. In the lower panel, the duration of each state is now four months so that at each interval, two stages are expressed simultaneously. MSID now equals 4 and the TSD 3. See text for details.

use the formula above to calculate the mean stage interval diversity (MSID) which is a measure of the diversity in duration of each life history stage; and the temporal stage diversity (TSD) which is a measure of the diversity of life history stages as a function of not only their duration, but also number and degree of overlap. Both of these measures may have considerable import for hormone mechanisms (see below). Some hypothetical examples will now show how MSID and TSD vary.

If we assume six life history stages each of two months duration (indicated by the shaded circles corresponding to each stage and interval in Fig. 2.2 upper panel) and with no overlap, then this gives us a MSID of 2 (*i.e.* all are the same - two months duration) and a TSD of 6 (there are 6 life history stages with no overlap). Note also that in these finite state machines, the organisms must be in at least one life history state at all times (otherwise it obviously would not be alive). If we now assume six life history stages each with four months duration (lower panel of Fig. 2.2), then we get a MSID of 4 (*i.e.* all stages have the same duration of 4 months) and a TSD of 3 (there is 50% overlap of all life history stages with the organisms expressing two in each interval).

We can go on to more complex hypothetical examples. The top panel of Fig. 2.3 is the same as that for Fig. 2.2 (for comparison, six life history stages each with two months duration). If we then assume six life history stages with unequal duration (one to four months, Fig. 2.3 middle panel) then we get a MSID of 2.7

$$\text{MSID} = 2$$

$$\text{TSD} = 6$$

| Stage | Interval | | | | | | | | | | | |
|-------|----------|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | ● | ● | | | | | | | | | | |
| 2 | | | ● | ● | | | | | | | | |
| 3 | | | | | ● | ● | | | | | | |
| 4 | | | | | | | ● | ● | | | | |
| 5 | | | | | | | | | ● | ● | | |
| 6 | | | | | | | | | | | ● | ● |

$$\text{MSID} = 2.7$$

$$\text{TSD} = 4.5$$

| Stage | Interval | | | | | | | | | | | |
|-------|----------|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | ● | ● | | | | | | | | | | |
| 2 | | | ● | ● | ● | | | | | | | |
| 3 | | | | | | ● | | | | | | |
| 4 | | | | | | | ● | | | | | |
| 5 | | | | | | | | ● | ● | ● | ● | |
| 6 | | | | | | | | | | | | ● |

$$\text{MSID} = 4.5$$

$$\text{TSD} = 2.7$$

| Stage | Interval | | | | | | | | | | | |
|-------|----------|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | ● | ● | ● | ● | ● | ● | ● | | | | | |
| 2 | | | | | | | | ● | | | | |
| 3 | | | | | | | | | ● | | | |
| 4 | | | | | | | | | | ● | | |
| 5 | | | | | | | | | | | ● | |
| 6 | | | | | | | | | | | | ● |

Figure 2.3 - Schematic representation of finite state diversity. Each column represents an interval in a temporal sequence of hypothetical life history stages. In this example the intervals are months in a year. The rows represent hypothetical life history stages; in this example we have six. The hatched circles indicate in which interval each life history stage is expressed by an organism in a given population. In the top panel, each stage has a duration of 2 months and there is no overlap (identical to the top panel of Fig. 2.2 for comparisons). In the middle panel, the duration of each stage is variable but there is no overlap. Here $\text{MSID}=2.7$ and $\text{TSD}=4.5$. In the lower panel we have an extreme hypothetical example where one stage has a duration of seven months and the rest have a duration of one month each. MSID is now 4.5 and TSD is 2.7. See text for details.

and a TSD of 4.5. In the lower panel of Fig. 2.3 we have six life history stages with an extremely skewed duration. One stage covers seven months (intervals) and the other 5 are of one month (interval) each with no overlap. In this case the MSID = 4.5 and TSD = 2.7. Clearly the diversity of life history stages is not simply a function of how many there are, but also functions of duration of each stage and the degree of overlap with other stages. Is this variation in finite state diversity reflected in natural examples?

If we now go to our avian examples from Fig. 2.1 and apply the diversity indices, once again we see considerable variation (Fig. 2.4). In the rock dove the breeding stage is found in up to 10 months and the molt in six months. This gives a MSID of 8.5 (both are relatively long in duration) and a TSD of 1.3 (very few stages and with considerable overlap). In the white-crowned sparrow (mid panel of Fig. 2.4), the four life history stages are of shorter duration with no overlap giving a MSID of 3.8 and a TSD of 3.1. The rock ptarmigan (lower panel of Fig. 2.4) has seven life history stages with varying degrees of overlap. In this case, MSID = 3.4 and TSD = 3.4. From these data it now becomes clear that although the application of the Shannon index is useful for comparing finite state diversity across taxa, it has a drawback in that in some cases very different temporal sequences of life history stages can give similar MSID and TSD values. Compare the number and temporal sequence for the white-crowned sparrow and rock ptarmigan in Fig. 2.4, they appear very different but the MSID and TSD values are

Rock Dove

MSID = 8.5
TSD = 1.3

| | Interval | | | | | | | | | | | |
|-------|----------|---|---|---|---|---|---|---|---|----|----|----|
| Stage | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | ● | ● | ● | ● | ● | ● | ● | ● | ● | | | |
| 2 | | | | | | | ● | ● | ● | ● | ● | ● |

Nuttall's White-crowned Sparrow

MSID = 3.8
TSD = 3.1

| | Interval | | | | | | | | | | | |
|-------|----------|---|---|---|---|---|---|---|---|----|----|----|
| Stage | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | ● | ● | | | | | | | | ● | ● | ● |
| 2 | | | ● | | | | | | | | | |
| 3 | | | | ● | ● | ● | ● | | | | | |
| 4 | | | | | | | | ● | ● | | | |

Rock Ptarmigan

MSID = 3.4
TSD = 3.4

| | Interval | | | | | | | | | | | |
|-------|----------|---|---|---|---|---|---|---|---|----|----|----|
| Stage | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | ● | ● | ● | ● | | | | | | | ● | ● |
| 2 | | | ● | | | | | | | | | |
| 3 | | | | ● | ● | | | | | | | |
| 4 | | | | | | ● | ● | | | | | |
| 5 | | | | | | | ● | ● | | | | |
| 6 | | | | | | | | | ● | ● | | |
| 7 | | | | | | | | | ● | | | |

Figure 2.4 - Finite state diversity for the three avian species in Fig. 2.1. Each column represents an interval in a temporal sequence of hypothetical life history stages (i.e. months of the year). The rows represent life history stages that vary according to species. The hatched circles indicate in which interval each life history stage is expressed by an individual in a given population. In the rock dove (top panel) there are two life history stages with relatively long durations and considerable overlap. MSID is high and TSD low. In the white-crowned sparrow and rock ptarmigan, there are more life history stages with shorter durations and varying degrees of overlap. Here the MSID and TSD values for each species are similar. See text for details.

very similar. Exactly the same problems have been encountered by ecologists when using this formula, and others, to determine species diversity (Pielou, 1969, 1975). For these reasons community ecologists have largely abandoned this approach. However, in this case, we feel that this property of the method may indicate useful information. Here we use the technique to describe the finite state diversity not define it. Thus is it possible that although the number and temporal sequence of life history stages in rock ptarmigan and white-crowned sparrows appear to be different superficially, the MSID and TSD values suggest they are very similar. Does this mean that the hormonal mechanisms underlying control of each stage are also similar? Conversely we could argue that since the finite state diversity of these species are very different from those of the rock dove, then the latter species may have very different control mechanisms. These hypotheses generated from the diversity index are eminently testable and will allow us to quickly identify extremes within taxa and similarities across widely diverse taxa. We can then test whether mechanisms differ or show convergence accordingly. This has the potential to provide a basic framework for environmental endocrinology.

Before going we should also point out that we are aware that the Shannon index should be ideally applied to an infinite population (or collection of stages and intervals, Pielou, 1969, 1975). This is obviously not the case for finite state machines with finite numbers of life history stages and intervals in which they are

likely to occur. We also calculated the Brillouin index for a finite population (Pielou, 1969, 1975) and compared the MSID values using the two indices for finite state machines from several species and populations of species (Fig. 2.5). Clearly the MSID values using the Shannon Index and Brillouin index in relation to the number of phenotypic stages (*i.e.* life history stages) are superimposable. Because the Brillouin index poses program problems owing to factorials within the formula, we use the Shannon index throughout.

Finite State Diversity in Different Taxa

Natural histories for several species and populations within species were used to determine the number of phenotypic stages (life history stages) and their estimated duration in months. From these data the MSID and TSD were calculated and plotted against the number of phenotypic stages (Fig. 2.6). Species from which data were compiled are named in the legend to Fig. 2.6 with references. MSID decreases as the number of phenotypic stages increases (Fig. 2.6, upper panel). This is expected as the duration of each stage must go down as the number of stages increases. However, this relationship is not a simple straight line. There can be considerable variation in MSID for species with similar phenotypic stages (*e.g.* stages 2 and 3 in the upper panel of Fig. 2.6). Similarly, there can be wide variation in phenotypic stages for a particular MSID (*e.g.* see the range of stages for the MSID value of 3.5-4.5 in Fig. 2.6).

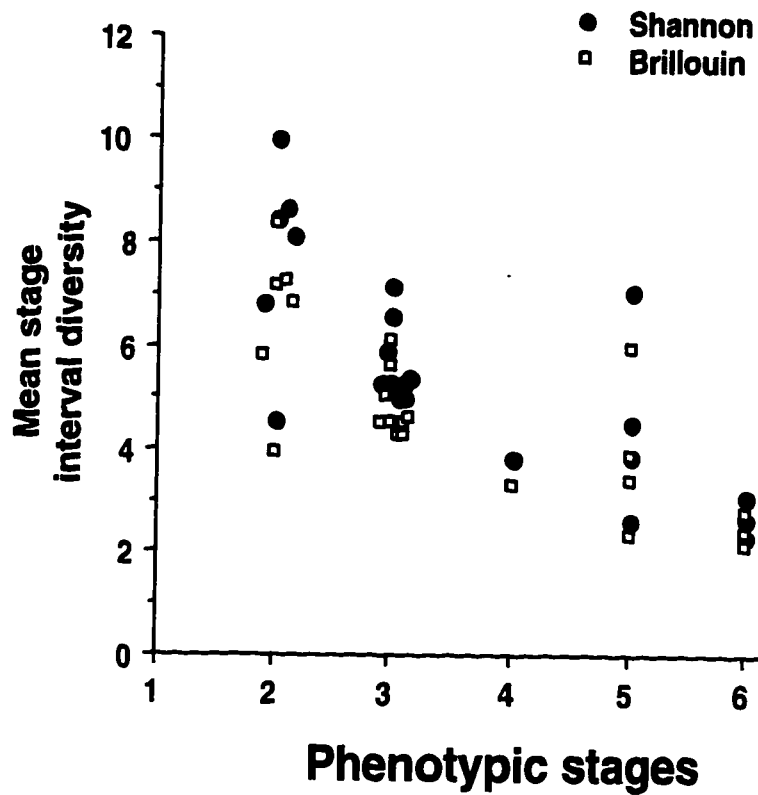


Figure 2.5 - A comparison of mean stage interval diversity calculated by the Shannon or Brillouin indices in relation to life history stages (phenotypic stages) in various avian taxa and populations. See legend for Fig. 2.6 for list of species. Values from the two methods are superimposable.

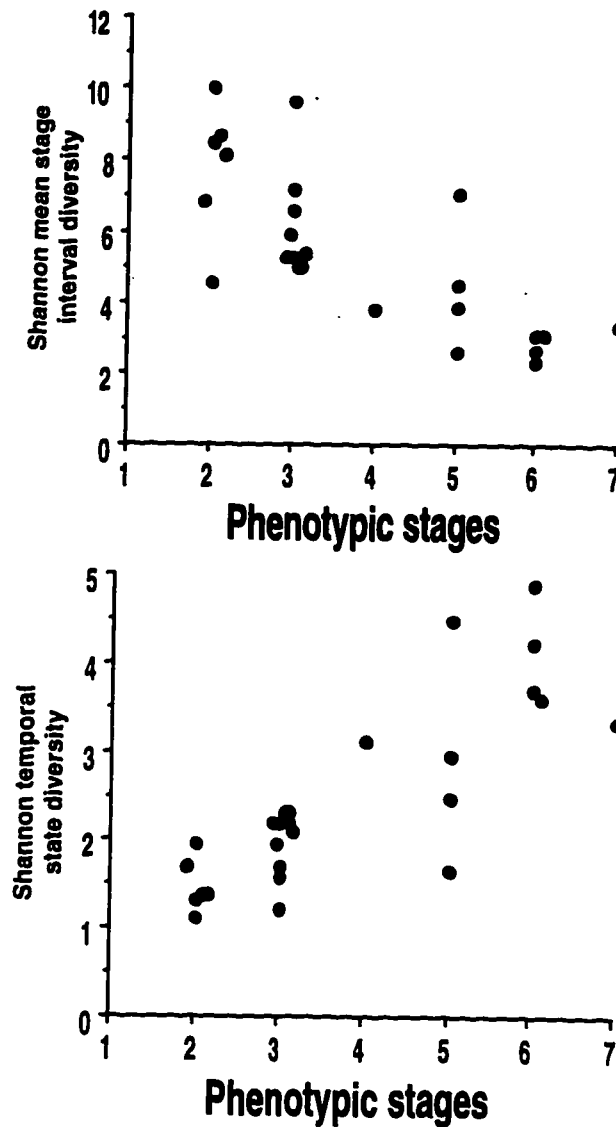


Figure 2.6 - Mean stage interval diversity (upper panel) and temporal stage diversity (lower panel), in relation to phenotypic stages (life history stages) of several taxa and sub-populations. These diversities were calculated from the Shannon index. Data were compiled from the following species: masked booby, *Sula dactylatra*, (Anderson, 1992); Smith's longspur, *Calcarius pictus*, (Briskie, 1992); European starling, *Sturnus vulgaris*, (Cabe, 1992). sooty tern, *Sterna fuscata*, (Chapin, 1954); semipalmated sandpiper, *Calidris pusilla*, (Gratto-Trevor, 1992); rock ptarmigan, *Lagopus mutus*, (Holder and Montgomerie, 1992); rock dove, *Columba livia*, (Johnston, 1992); house sparrow, *Passer domesticus*, (Lowther and Cink, 1992); Cassin's auklet, *Ptychoramphus aleutica*, (Manuwal and Thoresen, 1992); rufous collared sparrow, *Zonotrichia capensis*, (Miller, 1962); Inca dove, *Scardafella inca*, (Mueller, 1992); Laysan albatross, *Diomedea immutabilis*, (Whittow, 1992); white-browed sparrow weaver, *Plocepasser mahali*, (Wingfield et al., 1991); western gull, *Larus occidentalis wymani*, (Wingfield et al., 1982); zebra finch, *Poephila guttata*, (Zann et al., 1995); red crossbill, *Loxia curvirostra*, (Hahn, 1993); snow bunting, *Plectrophenax nivalis* (J.C. Wingfield and L.M. Romero unpublished); and several populations of white-crowned sparrow, *Zonotrichia leucophrys*, and song sparrow, *Melospiza melodia*, (Blanchard, 1941; Blanchard and Erickson, 1949; Mewaldt and King, 1977); Wingfield and Farner, 1978a,b; Wingfield and Hahn, 1994).

For TSD the trend is opposite to MSID as we would expect (lower panel of Fig. 2.6). As the number of phenotypic stages increases then TSD should also increase but again this is not a perfect straight line. There is wide variation in TSD for species with identical numbers of phenotypic stages (e.g. stage 5 in Fig. 2.6 lower panel). Additionally, TSD may be similar over a range of phenotypic stages (e.g. TSD value of 1.5-2.5 in lower panel of Fig. 2.6). These data suggest that calculating TSD and MSID can indicate much about variation in finite state diversity for different species. Are the mechanisms underlying control of the temporal sequence of phenotypic stages the same for MSID values of 10 and 4 when only two phenotypic stages are expressed (e.g. Fig. 2.6 upper panel) or for TSD values of 4.5 and 1.5 when 5 phenotypic stages are expressed? Conversely, are the mechanisms underlying the regulation of transitions from stage to stage similar when MSID values are similar across several species with different numbers of phenotypic stages? These kinds of analyses can indicate which comparisons can provide the most critical experiments to determine how individuals orchestrate morphological, physiological and behavioral adjustments throughout their life cycles.

It is also possible to conduct these kinds of analysis at different taxonomic levels. Comparisons of closely related species within a sub-family, e.g. the emberizinae (Fig. 2.7) show that the relationships of MSID, TSD and numbers of phenotypic stages are more linear. This is even more striking if a single genus is

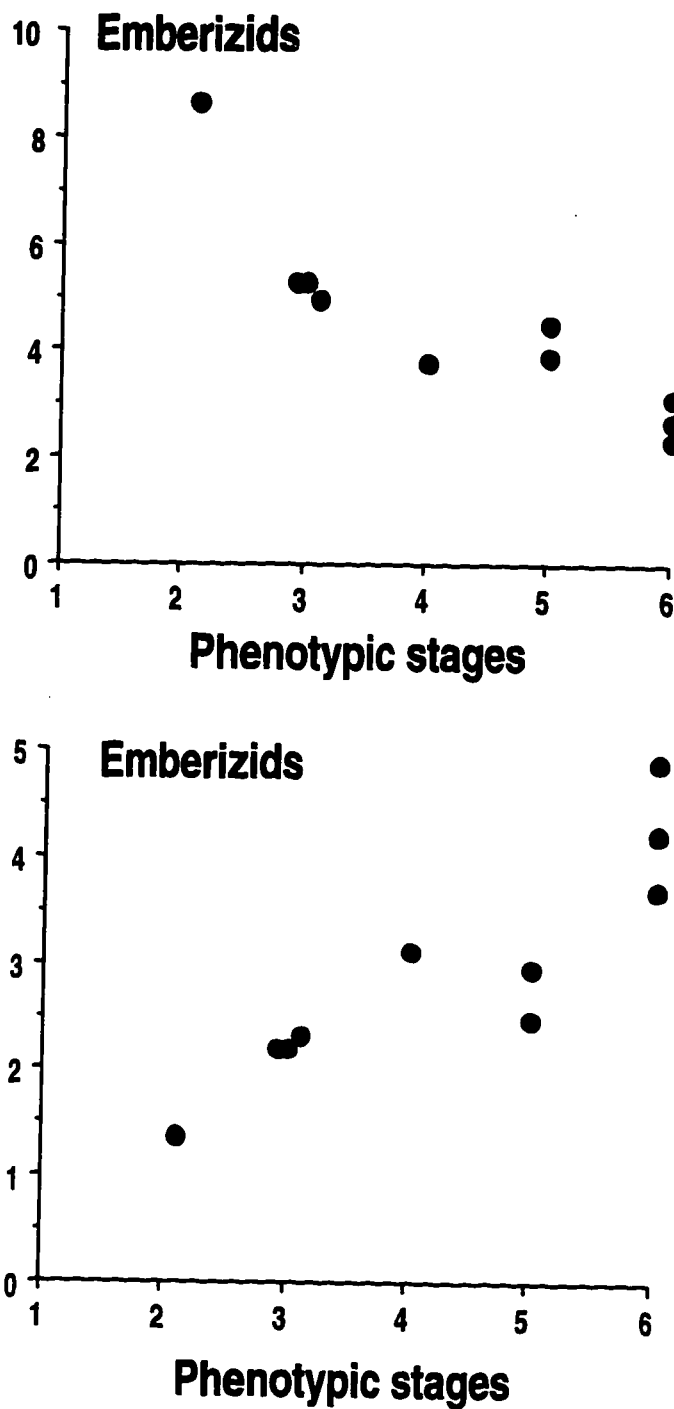


Figure 2.7 - Mean stage interval diversity (upper panel) and temporal stage diversity (lower panel), in relation to phenotypic stages (life history stages) of several taxa and sub-populations. Mean stage interval diversity and temporal stage diversity in relation to the number of phenotypic stages in a sub-family of passerines (Emberizinae). See legend for Fig. 2.6 for species and references.

compared (e.g. *Zonotrichia*, Fig. 2.8). Here then it is possible to conduct experiments on congeners to determine how mechanisms differ along a defined array of finite state diversities. Going one step further to look at populations of a single taxon of song sparrow (*Melospiza melodia morphna*), we can see that there is little change in MSID or TSD even though this one population has representatives with two different phenotypic stages (Fig. 2.9). Does this indicate that the mechanisms underlying transition from one stage to another are identical within these sub-populations? Clearly these methods allow us to analyze finite state diversity in a way that provides clear hypotheses and identifies which taxa will provide the best comparisons - even among very closely related taxa.

Assessing the Complexity of Sub-stages Within Each Phenotypic Stage.

Each phenotypic stage has a unique set of sub-stages (Fig. 2.1). Whereas phenotypic stages are expressed in a fixed temporal sequence, the sub-stages can be expressed in variable sequences or sets. From Fig. 2.1 it is clear that the number of sub-stages within each phenotypic stage can vary greatly from species to species and from stage to stage within an individual. Since the activation of these stages requires hormonal and neural input, probably in response to appropriate environmental cues, then the control mechanisms may vary from species to species within a type of stage as well as among different stages within an organism. Thus, there is a need to formally analyze the degree of complexity of sub-stages within

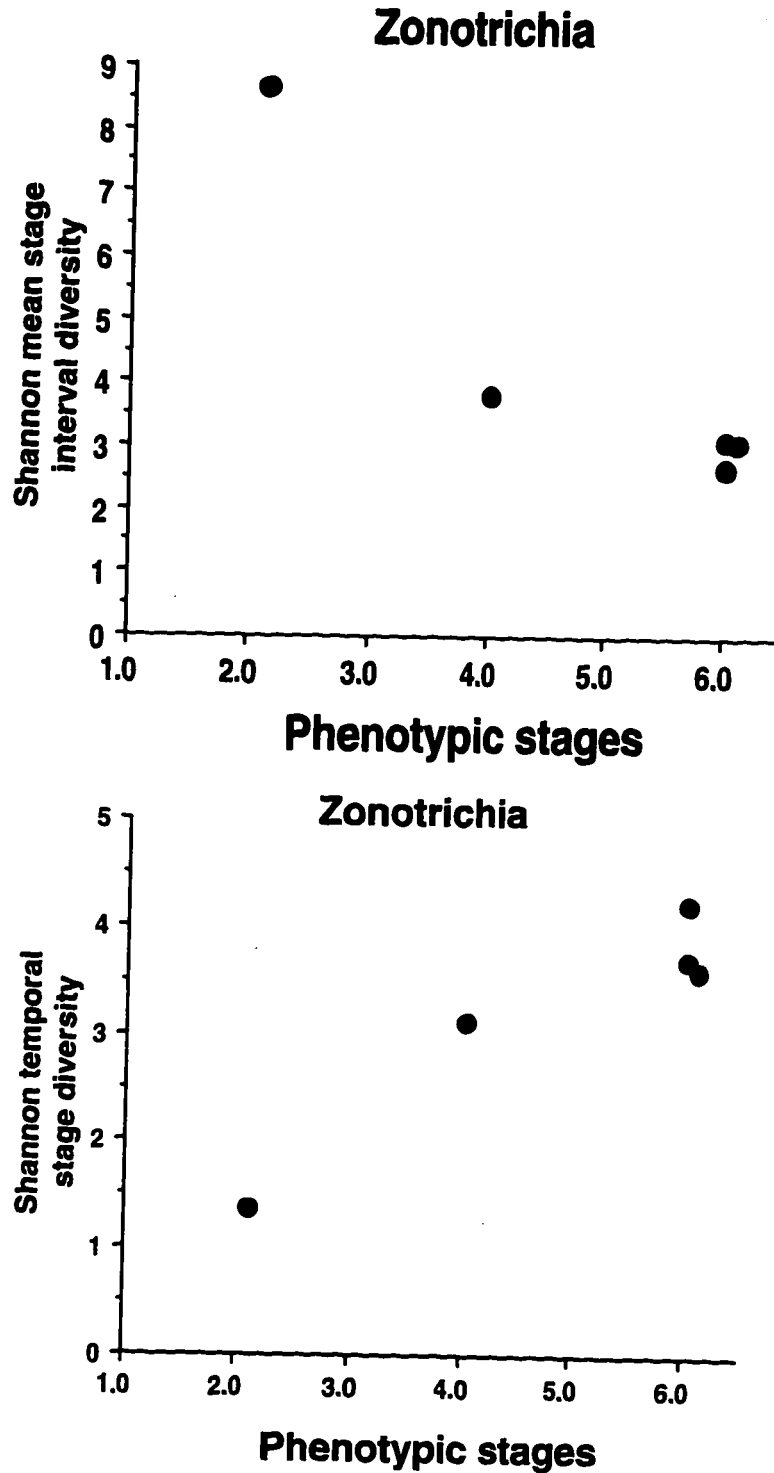


Figure 2.8 - Mean stage interval diversity and temporal stage diversity in relation to the number of phenotypic stages within a single genus, *Zonotrichia*. Taxa are: *Z. capensis*; *Z. leucophrys gambelii*; *Z.l. nuttallii*; *Z.l. pugetensis*, and *Z.l. oriantha*. See legend for Fig. 2.6 for references.

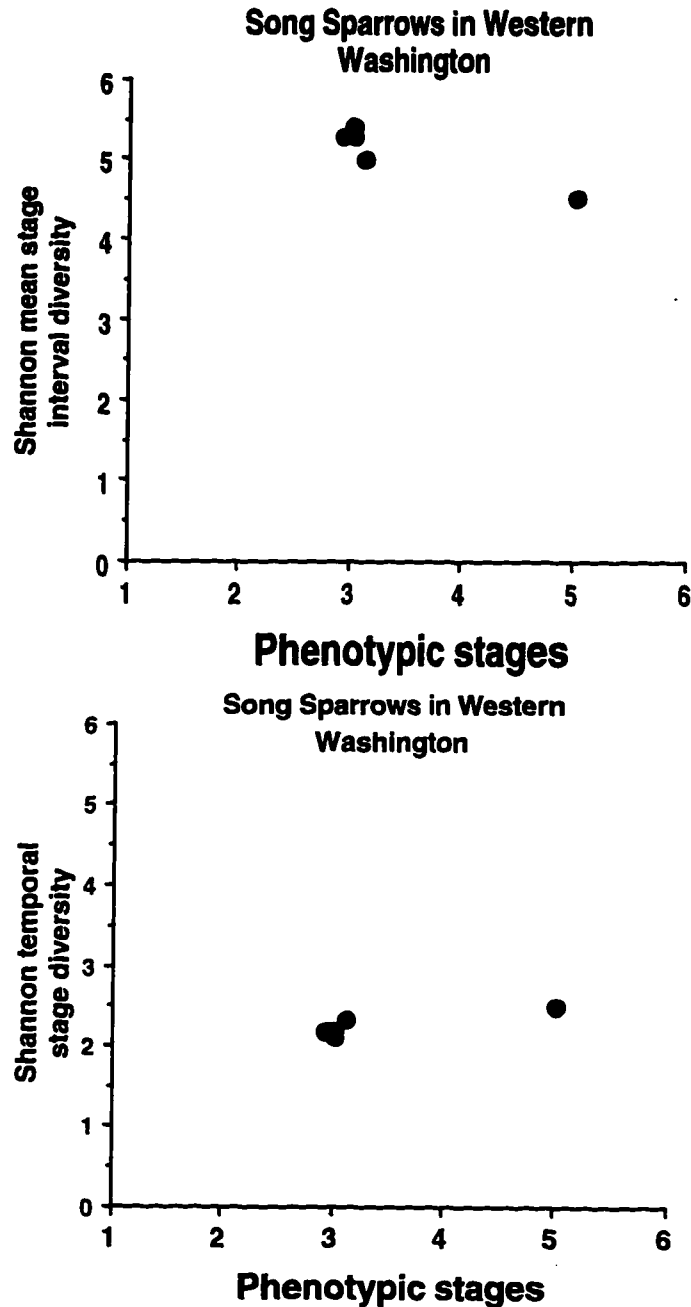


Figure 2.9 - Mean stage interval diversity and temporal stage diversity in relation to the number of phenotypic stages within a population of song sparrow, *Melospiza melodia morphna*, in western Washington State. These sub populations were studied at a sites on the Pacific Coast, lower Puget Sound, San Juan Islands, Mid and high elevations of the Cascade Mountains. All were at similar latitude (47-48° N). One of these populations (mountain) has two extra phenotypic stages - spring and autumn migrations between the lowlands and mountain passes (from Wingfield and Hahn, 1994; J.C. Wingfield unpublished).

each phenotypic stage, not only the number of sub-stages but also how each is connected to other sub-stages.

An illustration of variation in complexity of sub-stages is given in Fig. 2.10 for the migratory white-crowned sparrow, *Zonotrichia leucophrys gambelii*. The breeding stage (Fig. 2.10a) is complex and consists of at least eight distinct sub-stages. Firstly gonad development is necessary and then several sub-stages can follow such as establishment of a breeding territory, courtship and pair-bonding, nest building, and ovulation/copulation/egg-laying. Following this sexual phase then several parental sub stages can be expressed such as incubation, feeding nestlings and fledging. There is a temporal sequence in broad terms but note that if the nest or eggs are lost to predator then the various sexual sub-stages can be expressed again. Also if the population is multiple brooded then sexual and parental sub-stages can be repeated. This does not mean, however, that all sub-stages are equally connected with other sub-stages. In Fig. 2.10a we show the main known connections of sub-stages. The maximum number of connections is calculated from the formula $n(n-1)$. In Fig. 2.10a the maximum number of connections possible is 56 but only 19 (ratio of 0.34) have been identified in field studies of white-crowned sparrows (from Blanchard, 1941; Blanchard and Erickson, 1949; Mewaldt and King, 1977; Wingfield and Farner, 1978a,b).

The migration stage is less complex (Fig. 2.10b) consisting of a migratory readiness (zugdisposition) followed by periods of hyperphagia and fattening and a

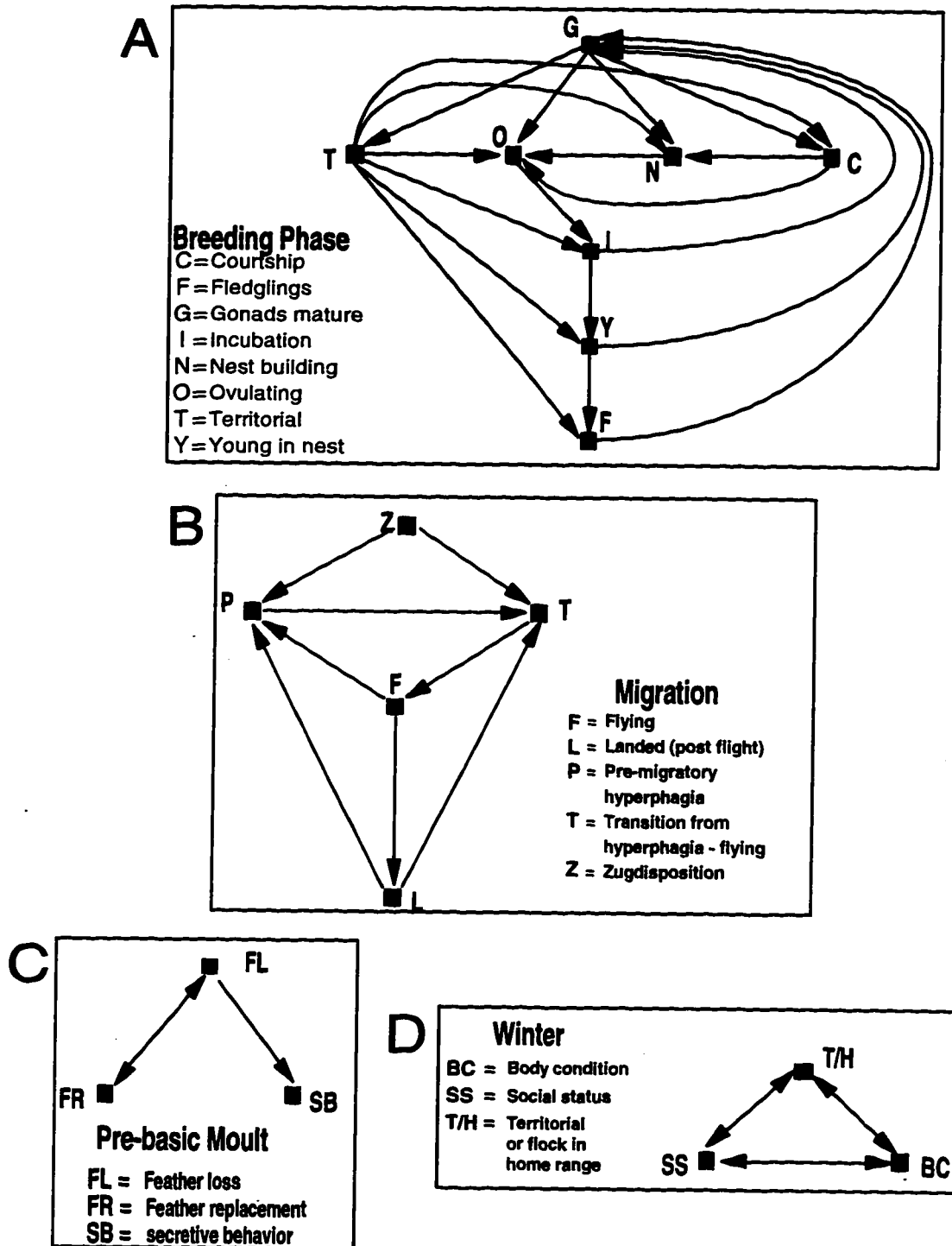


Figure 2.10 - Schematic representation of connectivity of sub-stages within life history stages of the migratory white-crowned sparrow, *Zonotrichia leucophrys gambelii*. Note that not all interconnections of sub-stages may actually be possible. Also, note that the diversity of sub-stages varies markedly with life history stage even within a population.

transition period before an actual flight. After landing there is another transition back to hyperphagia and fattening (see Wingfield *et al.*, 1990). Here the maximum number of connections is 20, but only 8 (ratio of 0.4) are demonstrated (Fig. 2.10b). For the pre-basic molt stage (Fig. 2.10c) there are only three sub-stages and the ratio of connections is 0.5. For the winter stage there is also a smaller number of sub-stages but connectivity is maximal (*i.e.* 1.0. Fig. 2.10d). Clearly connectivity varies not only as a function of the number of sub-stages but also as a function of known relationships and sequences of events within a phenotypic stage.

We can also compare the sub-stages within the same phenotypic stage across different taxa. For example, the connections of sub-stages in the breeding stage are different depending upon whether the species is single brooded (Fig. 2.11a) or double brooded (Fig. 2.11b). The potential number of connections is 56 in each case, but the number of actual connections is only 16 (ratio of 0.29) for single brooded and 0.34 for multiple brooded. For a brood parasite (*e.g.* the brown-headed cowbird, *Molothrus ater*, Dufty and Wingfield, 1986a,b) there is no parental phase at all because the females lays her eggs in the nests of other species. Here there are only 4 sub-stages (Fig. 2.11c) with a connectivity of 0.5. One can also compare individuals within a population but with different breeding stages (Fig. 2.12). Breeding females that show parental care will have a connectivity ratio of about 0.34 as in Figs. 2.10 and 11 (Fig. 2.12a). However, many males show no

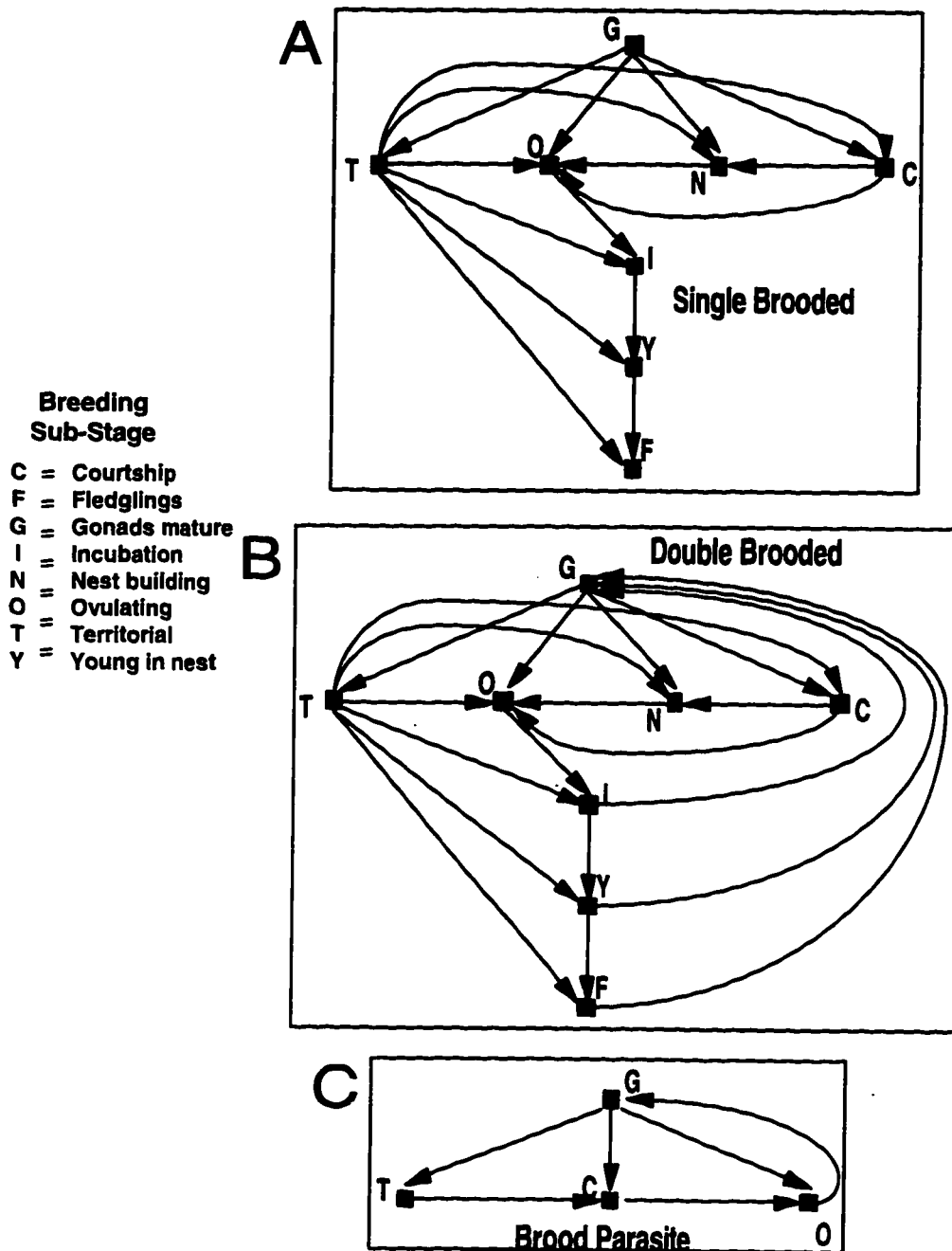


Figure 2.11 - Schematic representation of connectivity of sub-stages within a specific life history stage in different taxa of birds. In A, connectivity of single brooded populations when in the breeding stage is not as complex as in a double-brooded population (B). The breeding stage is even more simplified in terms of number and connectivity of sub-stages in a brood parasite that has not parental sub-stages (C).

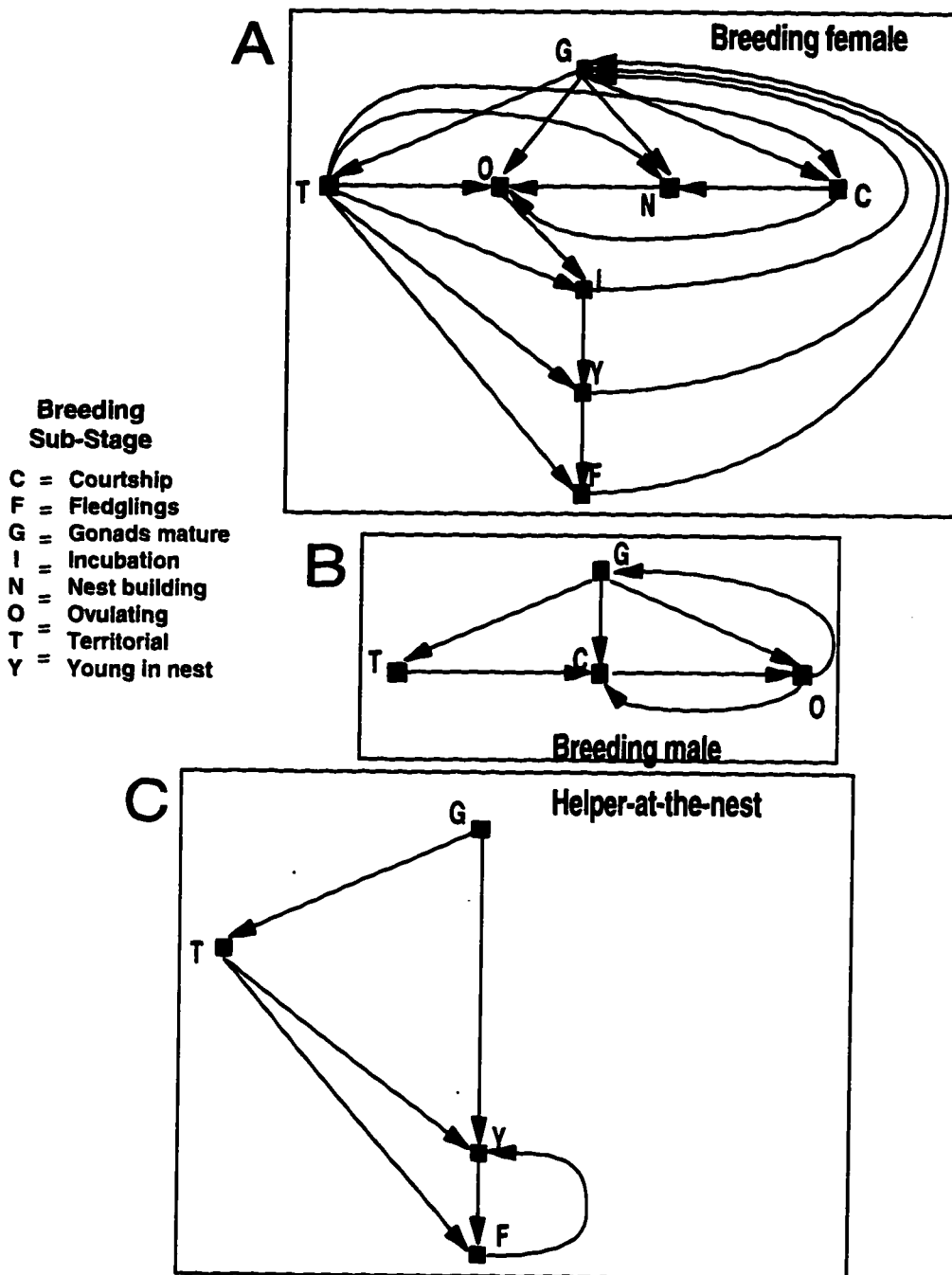


Figure 2.12 - Schematic representation of connectivity of sub-stages within a specific history stage within a population. In A, connectivity of sub-stages in a breeding female may be much more complex than for that of males which show no parental care (B). In cooperatively breeding species, the number of sub-stages and their connectivity may be even more simple. In this case individuals show some parental sub-stages, but no sexual sub-stages (C).

parental behavior and they show connectivity of 0.58 (Fig. 2.12b). In some cooperatively breeding species such as the white-crowned sparrow weaver, *Plocepasser mahali*, (Wingfield *et al.*, 1991) there is a dominant male and a dominant female who provide parental care and also have a sexual phase (connectivity thus equals 0.34 as in Fig. 2.12a), but there are also other individuals in the group that have no sexual phase but do feed young (Fig. 2.12c). Here there are only 4 sub-stages in the breeding stage with a connectivity of 0.5. These variations in numbers of sub-stages within the same phenotypic stage of individuals within a population and their connectivity could have fundamental influences on the neural and hormone control mechanisms. Such analysis as this may allow meaningful comparisons to be made when conducting experiments.

Implications for Hormone Control Mechanisms.

Endocrine secretions regulate many aspects of homeostasis as well as transitions in morphology, physiology and behavior in relation to predictable changes in the environment. Since most vertebrates live in changing environments, they change their physiological state to maximize survival at different times of year. These changes can be summarized as a finite state machine consisting of a temporal sequence of life history stages (phenotypic stages). The overall benefit of these transitions is to maximize life time fitness (see chapter one). However, the number of life history stages, their duration and the degree of overlap with other stages varies from species to species, and from

population to population. As hormones regulate the development of each state and its termination, then the number of stages and the degree of overlap with other stages could have a major influence on endocrine mechanisms and their control by environmental cues. For example, in Fig. 2.1 we see that the rock dove has only two life history stages with considerable overlap (Fig. 2.4) whereas the rock ptarmigan has seven with less overlap. The need for accurate timing of the transitions from one stage to the next in ptarmigan is clearly more important than in the rock dove. It is known that annual changes in photoperiod acting through neuroendocrine and endocrine secretions regulate at least some of the life history stages of the rock ptarmigan (e.g. Stokkan *et al.*, 1985, 1986). On the other hand, regulation of breeding and molt appear to be independent of photoperiod in the rock dove (Lofts and Murton, 1968). The analysis using diversity indices may help identify where possible differences, or similarities, in environmental and endocrine mechanisms may exist. The analysis provides an objective framework on which to make broad comparisons across taxa or even populations within taxa.

Each stage has a unique set of sub-stages that can be activated in complex sequences or combinations. Analysis of connectivity allows us to compare not only number of stages but also the complexity of interrelationships of that set of sub-stages. For example in figure 2.12 we can see that the neuroendocrine and endocrine mechanisms activating sexual sub-stages in a cooperative breeder, the white-browed sparrow weaver, may be in operation in dominant breeding males

and females but not necessarily in the helpers-at-the-nest that only express parental sub-stages. Does this mean that the helpers are incapable of expressing sexual sub-stages? Certainly it appears true that brood parasites such as the cowbird can activate sexual sub-stages (Fig. 2.11c) but have lost the ability to express parental sub-stages even if given hormones known to activate parental behavior in other species (see Dufty and Wingfield, 1986a,b).

Other comparisons across phenotypic stages show that these sub-stages are truly unique to their phenotypic stage. For example, in song sparrows, males in the winter stage (e.g. Fig. 2.10d) were unable to respond with sexual sub-stages typical of the breeding stage (Fig. 2.10a) when presented with females made sexual receptive experimentally. However, these males responded normally once increasing photoperiod had triggered the development of the breeding stage (Wingfield and Monk, 1994). Connectivity may be a useful way to compare complexity of expression of sub-stages in many other combinations of taxa or populations in diverse habitats. The potential for investigating endocrine mechanisms and environmental control is very high.

Chapter III:

Using a Finite State Machine Model to Understand Variations in Organismal Responses to Unpredictable Events in the Environment

Introduction

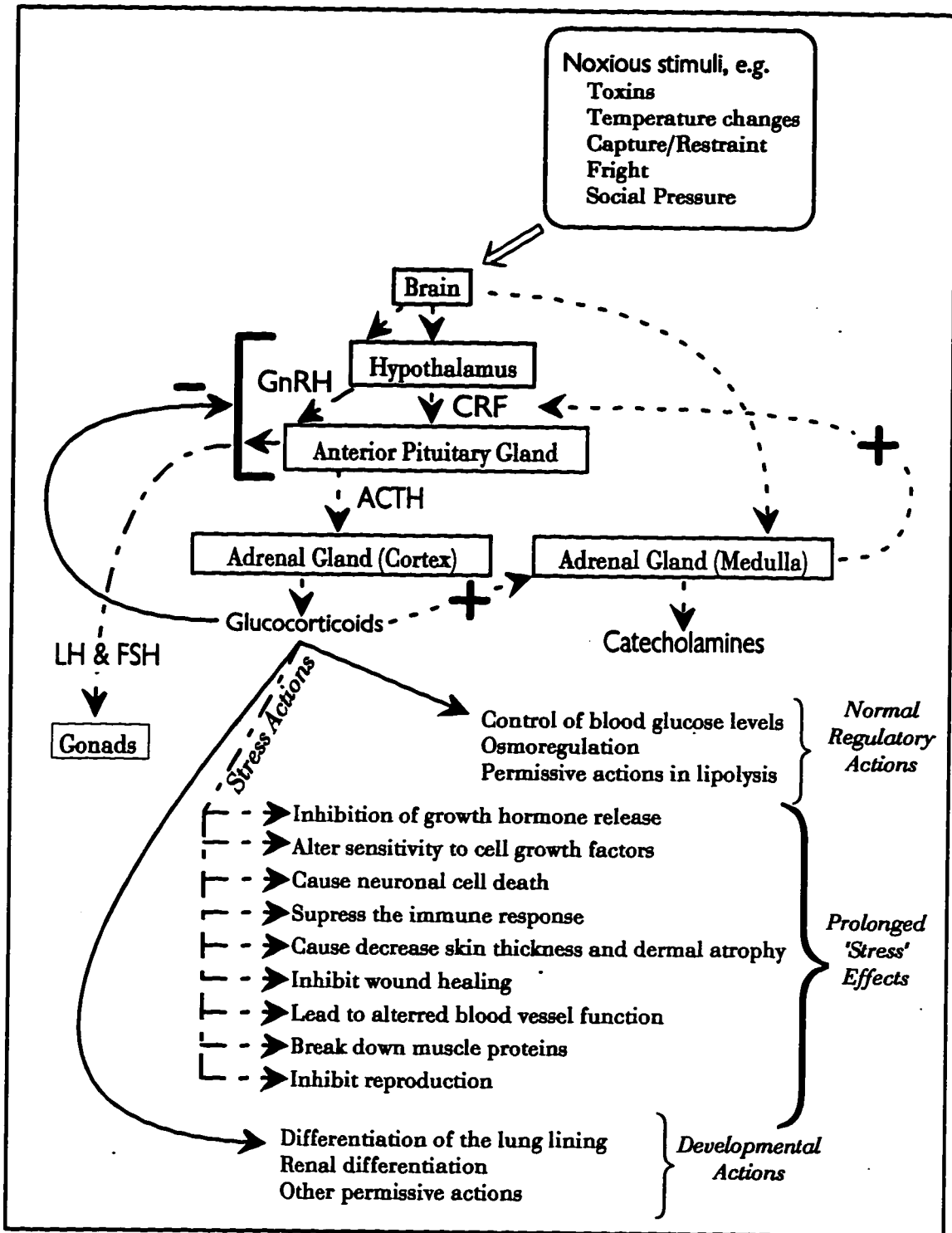
In the mid-1930's, Hans Selye studied the effects that a wide variety of noxious agents had on the physiology of mammals (see Selye, 1946 for a review). During this work on pathological and biochemical changes caused by noxious agents, he was

“struck by the fact that certain manifestations are always the same, irrespective of the specific nature of the eliciting damaging agent.” (Selye, 1946)

These “manifestations”, which involve a cascade of hormones and a series of physiological changes within the animal (see figure 3.1 and Hadley, 1996), have been shown in every vertebrate examined. He called this suite of physiological responses to noxious stimuli the General Adaptation Syndrome (GAS) (Selye, 1936). Although Selye's extension of the GAS into the so called “diseases of adaptation” (Selye, 1946) proved to be wrong in many cases (see Munk *et al.*, 1984), his description of the generalized reaction of animals to “stressors” has formed the foundation for most work on stress in vertebrates.

This nearly stereotypical response to noxious stimuli is now called the “stress response”. The stress response occurs in response to an extremely diverse

Figure 3.1 - Diagram of the response of vertebrates to noxious stimuli. The brain integrates responses to the stimuli, and triggers two hormonal responses: One leading to elevated catecholamines and the other to elevated glucocorticoids (GCs). Simulation of the adrenal medulla by the sympathetic nervous system leads to a nearly immediate increase in circulating catecholamines. These catecholamines, in addition to stimulating the "fight or flight" response, stimulate the hypothalamus to secrete Corticotropin Releasing Factor (CRF). At the same time, the hypothalamus is secreting CRF in response to the noxious stimulus. CRF passes through the hypothalamo-hypophyseal portal system to the anterior pituitary, where it stimulates the release of Adrenocorticotrophic hormone (ACTH). ACTH then flows through the general blood supply and stimulates the adrenal cortex to release glucocorticoids (GCs). The GCs have many beneficial effects (such as the regulation of blood glucose and a role in osmoregulation), but prolonged exposure causes many negative effects. GCs can act on the hypothalamus to inhibit the releases of Gonadotropin Releasing Hormone (GnRH) and on the anterior pituitary to inhibit the release of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). Several developmental processes also require the action of glucocorticoids.



suite of noisome factors including infectious diseases (Judd, 1917), capture/restraint by a "predator" (e.g., Holmes and Phillips, 1976; Siegel, 1980; Harvey, 1984; Wingfield *et al.*, 1982), bacterial toxins (Olitzki *et al.*, 1942), sudden exposure to cold (Alexandrova and Farkas, 1992) or heat (Wang and Edens, 1993), traumatic shock (Best and Solandt, 1940) and X-rays (Moon *et al.*, 1940)). Virtually any facet of the environment for which the animal is either quantitatively or qualitatively not prepared can act as a stressor, and trigger a stress response. A number of studies have shown that the proper activation of the stress response greatly decreases mortality to some severe stresses (e.g., Carey *et al.*, 1971; Finlay and McKee, 1982; Sibbald *et al.*, 1977). While the many aspects of the stress response are nearly stereotypical, there can be large differences in the responses of individuals to the same stress (e.g., Camp and Robins, 1988; Sapolsky, 1985; Sapolsky, 1986; Sapolsky, 1988; Swendsen, *et al.*, 1995; Meaney *et al.*, 1993a; Meaney *et al.*, 1993b). Some of this variation is due to genetic differences in the individuals (e.g., Flaherty and Rowan, 1989; Freund *et al.*, 1988; Satterlee and Johnson, 1988; Carsia and Weber, 1986; Gross and Siegel, 1985), but the rest of the variation appears to be associated with differences in the physiological condition and/or reproductive status of the animals (Wingfield *et al.*, 1994a, 1994b).

Munk and coworkers (1984) argued that the function of the stress response was not to increase the animal's resistance to stress, but rather as a feedback

mechanism to keep the animal's defense systems (immune system and inflammation) in check, such that they do not over-shoot and thus endanger homeostasis. While there is definitely a complex interaction between the immune system and the hypothalamo-pituitary-adrenal axis (see review by Bateman *et al.*, 1989), this safety-valve action is only a small portion of the reactions which occur in response to noxious stimuli.

In chapter one, I developed a finite state machine model for describing and understanding how animals respond to changes in their environment. The animal (as a finite state machine) conforms to pair of functions - the response function and the state function:

$$\begin{aligned} response_i &= f_{response}(excitation_i, state_i) \\ state_{i+1} &= f_{state}(excitation_i, state_i) \end{aligned}$$

As these equations indicate, both the actions of the animal (*response_i*) and the state the animal will occupy during the next time interval (*state_{i+1}*) are based only upon the current state (*state_i*) of the animal, and the stimuli (*excitation_i*) during the previous time interval. In any finite state machine model, there are three kinds of variables: excitation, response, and intermediate. In the animal finites state machine model (from chapter I), the excitation variables are cues from the environment, and the response variables are the animal's responses to those cues. The intermediate variables act within the machine (animal). While the intermediate variables were ignored in chapter one, they will be dealt with in

detail here as they are important in determining the state of the animal, and hence the animal's response to unpredictable events in the environment (see below). The effect of two intermediate variables (energy reserves and number of groups of progeny in a lifetime) on responses of individuals to unpredictable events in the environment will be examined.

Adult Responses to the Unpredictable

The sudden exposure of an animal to a noxious stimulus triggers a series of hormonal responses. The brain integrates cues from the environment, stimulating the adrenal medulla via the parasympathetic nervous system (Figure 3.1 and Hadley, 1996). The adrenal medulla begins releasing catecholamines into the blood. The brain also stimulates the hypothalamus to release Corticotropin Releasing Factor (CRF) from the median eminence into the hypothalamo-hypophysial portal system. CRF stimulates the anterior pituitary to release Adrenocorticotrophic Hormone (ACTH) into the general bloodstream. Arginine vasotocin, Arginine vasopressin, and oxytocin can also be released from the median eminence to stimulate the release of ACTH (*e.g.*, Romero *et al.*, 1993; Meaney *et al.*, 1996). ACTH then stimulates the adrenal cortex to produce glucocorticoids. These glucocorticoids stimulate the mobilization of amino acids from muscle proteins for use in gluconeogenesis, and through permissive actions tend to stimulate release of free fatty acids from adipose tissue. Glucocorticoids also trigger behavioral changes in animals. The combined responses triggered by

the catecholamines and glucocorticoids help the animal to survive the “stressful” event (Dunlop, 1963). The glucocorticoids also shut down many processes not necessary to the emergency state. Inflammation and the immune system are inhibited, preventing the animal from being immobilized by its own defenses (Bateman *et al.*, 1989). Other energy demands, such as reproduction (Collu *et al.*, 1984) and growth (Slotkin *et al.*, 1992), are also inhibited by the glucocorticoids, providing more available energy to help maximize the animal’s chances of surviving the emergency.

Though elevated glucocorticoids in the blood allow an animal to survive many “stressful” events, there is an extravagant cost for prolonged maintenance of high blood glucocorticoid levels. Prolonged exposure to elevated glucocorticoids results in a vast array of negative effects such as inhibition of reproduction (*e.g.*, Krulich *et al.*, 1974), inhibition of growth (*e.g.*, Slotkin *et al.*, 1992), suppression of the immune response (*e.g.*, Dohms and Metz, 1991; Irwin, 1994; Wilckens, 1995), neuronal cell death (*e.g.*, Virgin *et al.*, 1991), and wasting of muscles (*e.g.*, Dardevet *et al.*, 1995). With such extreme costs for extended exposure to elevated glucocorticoids, the “stress” response system only increases the fitness of the animal during relatively short-term disturbances, and is detrimental to the animal during protracted challenges to homeostasis.

Terminology

Unfortunately, the term stress has been applied to an extremely broad

range of physiological states such as exercise stress, stress of reproduction, fasting stress, emotional stress, and stress from noxious stimuli. Though there are some similarities in the physiological responses to all of these stresses, they are clearly not identical. There appear to be different pathways for stimulating the stress response, depending on the nature of the stress (see Romero *et al.*, 1993; Meaney *et al.*, 1996). Exercise stress, unless it is excessive and beyond the animal's ability to respond, does not endanger the animal's homeostasis. Birthing stress, while extreme in humans, is a normal physiological process, and is prepared for by the animal during pregnancy. In some animals, such as Emperor Penguins, fasting is also a normal part of the animal's breeding season and is prepared for with the deposition of fat (Stonehouse, 1953). In most other animals however, fasting is not self-imposed and not necessarily predictable, and thus has very different physiological responses. Emotional stress, and the stresses from noxious stimuli such as cold, excessive heat and infections are either not predictable, or are not within the animal's ability to control its environment.

As the word stressor and the concept of stress have become associated with a confused morass of different meanings, we need to have a new set of terms which define specifically the emergency response to challenges to homeostasis. These challenges are normally short-lived under natural conditions (or the animal will die, or move away from the challenge), and they stimulate an emergency state within the animal which is not within the normal progression of physiological

states (see chapter I for an in-depth discussion of physiological states). I will use the term *Transient Perturbation Factors* (TPF) instead of stressors, since TPF implies that the factor is of limited duration, and that it perturbs the normal cycle of physiological states in the animal's life cycle. A *Transient Perturbation Factor* will be defined as any noxious stimulus to which the animal is either qualitatively or quantitatively not adapted in its current physiological state, and the occurrence of the stimulus is either unpredictable, or the animal cannot normally express an appropriate response to the stimulus. It is important to keep in mind that even though a stimulus seems noxious to an investigator, it may not be perceived as such by the study animal. The noxious stimulus must be outside the normal range of the animal's ability to cope with the stimulus. A human with a body temperature of 43°C would soon die, but this same "excessive" temperature is often observed in desert iguanas (*Dipsosaurus dorsalis*) when active during the heat of the day (Swezey and Somero, 1982) and produces no ill effects in these lizards.

An animal's ability to cope with a particular stimulus can vary over time. For example, a temperature of 4°C is not a noxious stimulus to goldfish acclimated to cold temperatures during the winter, but it is often lethal to goldfishes acclimated to warm summer temperatures (Fry *et al.*, 1942). The stimulus must either be unpredictable, such as a sudden storm, or capture by a predator, or the stress is uncontrollable by the animal (that is it has no way of dealing with the stimulus). If the noxious stimulus is predictable, and the animal can "prepare"

itself, either by alterations in behavior or physiology, the stimulus ceases to be a TPF. Some stimuli are uncontrollable even if they are predictable. Social stress in a baboon troop may be relatively predictable by the lower order males, but as they are usually smaller and/or weaker than the dominant male, they can not change their social status (Sapolsky, 1982; Sapolsky, 1983; Sapolsky, 1990). Their only possible active response is to leave the troop, and this is not a viable option. Many experimental stress experiments fall into this uncontrollable class: the stimulus may occur at predictable intervals, but there is no way for the animal to deal with the stimulus (*e.g.*, electric shock, hypertonic saline injections).

In keeping with the transitory nature of the stimulus and the response, the animals will be said to adopt a *Transitory Emergency State (TES)* in response to transient perturbation factors. A TES is characterized by the inhibition of systems not "required" during a short duration emergency (such as the immune system and the reproductive system), the mobilization of some energy reserves, and the alteration of behavior to those appropriate to dealing with the TPF. These alterations in the physiological state of the animal often allow the animal to cope with the emergency situation. A TES is obtainable from almost any physiological state in the animal's life cycle and by its very nature interrupts the normal progression of physiological states.

Every system within an organism has some sort of effect on the survivorship of the organism. The *adaptive value* for a system will be defined here as the effect

the system has on the survival of the animal, and on its potential for production of offspring. This adaptive value may be zero, or possibly slightly negative, as in the case of the human appendix which has no apparent function (see Romer, 1962). Most systems within the animal will have high, positive adaptive values. Obviously organ systems such as the circulatory system have adaptive values which are large and positive, their benefit greatly outweighing the cost of producing and maintaining the organ system.

The adaptive value for a system can change over time. In the larvae of an aquatic salamander, the gills have a large positive adaptive value as they greatly increase the larva's ability to extract oxygen from the water. If these gills were to persist in the terrestrial adult, the fact that the gills could be easily damaged, could increase the animal's water loss rate and are essentially useless as a terrestrial respiratory organ would give the gills a negative adaptive value in the adult stage. The adaptive value of bright plumage and showy displays may be high for a bird during the breeding season, but flamboyance has a tendency to attract predators and will generally have a negative adaptive value during the non-breeding seasons.

If the positive adaptive value of a system drops enough it will reach a *critical adaptive value*, where it has neither a positive or a negative effect on the fitness of the animal. If the adaptive value for the system drops below the critical adaptive value, the system is reducing the fitness of the animal. If the adaptive value for a system is above the critical adaptive value the system is increasing the

fitness of the animal. The critical adaptive value will act as an intermediate variable in the finite state machine model for animals, as developed in chapter one, and will be pivotal in determining how animals respond to TPFs.

In natural conditions in the wild, an animal will exist in one of a finite number of physiological states (see chapter one) which are determined by the animal's starting state, and the signals/cues it has received. Responses to unpredictable events will follow these same state-dependent rules. When the animal is exposed to a TPF, it will attempt to adopt a TES based on the state of the animal before the TPF, and appropriate to the set of cues at that time (see figure 3.2). The cues that determine the particular TES adopted include the normal environmental cues (such as initial predictive cues and supplementary cues; see chapter one), as well as the TPF. The TPF can over-ride the effects of many of the other cues, but the normal cues can be important in determining the particular TPF adopted. For example, if food seed supplies in the territory of a bird are large (a supplementary cue), the bird may choose to stay on its territory during a snow storm if it can collect enough seeds through the snow cover, but may abandon its territory if the food supplies were poor. If the animal can not attain a proper TES or the TPF is too severe, the animal will die. The normal environmental cues are certainly important in determine where the animal will re-enter the normal cycle of physiological states when the TPF is removed. When the TPF is removed, the animal will re-enter the normal progression of states at a state

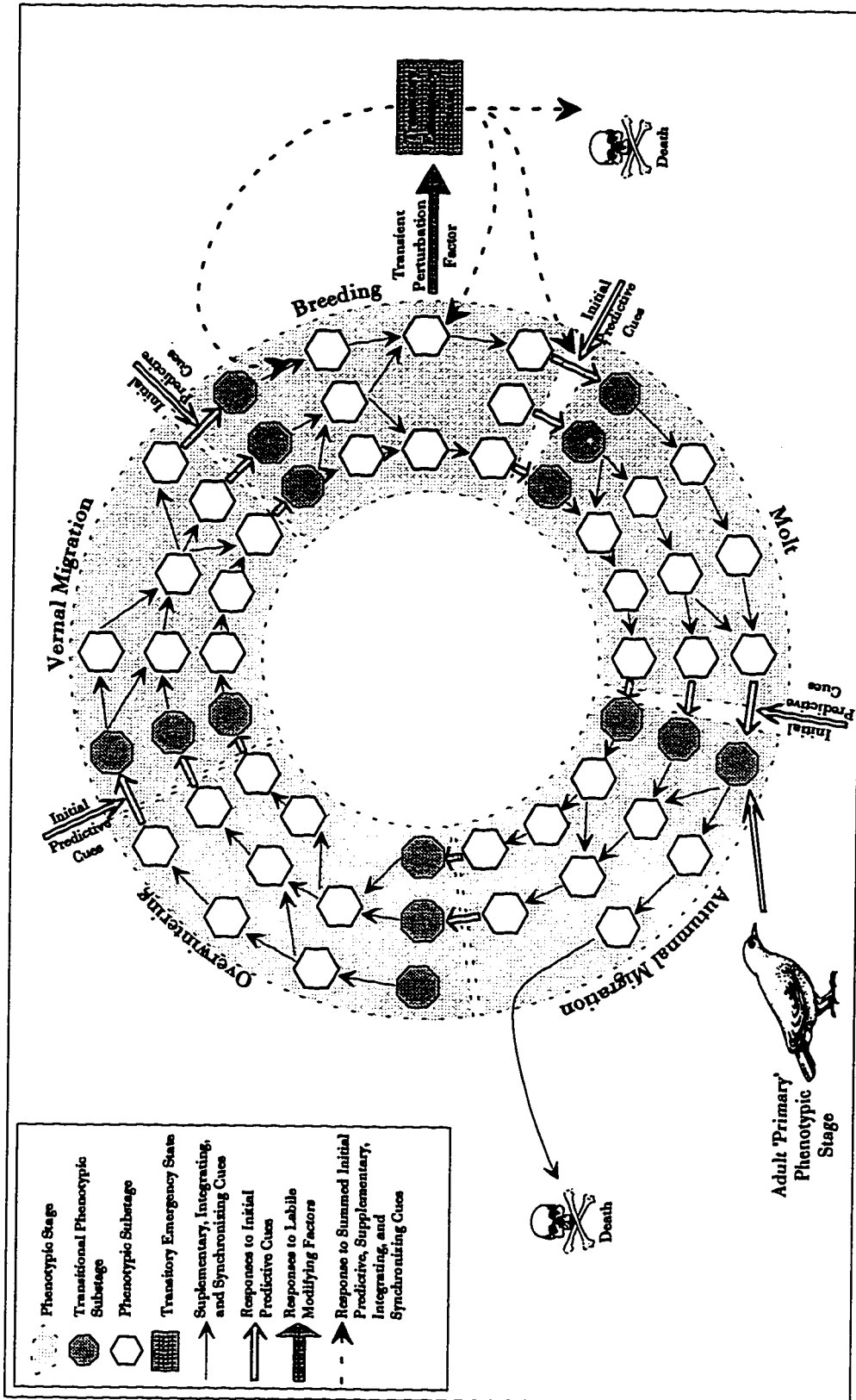


Figure 3.2 - A finite state machine model of the life cycle of an adult bird showing the effects of transient perturbation factors (TPFs). Death can occur at any time during the cycle. A TPF will normally shunt an animal from the normal cycle of states into a transitory emergency state. After the TPF is removed, environmental cues determine the state the animal will express next. If the TPF is too intense, or is not removed, the animal will die.

determined by the environmental cues the animal receives at that time and the TES the animal is in at the time the TPF is removed (including the effect the TPF had on the animal's state). If for instance, a bird was feeding young at the time of a severe storm, and the nest was destroyed, the bird can not return to the state of feeding the young when the storm passes. It is early enough in the season (that is the cues are still correct for breeding) the bird can renest and produce another brood, but if it is too late in the year for breeding, the bird will begin to molt its nuptial plumage, preparing for the autumnal migration. If the storm did not harm the nestlings or the nest, the bird could return to its original state (assuming it was not otherwise injured), and continue to feed the young.

The situation may be very different in laboratory conditions (see figure 3.3). Under natural/field conditions (see Figure 3.3, upper panel) an animal can be in a fairly large number of states triggered by a variety of environmental cues. Exposure to TPF will trigger a one of many possible TESs (or the animal may move away from the TPF). In the laboratory situation (Figure 3.3, lower pane), most of the cues an animal would receive in the wild have been eliminated, and hence the number of possible physiological states available to the animal is reduced (as the state adopted during the next time interval is determined by the current state, and the stimuli detected during the current time interval). Some of these possible laboratory states may not normally exist in the wild as they result from cues not normally available in the wild. Animals in the lab are usually in

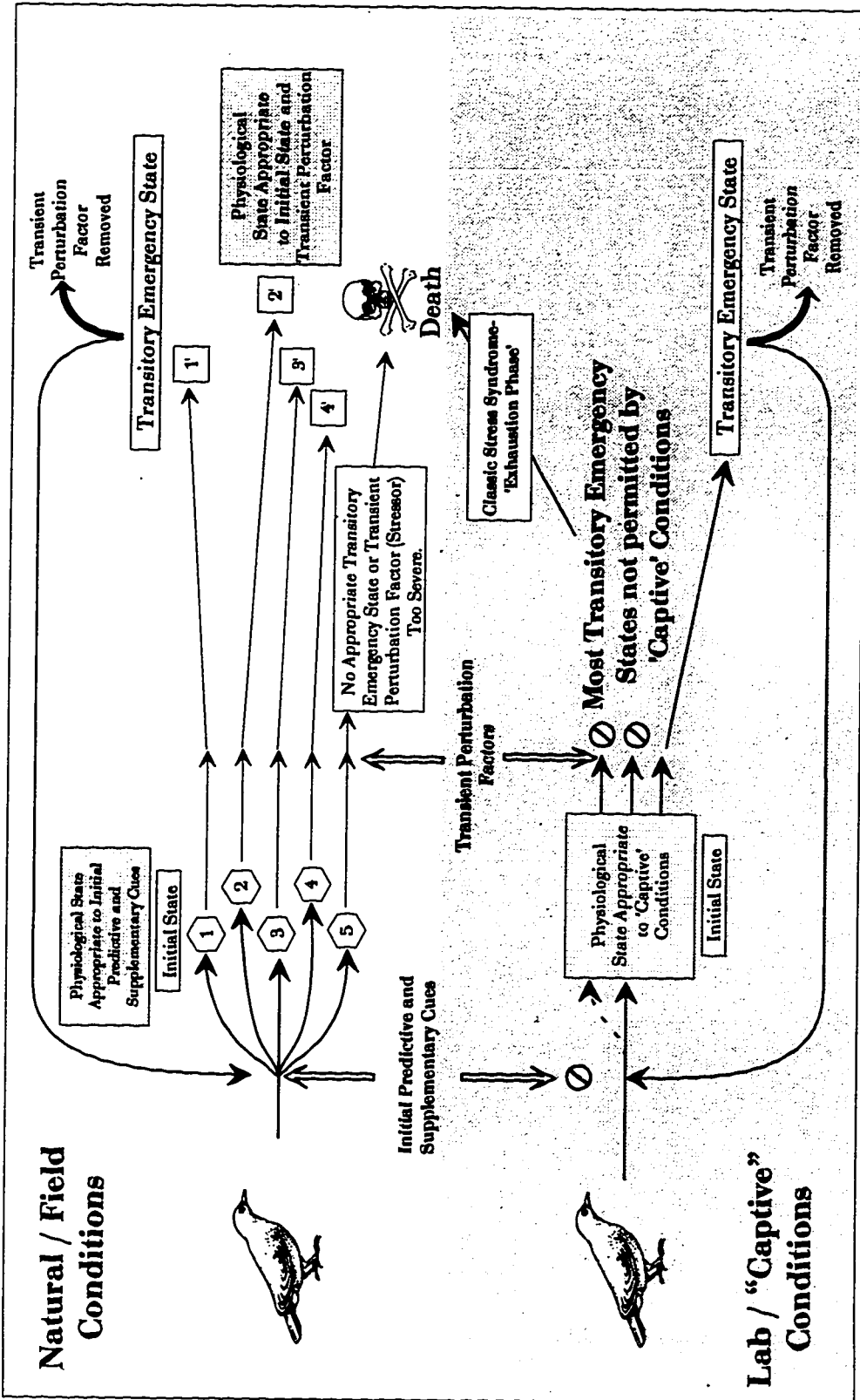


Figure 3.3 - Responses to transient perturbation factors (TPFs) under natural and laboratory conditions. At any time, an animal will be in some physiological state determined by its previous state, and the cues it has received. When exposed to a TPF, it will normally alter its state into one of the transitory phenotypic states in order to respond to the TPF. Lab conditions limit most responses, and the animal usually cannot respond appropriately.

cages, so this alone is a major change in their environment, and hence a major change in their physiological state. Thus when exposed to a TPF, the possible reactions of the laboratory animal are severely constrained. The animal can not move away from the stress, and the constraints imposed on the animal by laboratory conditions severely limits the possible TESs available to the animal. Such limitation of the possible TESs can lead to death, as it may not be possible to adopt an appropriate TES for the particular TPF. The laboratory TPF itself may not exist in the wild, and hence the animal may have no way of dealing with the TPF. The life span of many animals kept in captivity is greatly reduced, possibly resulting from "unnatural" TPFs which lead to prolonged exposure to elevated glucocorticoids, or from TPFs which could be dealt with in the wild, but cannot be coped with in captivity due to limitations imposed by captivity on the possible TESs.

Modulation of the TPF response during the breeding season.

Breeding is the most crucial phase of an organisms life cycle, at least in evolutionary terms. Since fitness can be considered a function of the number of offspring produced into the next generation, the breeding season is pivotal to the animal's fitness. Maximizing lifetime reproductive success is very important to all organisms, so physiological process which interfere with breeding are usually inhibited.

The TPF response can inhibit reproduction, either by altering behavior, or inhibiting the hypothalamo-adenohypophysial-gonadal axis (Collu *et al.*, 1984; Greenburg and Wingfield, 1987). The number of possible attempts an animal has to breed in a lifetime is an important intermediate variable, and one that can regulate responses to TPFs. In short lived vertebrates, which may produce only a single group (brood, litter, etc.) of offspring, there will be a large reduction in the adaptive value of the TPF response during the breeding season as the TPF response would inhibit the one chance they have at producing offspring (see Figure 3.4, bottom panel). This drop in adaptive value may be large enough to cause the adaptive value to drop below the critical adaptive value. In such situations, the TPF response would either be inhibited or muted, so as not to interfere with the animal's limited chance for reproduction. Vertebrates which produce many sets of offspring in a lifetime will have only a small drop in the adaptive value during the breeding season (see figure 3.4, top panel). By plotting the effect on the adaptive value of a TES versus both time of year and the number of broods in a lifetime, we get a landscape as show in Figure 3.5. For most of the year, there is no effect of the number of broods in a lifetime on the adaptive value of the TES. Yet during the breeding season we see a distinct valley, becoming increasingly deeper as the number of lifetime broods decreases. At low numbers of lifetime broods, the adaptive value of the TES drops below the critical adaptive value, indicating that animals with low number of lifetime broods may suppress their response to TPFs

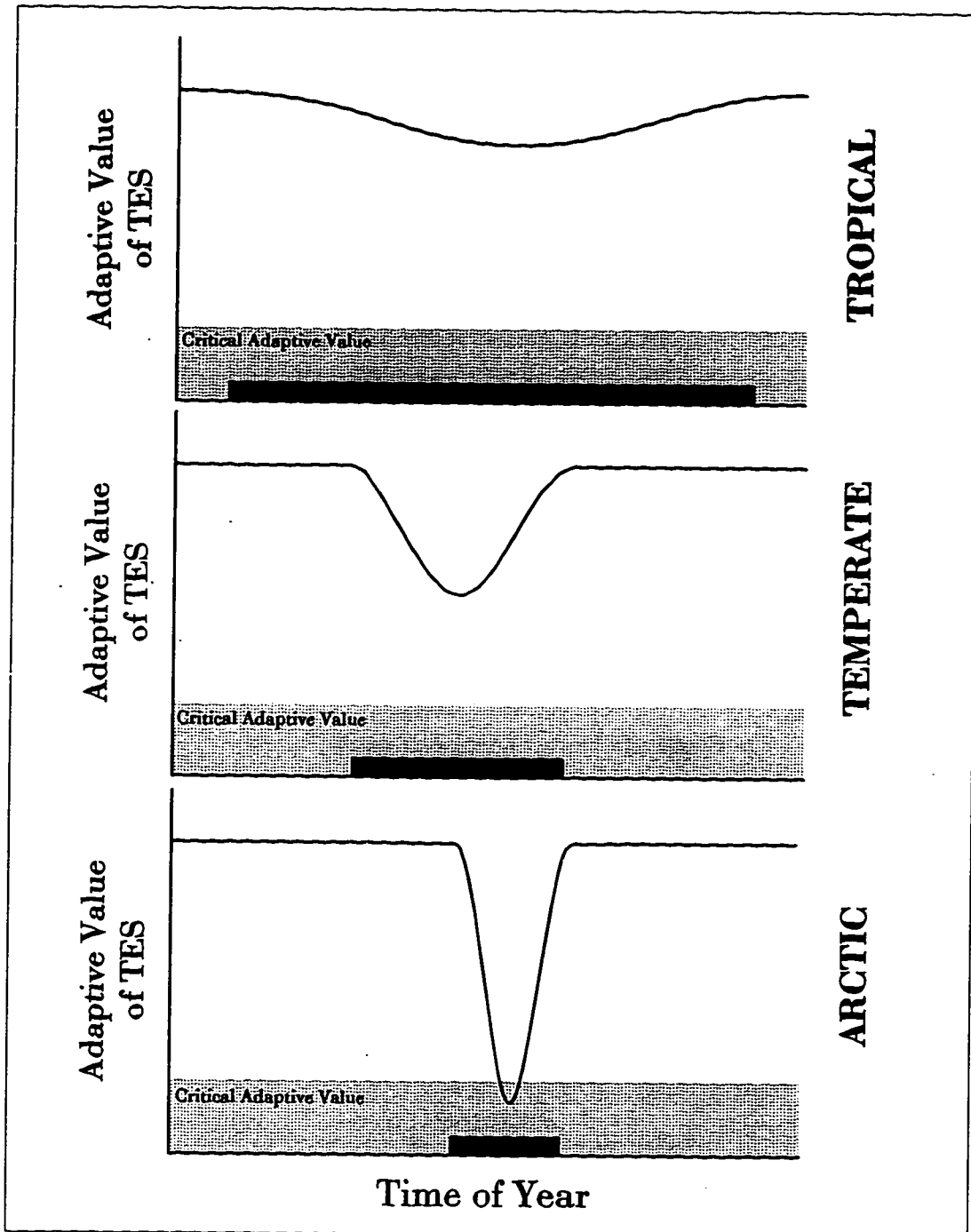


Figure 3.4- Model of breeding season depression in the adaptive value of a transitory emergency state. Going from low latitudes to high latitudes, there is a decreasing length of the breeding season (black bars). As the length of the breeding season decreases, the adaptive value of expressing a TES during the breeding season decreases.

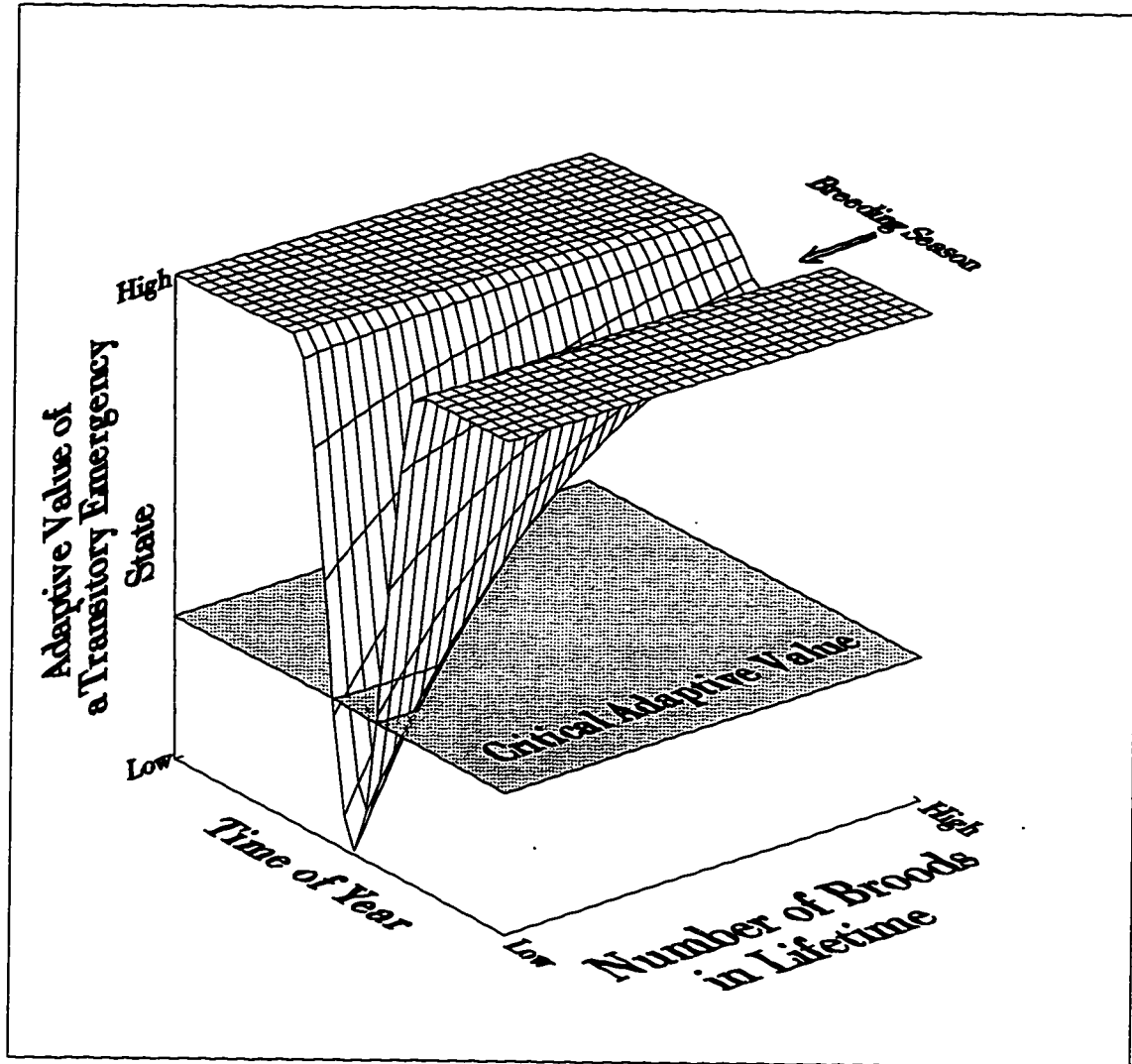


Figure 3.5 - Effect of time of year and number of broods in a lifetime on the adaptive value of a transitory emergency state (TES). The grey plane represents the critical adaptive value. During most of the year, the number of lifetime broods has no effect on the adaptive value of the TES. During the breeding season, there is a very large pressure to complete reproduction if the number of lifetime broods is small.

during the breeding season.

Of course, simply the number of offspring in a life time will not be the sole determining factor determining an animal's response to a TPF. The response of an animal to a given stimulus (including TPFs) will be determined by the animal's current state, and the integration of all stimuli at that time. One intermediate variable important in determining an animal's physiological state is the energy reserves of the animal. High energy reserves help an animal in a number of ways, including providing energy for such tasks as reproduction, growth, and migration. Energy reserves can also help an animal survive many TPFs (unpredictable emergencies).

We can visually examine the interaction two variables have on the adaptive value to a TES by creating three dimensional graphs. Plotting a series of these 3-D graphs along a series of a third variable gives us a feel for how three different variables interact. We will look at the effect an intermediate variable (energy reserves) and an excitation variable (time of year) have on the adaptive value of a TES in short-lived birds, and compare these effects in environments of different latitudes. As an animal's energy reserves increase, the need to adopt a TES in response to many TPFs becomes less, as the animal is better able to "sit-out" the emergency. For an animal with a moderate number of lifetime broods, this slight drop in the adaptive value associated with elevated energy reserves does not bring the adaptive value any where near the critical adaptive value, even when coupled

with the decrease in adaptive value associated with the breeding season, and hence has little or no effect on the expression of a TES (Figure 3.6). If however, the animal has only a relatively small number of lifetime broods, this slight drop in the adaptive value of a TES due to elevated energy reserves also has minimal effects on the adaptive value of the TES for most of the year, but may cause the adaptive value of the TES to drop below the critical adaptive value during the breeding period (see Figure 3.7). In such a situation, animals with low energy reserves would show the normal response to a TPF during the breeding season, and those with high energy reserves would show a muted response (or possibly no response) to TPFs during the breeding season.

Example I. Attenuation of the TPF response in high latitude breeding birds.

Birds which migrate to Arctic regions in order to breed are under fairly severe time constraints. They must breed early enough in the summer such that the resulting offspring are fledged and ready for the autumnal migration. This time constraint means that during years when spring comes late, the birds must begin setting up territories while there is still a strong possibility of bad weather and snow fall. There is only a very narrow window where re-nesting is possible if a brood is lost, as it soon becomes too late in the year to produce offspring which will survive and be able to migrate. Both of these constraints may act to reduce the adaptive value of the TPF response during the breeding season.

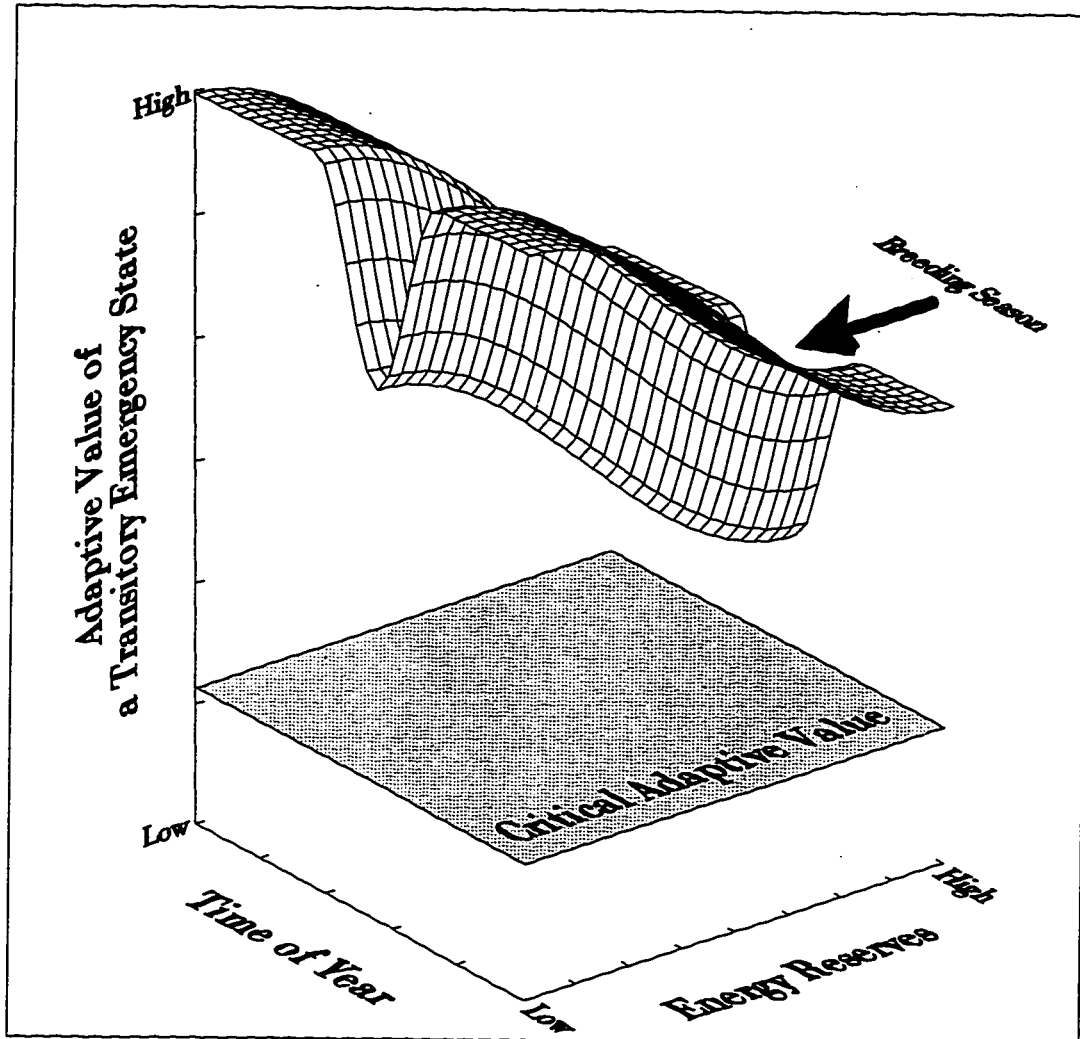


Figure 3.6- Model of the effect of time of year, and energy reserves on the adaptive value of expressing a transitory emergency state (TES) in a short lived animal that breeds in the temperate zone. The grey plane represents the critical adaptive value. The breeding season is shorter than in the tropics, but still long enough normally for multiple broods. There is a larger depression in the adaptive value of the TES associated with the breeding season than in the tropics, but it is still normally above the critical adaptive value.

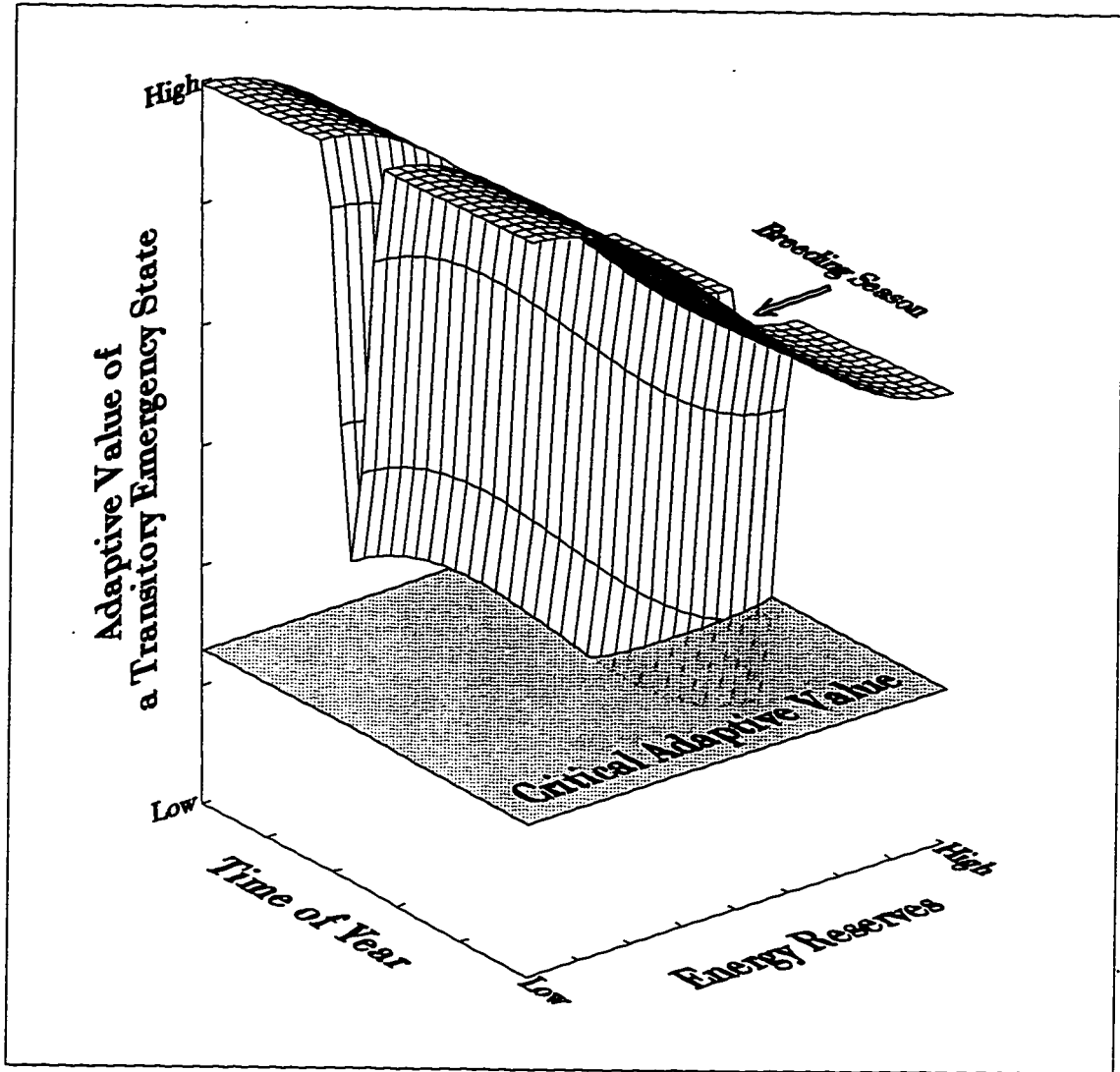


Figure 3.7- Model of the effect of time of year, and energy reserves on the adaptive value of expressing a transitory emergency state (TES) in a short lived animal that breeds at high latitudes. . The grey plane represents the critical adaptive value. The breeding season is shorter than in the tropics, but still long enough normally for multiple broods. There is a larger depression in the adaptive value of the TES associated with the breeding season than in the tropics, but it is still normally above the critical adaptive value.

Adopting a TES during usually leads to the elevation of plasma glucocorticoid levels, a conditions which in normally inhibitory to both reproductive behavior, and maintenance of the reproductive system (Greenburg and Wingfield, 1987). If the gonads begin to regress in response to elevated glucocorticoids, it may take on the order of weeks to recrudescence the gonads, making it too late in the season for beginning to re-nest. As the environmental cues may no longer be correct for gonadal recrudescence, it may not be possible to reverse the regression process once it has begun. Thus is should be in the interest of these small, short-lived, migratory birds to either repress the stress response, or limit the magnitude of the response such that it does not endanger their reproductive condition.

Common Redpolls (*Carduelis flammea*) are small finches which live in Arctic and sub-arctic regions during the entire year. When these birds breed north of the arctic circle, they are under the same time constraints as the long distance migrants during the breeding season. Throughout the entire year, Redpolls show a significant response to TPFs (as indicated by elevated plasma corticosterone levels; Wingfield *et al.*, 1994a). However, redpolls breeding near the north coast of Alaska at Barrow (71°N) showed a muted response to capture stress, when compared with redpolls breeding in the more southerly area around Toolik Lake (69°N). As expected (see figure 3.7), individuals at Barrow with larger fat reserves had smaller adrenocortical responses to capture and restraint (a TPF). It is

surprising that the redpolls breeding at Toolik Lake did not appear to down regulate their response to TPFs during the breeding season. This may be explained in part by the fact that day-time temperatures at Barrow Alaska are 10-20 C° lower than at Toolik Lake, causing the snow to melt off later in the summer, making the window for breeding and raising young even shorter than at Toolik Lake. Also surprising was that redpolls sampled during the winter at Fairbanks, Alaska (64°N) showed very different adrenocortical responses between January and February. In January, when the temperatures were very cold (-35°C), the response was very muted, similar to or less than the response at Barrow during the breeding season. However, the response of Redpolls during January when temperatures were a bit warmer (-15°C) showed adrenocortical responses to capture which were similar to, or larger than the responses of redpolls breeding at Toolik Lake. Such a rapid change (samples in January were taken only 16-18 days before the samples taken February) indicate that redpolls in the winter are operating in conditions where the adaptive value for the TPF response is near the critical adaptive value, as it appears that minor changes in the environment (such as changes in temperature) can alter the bird's responses to capture. Possibly winter conditions at Fairbanks are unpredictable enough that the unpredictability of the environment is lowering the adaptive value of the TPF response to near the critical adaptive value (see figures 3.8 and 3.12 and discussion of predictability below).

Snow buntings (*Plectrophenax nivalis*) and Lapland longspurs (*Calcarius*

lapponicus) are two passerine species which are abundant throughout the Arctic region during the breeding season. Since both these species arrive on their Arctic breeding grounds during the early spring when the weather may be quite severe, and since the Arctic summer is so brief, both of these species would be expected to show reduced responses to TPFs during the breeding season. When Lapland longspurs and snow buntings were examined at Barrow Alaska (71°N), neither of these birds showed the expected inhibition of the response to capture and restraint when compared with post breeding adrenocortical responses in the fall (Wingfield *et al.*, 1994b). Both longspurs and snow buntings showed individual variation to the TPF (capture and restraint), and this variation is inversely correlated with fat reserves: those with high fat reserves showed lower maximum responses to the TPF. While they show significant responses to capture and restraint, the magnitude of the increase in plasma corticosterone was 2-8 times less than that shown by white-crowned sparrows (Wingfield *et al.*, 1992). Lapland longspurs can show much stronger responses to TPFs during the breeding season (up to a 10 fold increase in corticosterone levels) when they have been exposed to a 3 day snow storm (Astheimer *et al.*, 1992). The inverse relationship of fat reserves with the maximal response to a TPF, and the ability to rapidly change (within 3 days) their response to TPFs indicate that both snow buntings and Lapland longspurs are operating with their adaptive value for the TPF response near the critical adaptive value during the breeding season.

Example II. Attenuation of the TPF response of some desert birds during the breeding season.

Birds breeding in the lower Sonoran zone of Arizona during the summer months can experience periods of intense heat, reaching up to 50°C, with limited or no access to open water (Wingfield *et al.*, 1992). High temperatures have been shown to elevate corticosterone levels in domesticated birds such as chickens and turkeys (Siegel, 1980; Harvey *et al.*, 1984). High temperatures, coupled with drought have been shown to elevate plasma corticosterone levels in bobwhite quail (Cain and Lien, 1985). While desert birds are undoubtedly more resistant to heat than turkeys, chickens, or bobwhite quail, they may still be exposed to unusually high temperatures during the summer which could disrupt breeding by triggering a TES. The length of the breeding season in the Sonoran desert does not put the same constraint on breeding as it does in the Arctic. However, the need to continue with reproduction during inclement heat waves may cause these birds to mute their responses to TPFs.

Wingfield *et al.* (1992), examined three species of Sonoran desert birds (black-throated sparrow, *Amphispiza bilineata*, cactus wren, *Campylorhynchus brunneicapillus*, and curve-billed thrasher, *Toxostoma curvirostre*) during both the breeding season (summer) and in the winter. Adrenocortical responses to capture and restraint for these birds was lower in the breeding season than in the winter. There was no correlation of basal corticosterone levels with air temperature, so the

TPF response system in the summer birds was not already “maxed-out”. Thus the birds breeding in the lower Sonoran zone have an adaptive value for the TPF response which is near, or below the critical adaptive value during the breeding season (similar to the lower panel of figure 3.4, only with a broader breeding season). Two other birds (Inca Dove, *Scardafella inca* and Albert’s towhee, *Pipilo alberti*) that breed in riparian habitats that are much more benign than habitats in the lower Sonoran zone were also examined in this study. These riparian birds showed the same adrenocortical responses to capture and restraint in both the breeding season and during the winter. The relationship of adaptive value of a TES to time of year would look like the middle panel in Figure 3.4.

Example III. The response of King Penguins (*Apentenodytes patagonica*) to the “stress” of fasting.

King penguins breed on peripheral Antarctic islands, and on islands in cool temperate Subantarctic regions (Stonehouse, 1970). Both adult and juvenile King Penguins must survive prolonged periods of fasting. Each year, after a period of one or two week period of fattening at sea, the adults come ashore and begin a four to five week pre-nuptial molt. During the molt, these penguins do not feed, and normally loose up about 50% of there pre-molt body mass (Stonehouse, 1956; Weimerskirch *et al.*, 1992). After completing the molt, they return to the sea for two to four weeks for a second period of fattening. After fattening, the birds return to land and begin a three to six week courtship, where neither the male nor female

feeds. After the single egg is laid, the females return to the sea to feed, and the male continues to fast while incubating the egg for the first watch. After 12-15 days, the female returns and takes over incubation of the egg and the male begins a period of feeding at sea. The pair trade off periods of incubation/fasting and feeding till the chick hatches. After hatching, the chick is fed regularly for 3-4 months, reaching 10-12 kg (Cherel *et al.*, 1987). During the austral winter, food supplies drop and feeding of the young becomes sporadic. The chicks must survive prolonged periods of fasting during the winter, losing up to 50% of their body mass. The chicks can tolerate a 4-6 month fast and up to a 70% loss in their body mass (Cherel and Le Maho, 1985).

The prolonged fasting periods of the King Penguins may seem very stressful (and extremely so in the case of the chick), yet there is normally no increase in plasma glucocorticoids during most of the fasting period (Cherel *et al.*, 1987; Le Ninan *et al.*, 1988). These birds (and the chicks in particular) are well adapted to predictable periods of fasting. Thus these prolonged fasts normally are not a TPF since they are both predictable and within the normal capacity of the animal to survive. The fasting can become a TPF if it is prolonged. If either parent is killed during the incubation period, the other parent will attempt to continue the incubation until it exceeds its normal capacity for fasting, at which point plasma glucocorticoids become elevated. These elevated glucocorticoids are indicative of a TES and may trigger a shift in the behavior of the adult penguin, shifting behavior

from brooding of the egg to feeding of the adult, causing the egg to be abandoned.

Modulation of the TPF response at the extremes of predictability

The TPF response system has been found in all organisms examined so far, from a wide variety of environments, so in most situations the TPF response system probably has a high adaptive value. There may however be environments where the TPF response drops below the critical adaptive value.

In a completely predictable environment, there would be no *transient perturbation factors* ("stressors"), as there would be no unpredictable events and thus no need for a response system to TPFs (*i.e.*, low adaptive value for the TPF response; see right side of figure 3.8). As the predictability of the environment decreases, the adaptive value of the TPF response increases, and probably reaches a plateau (see center region of Figure 3.8). As the predictability continues to decrease, the frequency of TPFs increases. Each TPF leads to an increase in glucocorticoids released in response to the TPF (bell shaped curves in Figure 3.5). If the frequency is relatively low, there will only be a small increase in the average blood glucocorticoid levels (Figure 3.9 - dashed line, upper panel). As the frequency increases, the average glucocorticoid level will be significantly above basal levels (Figure 3.5 - dashed line, middle panel). If the frequency becomes high enough, the average glucocorticoid will be at the maximum level (Figure 3.9 - dashed line, lower panel). In the latter two cases, the animal will begin to suffer

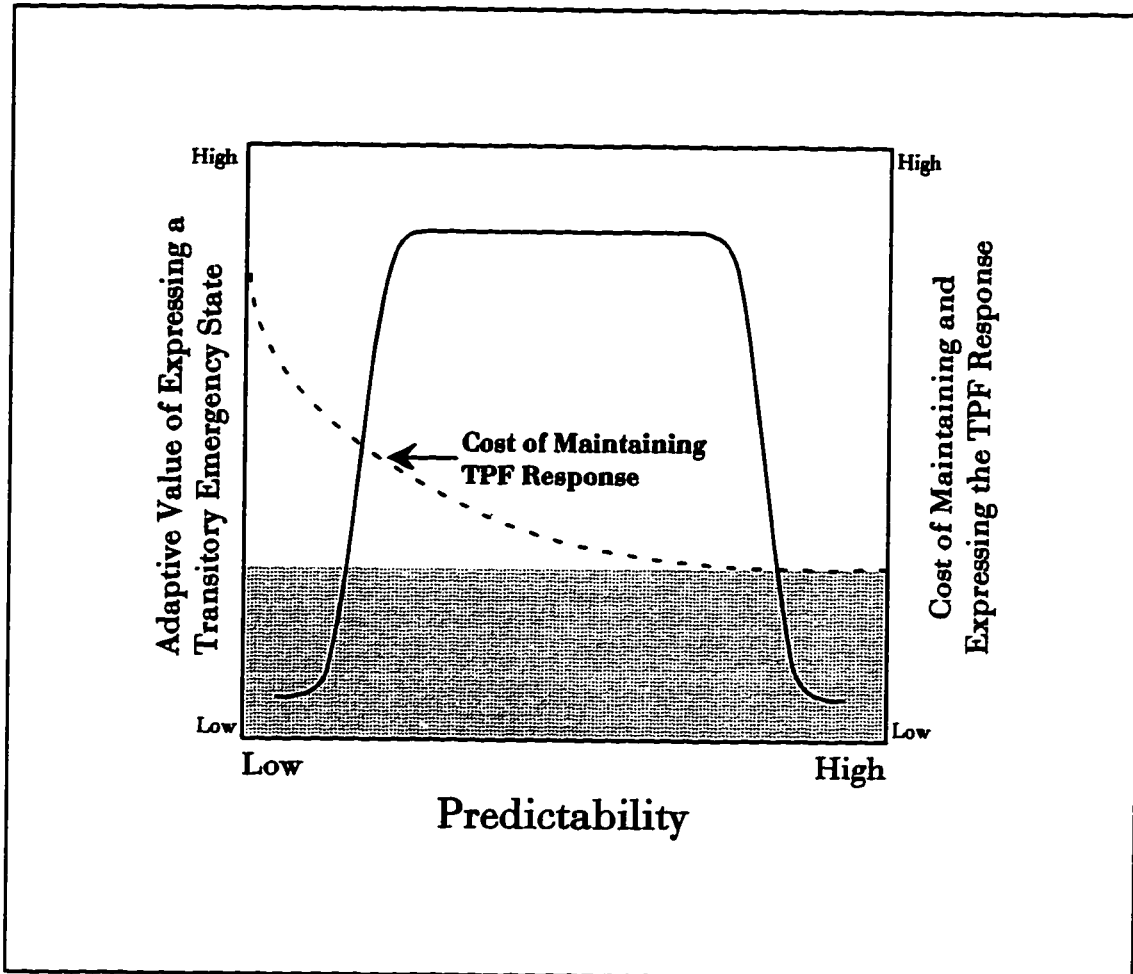


Figure 3.8 - Model of the effect of environmental predictability on the adaptive value of expressing a transitory emergency state (TES). Grey region represents the cost of maintaining the TPF response machinery (basal cost), and the dotted line represents the basal cost plus the cost of expressing a transitory emergency state. The thin solid line is the adaptive value of expressing a TES. At high predictability levels the TPF response is rarely, if ever expressed, and thus may have low adaptive value. At low predictability levels, the rate of TPFs is so high that the cost of expressing TES becomes too high. At both low and high predictabilities, the cost of maintaining the TPF response exceeds the adaptive value of expressing a TES.

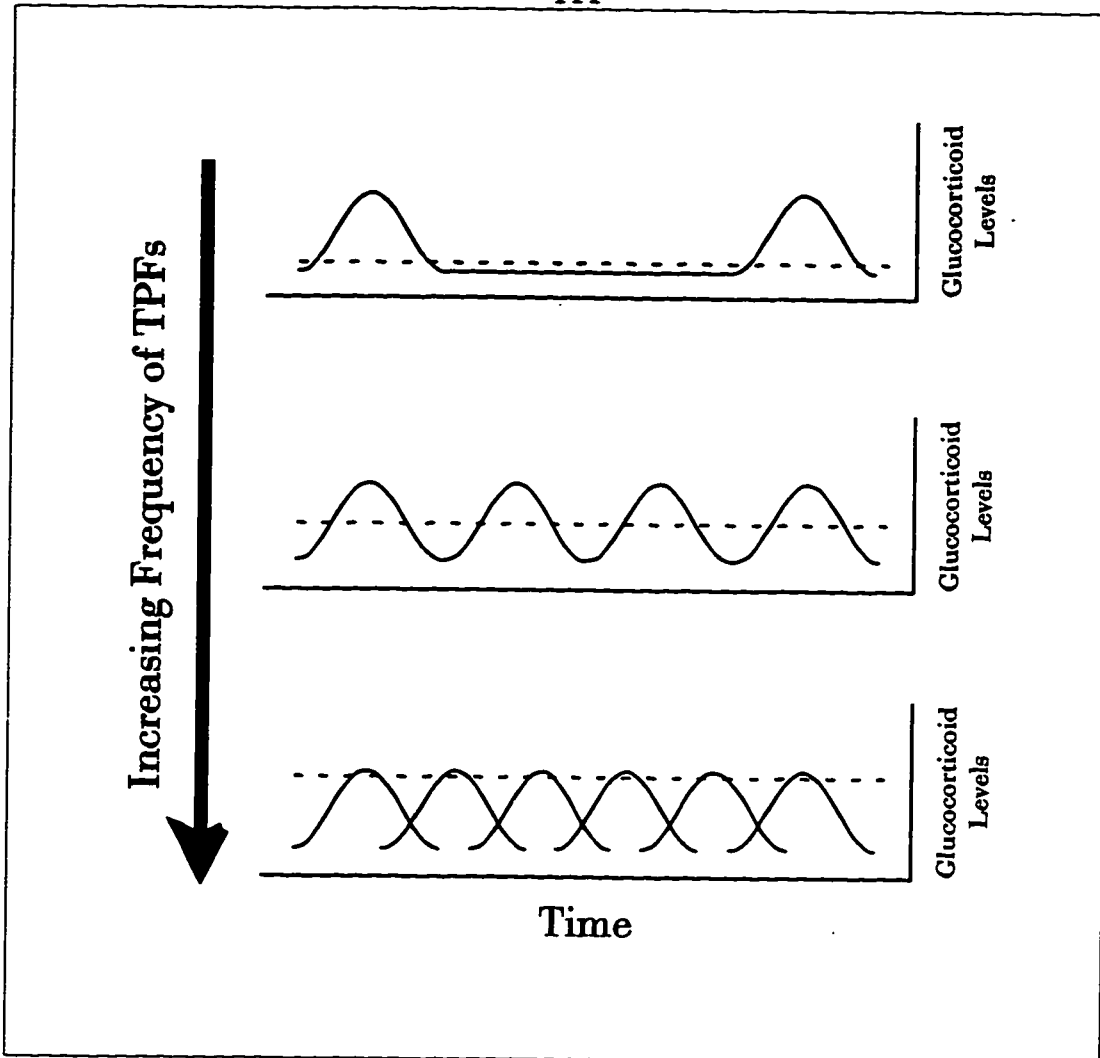


Figure 3.9 - Effect of the frequency of TPFs on basal average glucocorticoid levels in the blood. Solid line curves represent the glucocorticoid response for a single TPF. Dotted line represents the average plasma glucocorticoid level. As the frequency of TPFs increases, so does the average glucocorticoid level. At high frequencies, the average glucocorticoid level is far above basal level.

from the deleterious effects of elevated glucocorticoids. Thus in environments where the predictability is low, the adaptive value of the TPF response will be low since the maintenance of high glucocorticoid levels would drastically reduce the fitness of the animal. As the adaptive value at both high and low ends of the predictability range would be low, the resulting plot of adaptive value versus predictability would result in a large plateau with steep sides at the extremes of predictability (solid line - Figure 3.8). The curve need not be smooth or symmetrical, but should follow the general pattern shown in Figure 3.8. The response to TPFs is very complicated, involving several hormones, a large number of target organs and a complex distribution of glucocorticoid and mineralocorticoid receptors (see Meaney *et al.*, 1996). There will always be an energetic cost to maintaining any complex system (Figure 3.8, shaded region). On top of this base line maintenance cost for the TPF response system will be the cost associated with expressing the TES. As the predictability of the environment decreases, the frequency of TPFs increases, and so does the overall cost for required to respond to TPFs. At very low predictabilities, the cost of maintaining the TPF response system will be much higher than in highly predictable environments as there will be far more TPFs.

At the extremes of environmental predictability, the cost of maintaining the TPF response may exceed the adaptive value of the response (Figure 3.8 - areas where solid line drops below the dashed line). Under normal conditions, there

may be an attenuation of the stress response at either end of the predictability scale, particularly at the low predictability end of the relationship.

Though it may be expected that the TPF response will be attenuated in animals that live in either very predictable, or very unpredictable environments, predictability is hardly the only variable determining the physiological state of the animal. Many other factors (such as energy reserves and time of year) will be important in determining the animal's state, and hence its response to TPFs. Figures 3.10-3.12 show the effects of latitude, time of year, and predictability on the adaptive value of the TPF response for a short lived animal. In this example, birds breeding in tropical regions will show a broad but shallow depression in the adaptive value for a TES during the breeding season, and this drop will be well above the critical adaptive value at all times, except in environments which are either very predictable, or very unpredictable (Figure 3.10). At temperate latitudes, there would be a narrower but deeper dip in the adaptive value for a TES during the breeding season, but again it would not be near the critical adaptive value except in environments at the extremes of predictability (Figure 3.11). In the birds breeding at high latitude, the adaptive value will drop below the critical value at all times, regardless of the predictability of the environment (Figure 3.12).

Embryonic Responses to the Unpredictable

Animals use TESs to help increase survivorship in "stressful" situations, but

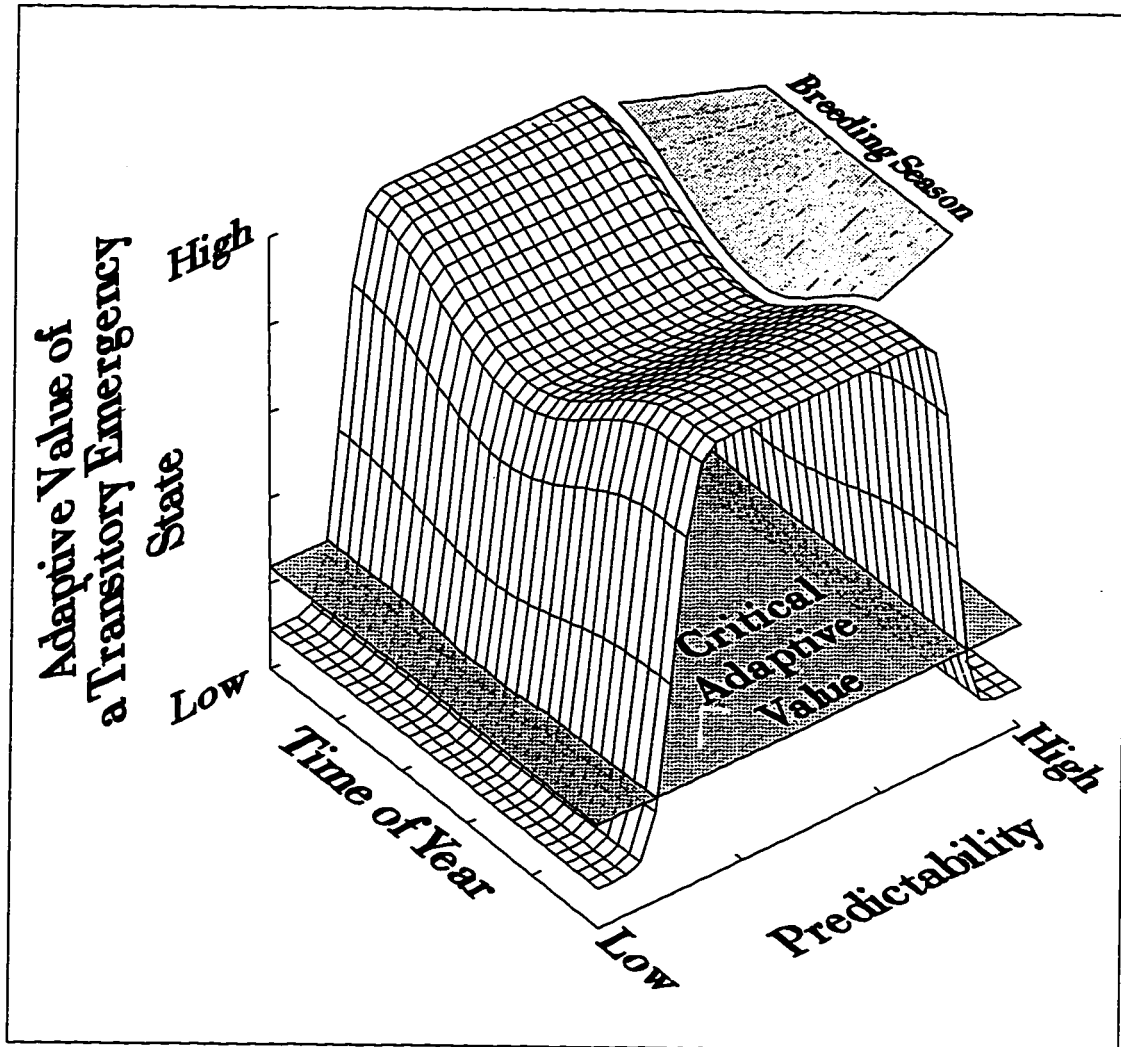


Figure 3.10 - Effect of time of year and predictability on the adaptive value of a transitory emergency state (TES) for a tropical breeding bird. The grey plane represents the critical adaptive value. As the breeding season is long, there is only a small depression in the adaptive value of the TES associated with the breeding season.

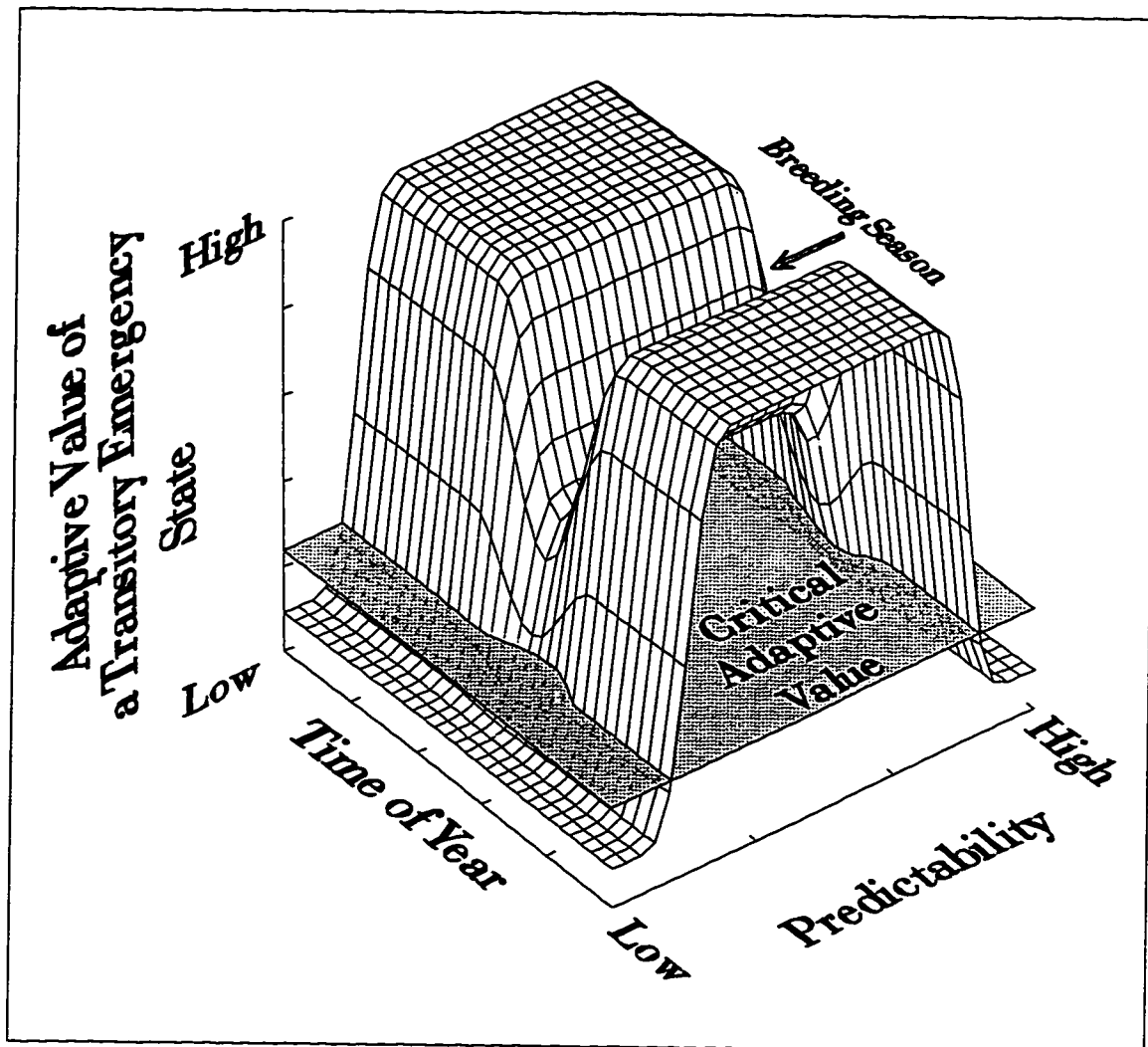


Figure 3.11 - Effect of time of year and predictability on the adaptive value of a transitory emergency state (TES) for a temperate bird. The grey plane represents the critical adaptive value. The breeding season is shorter than in the tropics, but still long enough normally for multiple broods. There is a larger depression in the adaptive value of the TES associated with the breeding season than in the tropics, but it is still normally above the critical adaptive value.

there are some situations where most TESs are not possible. Common laboratory conditions prevent an animal from responding in normal ways to unpredictable events. Animals have not been in captivity long enough to adapt completely to lab conditions, so there has been little or no change in the TPF response resulting from this constraint on their environment. There is however a naturally constrained portion of all animal's life cycles: during embryonic development.

The process of development is a very crucial, and fragile period of an animal's life. Cell division can be rapid, and many tissues must interact at proper times in order for proper development to occur. The release of growth factors and morphogens is important for pattern formation in many tissues and organs. The release of sex steroids is often important in development of primary sex characteristics. During a response to a TPF, glucocorticoids (GCs) become elevated, and these elevated GCs have been shown to inhibit many of the above developmental processes. The release and action of many growth factors is inhibited by GCs (Munk *et al.*, 1984), as well as the release of growth hormone (Slotkin *et al.*, 1992). GCs act on the hypothalamus and pituitary gland to inhibit the release of luteinizing hormone and follicle stimulating hormone (Collu *et al.*, 1988). Nerve cells, at least within some regions of the brain, suffer from neurotoxicity from GCs (Virgin *et al.*, 1991). All of these effects could be extremely deleterious during the process of development, potentially causing deviant development of some tissues, and the de-synchronization of the

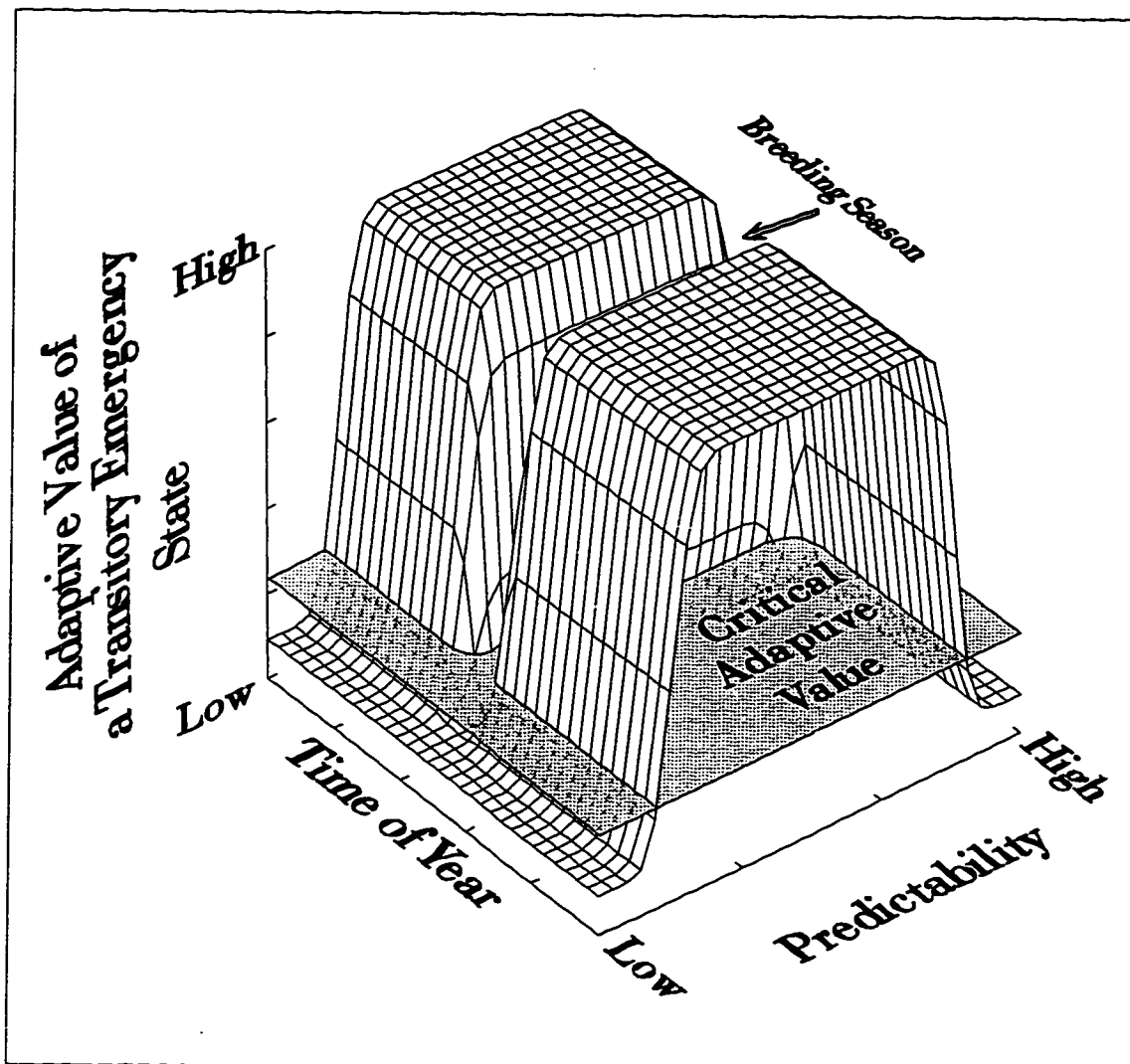


Figure 3.12 - Effect of time of year and predictability on the adaptive value of a transitory emergency state (TES) for a high latitude breeding bird. The grey plane represents the critical adaptive value. As the breeding season is short, there is a large depression in the adaptive value of the TES associated with the breeding season, causing the adaptive value of the TES to be below the critical value throughout the breeding season.

development of dependent tissues.

The developmental processes of an animal follows basically a linear sequence of states from zygote to juvenile, with few possible branching points in the sequence (see figure 3.13). The change of state is driven by a variety of developmental cues: tissue-tissue interactions, growth factors, and gradients of morphogens. In some animals, developmental cues can lead to changes in state leading to either male or female phenotypes, such as in the development of some reptiles (Janzen and Paukstis, 1991) and fishes (Ross, 1990).

The very linear, and generally non-branching nature of the developmental process does not normally allow for the interruptions such as *Transitory Emergency States (TES)* under the influence of perturbation factors, unless it is possible to return to the normal developmental pathway from the TESs. If they cannot return to the normal pathway, the embryo will embark on an abnormal developmental path, leading to a deformed/impaired juvenile, or death. (see bottom path of Figure 3.13).

Since most *transitory emergency states* lead to impaired fitness during hence elevated GCs). Developing embryos are also constrained since they develop within a "container" (egg, or placenta), so the possible responses of the embryo are also limited in much the same manner as in the laboratory animal, and since many of their response systems may not be functional within the embryo at the time (depending on their stage of development at the time of the TPF). Thus, embryos

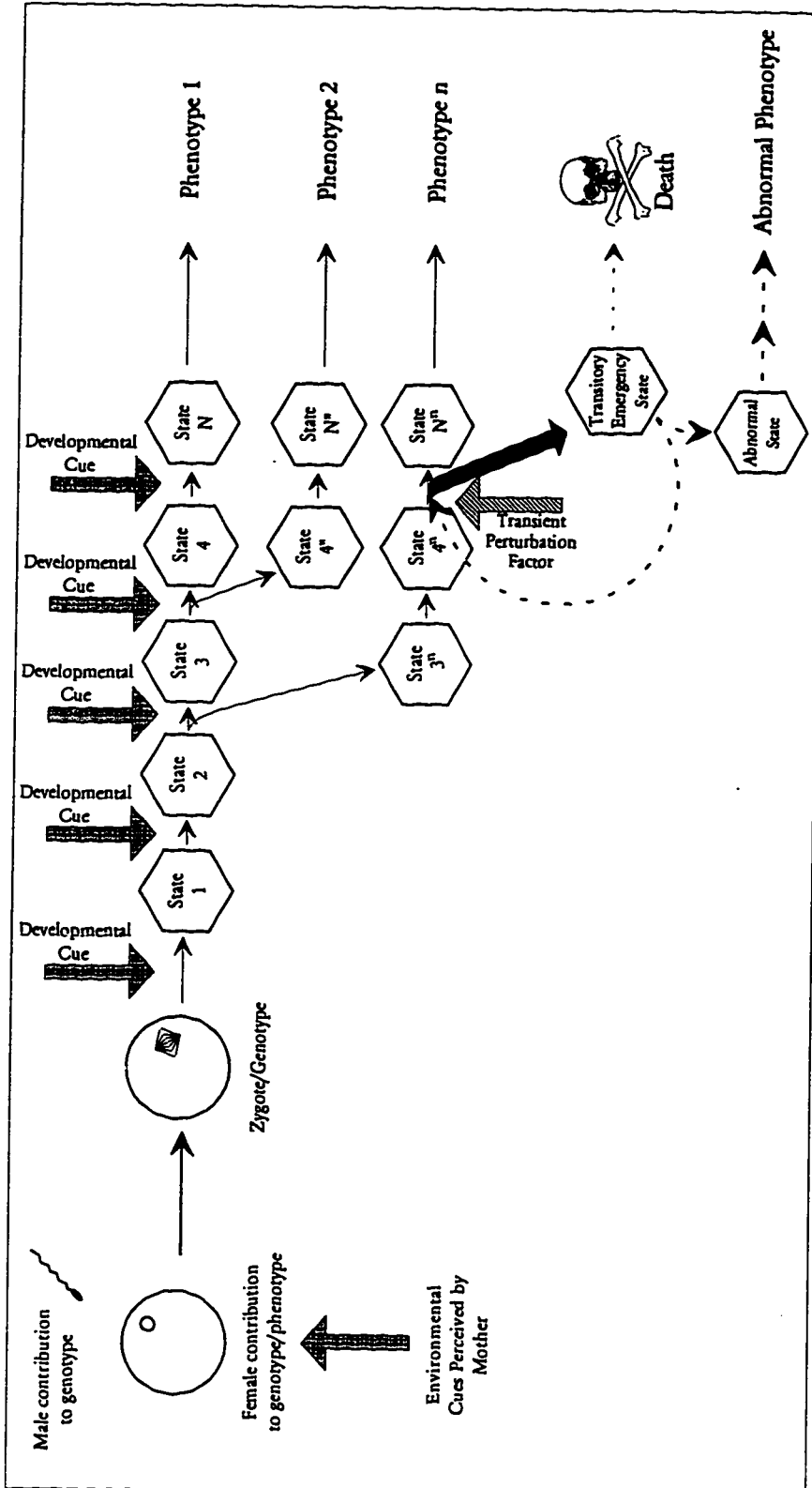


Figure 3.13 - Development viewed as a finite state machine. The developing organism progresses through a mostly linear array of states corresponding to developmental stages. Each stage is subjected to a number of developmental cues, usually from internal sources, but sometimes from the environment. These cues normally lead to the next state in the linear progression, but in some instances, they can lead to branches in the developmental pathway. If the cue is a TPF, the organism will be shunted into a Transitory Emergency State, and off the normal developmental pathway. Once the TPF is removed, the next state will be determined by the TES, and the cues preceived at that time. In many cases the embryo can not re-enter the normal developmental pathway, leading to death or abnormal development.

are constrained both by their environment, and the need to maintain the normal developmental pathway. We would thus expect to see severe limitations on the TPF response during development (see chapter four for some experimental evidence).

Intensity of TPF and Modulation of the Response to TPFs

As we have seen above, the response to TPFs is dependent on the animal's state at the time of the perturbation. The nature of the TPF is also a factor determining what kind of TES the animal will adopt, and also whether or not the animal will show a response to the TPF. The intensity of a TPF may be important in determining the adaptive value for the TPF response.

A short lived bird which only has a small number of possible broods in a lifetime, and has good energy reserves should normally down regulated its response to TPFs. If however, the TPF is perceived by the animal to be too disruptive, or if the TPF is persistent (such as during a heavy snow fall; see Astheimer et al., 1992) there may be an opposing effect which pushes the adaptive value of the TES back up again (See Figure 3.14). If the short-lived bird is exposed to most TPFs, including capture and mild storms, it will show a muted response to the TPF in order to attempt to continue breeding. If however, the animal is exposed to a severe storm (possibly the result of an early return of winter), the intensity of the TPF may push the adaptive value of the TPF back above the critical adaptive value, triggering the TPF response, causing the animal to

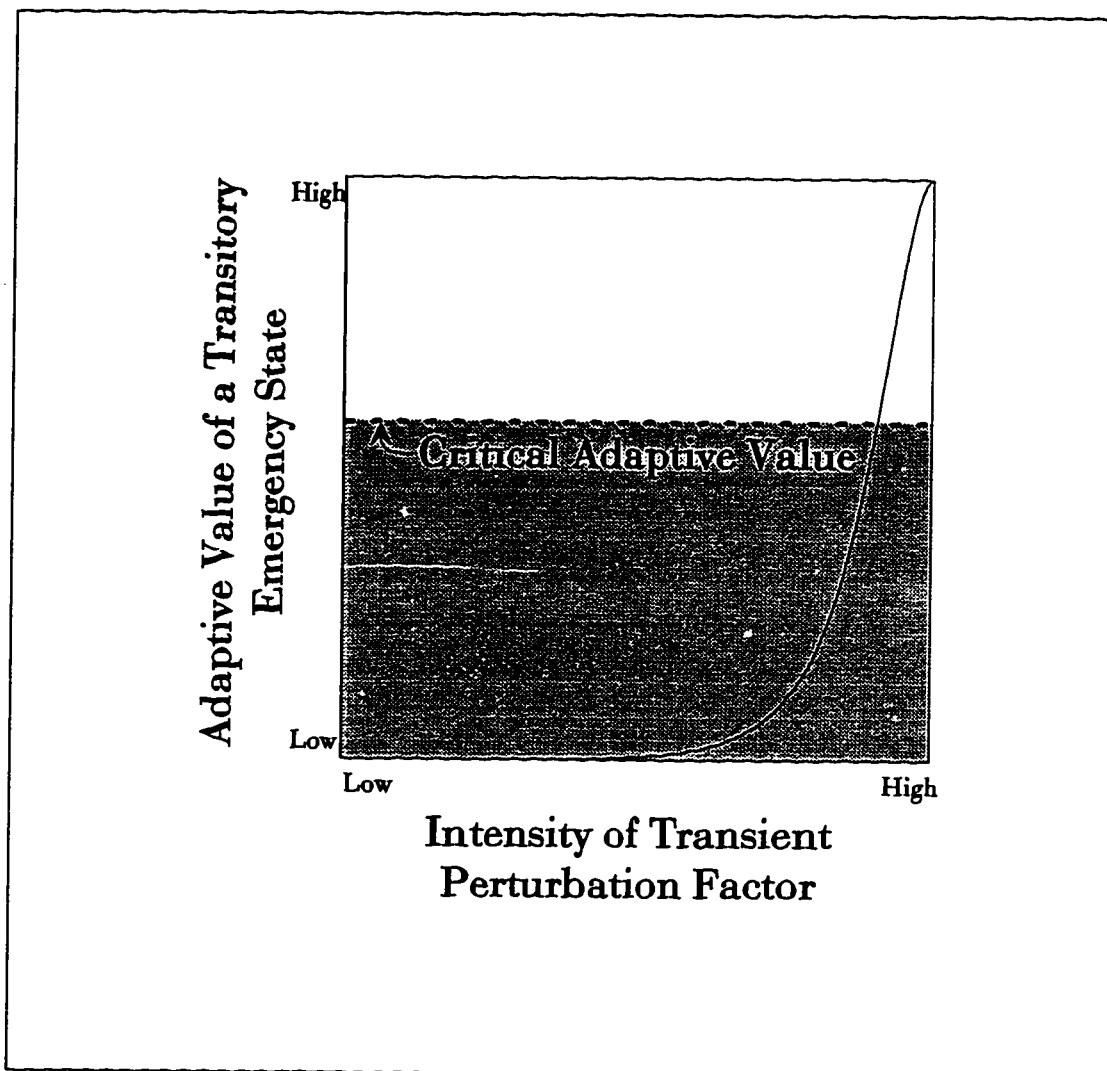


Figure 3.14 - Model of the effect of the intensity of a transient perturbation factor (TPF) on the adaptive value of expressing an transitory emergency state (TES) for an animal under environmental or life history constraints. As the intensity of the TPF becomes high, the adaptive value of the TES increases, and may eventually exceed the critical adaptive value. The critical adaptive value is the dividing line between where the positive aspects of the TES out weigh the negative effects of expressing an TES.

abandon its nest and attempt to save itself. Certainly, if the nest and young are destroyed by the TPF, the animal will no longer be constrained by the need to preserve its offspring.

Conclusions and Implications

The hypotheses generated using this finite state model allow the interpretation of the effects of a number of variables, both excitation variables (such as time of year) and intermediate variables (such as number of broods in a lifetime and energy reserves) on individual responses to TPFs. The dependency of the animal responses to TPFs on the state of the animal (and hence the intermediate variables) and the excitation variables (or environmental cues) indicates that the factors which determine the responses to TPFs may change over time as the animal's state and the environmental cues will change over time. As with the finite state machine model in chapter one, an investigator must know the physiological state of the experimental animal before beginning manipulations, as differences in the starting state of the animal can lead to differences in the responses of the animals.

Chapter IV:

Attenuation of the transitory emergency state (TES) response to transient perturbation factors (TPFs) in a constrained environment:

Developing Chicken Embryos

INTRODUCTION

The egg shell protects a developing avian embryo from many noxious stimuli present in the environment. Other transient perturbation factors (TPFs or “Stresses”) which effect adults, such as social stress and food limitation, are not applicable to developing embryos. However some factors which have only minor effects on the adult such as humidity, temperature, and oxygen levels all must be kept within relatively narrow limits in order for the embryo to develop properly. The normal incubation temperature of chicken embryos is 38.5°C and incubation temperatures of as little as 2 C° above or 4 C° below 38°C are fatal to the developing embryo (Romanoff *et al.*, 1938; See also figure 4.1). Care by the parental birds can limit the actions of TPFs on the embryos by choosing optimal nest sites and by alter the environment of the egg by warming (and in some cases cooling) the egg. Parental care can not eliminate all possible TPFs. The parent must leave the nest to forage and in species in which only one sex incubates, the eggs may be exposed to reduced temperatures if the weather is cold. Even if the

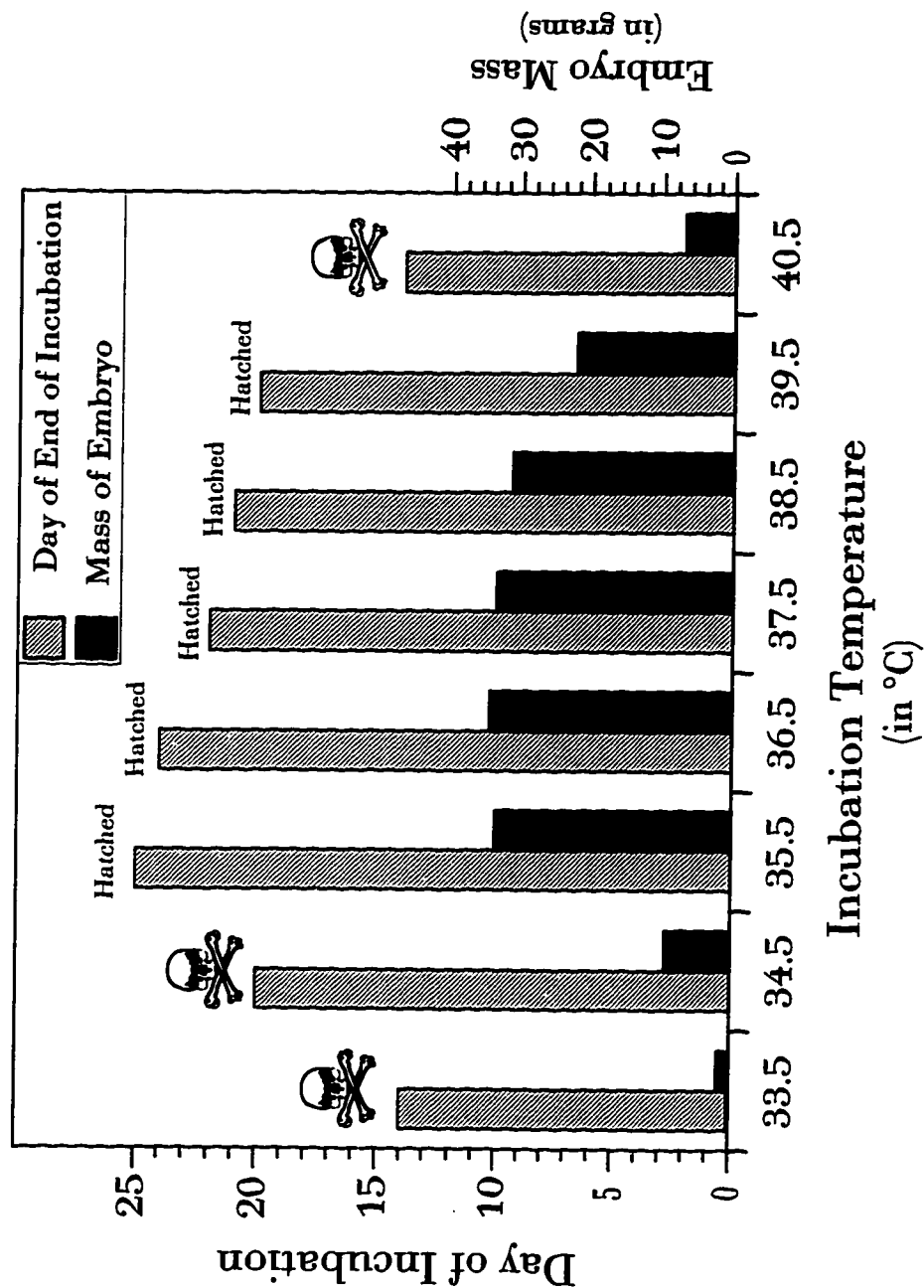


Figure 4.1: Relationship of incubation time and hatching mass to incubation temperature. Drawn from data taken from Romanoff, A.L., (1966). Eggs were incubated at various temperatures, and the date of hatching or death (hatched bars), and embryo mass at the end of incubation (solid bars) were recorded. Bars marked with a skull and crossed bones died before hatching, and those marked with 'Hatched' hatched normally.

parent is present, there is not much a bird such as a chicken can do to reduce the temperature of the egg if the air temperature gets too high.

Embryos are also under a number of constraints absent in adults. As the embryos are locked within a shell, they can not move to a better location to avoid a noxious stimuli, as adults often can. The embryo may not be able to wait for improved conditions, as the developmental can often not be paused, and TPFs such as heat will accelerate many aspects of development and can lead to abnormal development (Primmitt *et al.*, 1989). They are also constrained in that they must keep the developmental processes synchronized. Development involves the interactions of many cells and tissues and should the development of different parts of the animal becomes de-synchronized, these tissue interactions may not occur normally (see Gilbert, 1991).

The transitory emergency state (TES) response in adult animals normally leads to elevated levels of glucocorticoid hormones in the blood (*e.g.*, Gorbman, *et al.*, 1983). These steroids cause a number of major problems for the organism if they remain elevated for prolonged times: they can cause neuronal cell death (Virgin *et al.*, 1991) and the death of macrophages (Dohms and Metz, 1991), modify cellular responses to growth factors (Munk *et al.*, 1984), simulate break down of muscle proteins (Dardvet *et al.*, 1995), inhibit wound healing (Munk *et al.*, 1984), cause reduced skin thickness and dermal atrophy (Munk *et al.*, 1984), inhibit the immune response (Wilckens, 1995), and lead to altered blood vessel

function (e.g. DeKloet *et al.*, 1988). All of these effects would be detrimental to an embryo leading to abnormal development and/or death.

While many studies have examined the response of newly hatched and adult birds (e.g. Freeman and Flack, 1980; Ramade and Bayle, 1980; Saterlee, *et al.*, 1980; Freeman, *et al.*, 1984), studies of the embryonic development of adrenal function have been mostly limited to histological studies (Willier, 1930), examinations of levels of steroids in the adrenals (e.g. Kalliecharan and Hall, 1976) and the depletion of steroid precursors from the adrenal (Freeman, 1967). Three studies have examined the developmental time course of the corticosterone release during development of the chick (Wise and Fry, 1973; Kalliecharan and Hall, 1974; Idelman and Marie, 1985). Wise and Fry (1973) also looked at the response by embryos to a TPF, and showed that by day 15 of incubation, the embryos were capable of showing a corticosterone response to a TPF. In this experiment they used a TPF which might mimic a partial predation event: they broke the femur of one leg. Such a TPF is probably not physiologically relevant to the embryo, as they would not be able to survive such a "stress" in the wild.

In this investigation, the effect of temperature change on developing chicken embryos was examined. As the embryos are likely to experience temperature fluctuations during development in the wild, this is a physiologically relevant stimulus. The effects of both cold (22°C and 12°C) and heat (43°C) were examined, as well as the response when the embryos were returned to their normal

incubation temperature after cooling (as would happen when the hen returned to the nest after feeding during cold weather). Corticosterone levels in the plasma of the embryos were measured as an indicator of the degree of embryo's TES response.

METHODS

Study Animal

Cold-fertilized eggs of the domestic chicken, *Gallus gallus domesticus* (Nick Chick variety of the White Leghorn strain), were purchased from H&N International (Redmond, WA). The eggs were incubated at dry bulb temperature of 38°C ($\pm 1^\circ\text{C}$) and wet bulb temperature of 31°C in a David-Bradley forced air incubator. Eggs were manually turned once each day during incubation. The eggs were candled, at day 12, and the day before treatment, and randomly selected into pools for each time point.

Heating

Eggs incubated at 38°C for 14, 17, 18, 19, or 20 days were then transferred to a second incubator at 43°C. Eggs incubated at 43°C will not hatch (Romanoff, 1960; See also Figure 4.1). Each egg was bled one time.

Cooling

For the cooling experiments, the eggs incubated at 38°C were transferred to cardboard egg trays, then transferred to the appropriate temperature room. For

12°C, the eggs were placed in a 12°C cold room, and for 22°C, the eggs were left at room temperature. Eggs left at either of these temperature will not hatch (Romanoff, 1960; See also Figure 4.1).

Cooling then rewarming:

The eggs were incubated for 19 days at 38°C then transferred into a cold room at 12°C, where they were allowed to remain for 2 hours. The eggs were placed in a cardboard egg tray (with no lid) while they were incubating at 12°C. The eggs were then returned to the 38°C incubator after the 2 hours of cooling.

Egg Temperature Measurement

Measurement of the rate of temperature change within the chicken eggs was determined using a 55 gram unfertilized chicken egg. A hole was drilled in the egg using a 3/32" drill bit, and a micro-thermocouple was inserted into the middle of the egg and the thermocouple was sealed in the hole with melted paraffin.

Temperatures were recorded using a Physitemp Electronic thermometer (Model BAT-12, Physitemp Instrument Inc., Clifton, NJ).

Blood Sampling

In order to obtain quick samples, with relatively large volumes of blood, the eggs were cracked open into a 115x50mm preparation dish (08-807, Fisher Scientific) containing a paper towel, and the vitelline blood vessels were exposed where they exit the embryo's body. The vitelline artery was nicked with a pair of

iris scissors, and the blood was collected with heparinized capillary tubes using a mouth pipetting device (see figure 5.2). Blood sampling was completed from 1 to 5 minutes from opening the egg. The capillary tubes were flame sealed at one end, and stored at 4°C until they were centrifuged (2-4 hours). After centrifugation, the plasma was transferred to 500µl plastic microcentrifuge tubes, and stored at -20°C until they were assayed.

Sexing of Embryos:

After the blood sampling was complete and the embryos were euthanized, the embryos were sexed. An incision was made through the body wall from the cloaca to the sternum, and the viscera was pushed aside exposing the gonads. Examination of the gonads allowed positive identification of the sex on and after day 14 of incubation.

Corticosterone Radioimmunoassay:

The assay procedure was a modification (without chromatography) of the methods described by Wingfield and Farner (1975) and Ball and Wingfield (1987). All plasma samples (ranging in volume from 20-100 µl) were equilibrated with 2,000 cpm of tritiated corticosterone (New England Nuclear) and 0.3 ml of distilled water overnight at 4°C. They were then extracted in 5 ml of freshly redistilled dichloromethane. The organic phase was aspirated and dried under a stream of nitrogen at 40°C. Dried extracts were reconstituted in 0.55 ml of

phosphate-buffered saline and 200 μ l aliquots taken to duplicate assay tubes and 100 μ l to a scintillation vial (with 4.5 ml of scintillation fluid: 4 g Omnifluor, New England Nuclear, in 1 liter of toluene). The cpm of tritium in this vial for each sample provided an estimate of percent recovery following extraction.

The radioimmunoassay utilized an antibody to corticosterone (B21-42, Endocrine Sciences, Tarzana, CA). Of the steroids likely to be encountered in avian blood, progesterone showed 57.8% cross reaction with the antibody. However, progesterone has a low extraction in dichloromethane, and corticosterone is the main steroid assayed in this system. Reconstituted extracts were incubated with 100 μ l of antibody (diluted 1/100 from each vial) and 100 μ l of tritiated corticosterone (approximately 10,000 cpm) overnight at 4°C. Separation of bound and free cpm was achieved by addition of 0.5 ml of dextran coated charcoal for 12 minutes at 4°C. All samples were then promptly centrifuged at 2,000 rpm for 10 minutes at 4°C in a Beckmann TJ-6 refrigerated centrifuge. Supernatants (containing bound cpm) were decanted into scintillation vials, 4.5 ml of scintillation cocktail added, and equilibrated overnight before counting in a Beckmann LS3500 system (for 10 min or 2% accuracy).

With each assay, two solvent blanks and a standard sample (containing 1,000 pg of corticosterone) were taken through the entire assay procedure as a check on reliability criteria.

Data for the assays were collected from the scintillation counter by an IBM computer and plasma concentrations of corticosterone were calculated using the program in Appendix B.

Statistics:

All statistical analysis of the data was done using the computer program SYSTAT (Wilkinson, 1990). Corticosterone responses over time were analyzed using a one way ANOVA. Post hoc comparisons were made using routines for this purpose built into SYSTAT. Statistics for embryos treated at 38°C on day 18 were done using a Kuskal-Wallis nonparametric ANOVA, as the data variance was heteroscedastic. Pair-wise comparisons between untreated embryos and treated embryos and between males and females were made using an independent Student's t-test.

RESULTS

Changes in Basal Corticosterone Levels with Age of Development.

Basal corticosterone levels showed several changes during the later course of development (See Figure 4.2). Before day 16, basal corticosterone levels were essentially undetectable (<1 ng/ml). Between days 15 and 17, there was a significant rise in the basal corticosterone level ($F=8.820$, $df=1$, $P<0.003$) up to around 7 ng/ml. A second rise in basal corticosterone levels occurred between days 18 and 19, leading to a significant peak (12.4 ng/ml) in basal corticosterone

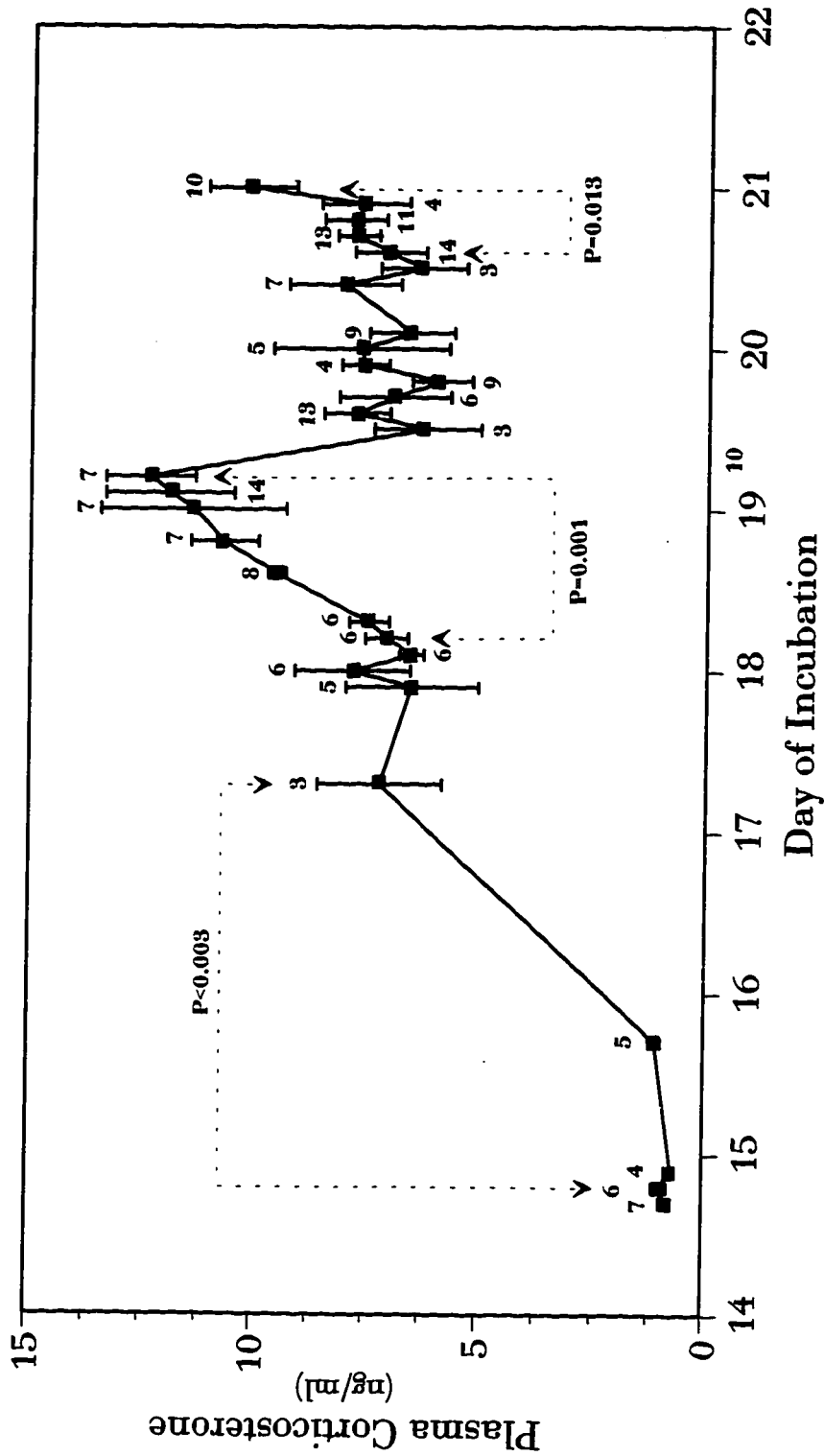


Figure 4.2 - Changes in basal corticosterone with days of incubation. Eggs were incubated continuously at 38°C, and blood samples were taken to determine plasma corticosterone levels at a particular time. Sample sizes are displayed near the time points. Error bars show +/- the standard error of the mean for each data point.

levels ($F=10.348$, $df=1$, $P=0.002$). This peak rapidly tailed off to approximately the day 17 levels (~7 ng/ml) by mid day 19 ($F=10.945$, $df=1$, $P=0.001$ and this level continued till almost day 21. Just prior to day 21 and hatching, there was again a significant increase in basal corticosterone levels ($F=6.295$, $df=1$, $P=0.013$) up to 10 ng/ml.

Temperature Changes in Chicken Eggs.

Heating: Using a 55 gram unfertilized egg, it takes 30 to 40 minutes to reach 43°C when transferred from a 38°C to a 43°C incubator (See figure 4.3). By 20 minutes, the internal egg temperature is nearly 41°C.

Cooling to 22°C: Temperature change from 38°C within the egg to 22°C took 80 minutes (See Figure 4.4). Internal egg temperature were well below normal incubation temperature within 20 minutes of the transfer to 22°C.

Cooling to 12°C: Cooling of the egg to 12°C took 120 minutes (see Figure 4.5). Internal egg temperatures dropped well below normal incubation temperatures within 10 minutes of the transfer.

Rewarming from 12°C to 38°C: Rewarming in the incubator was rapid, taking 50 minutes. Temperature within the egg were in the low end of the incubation range within 30 minutes of the transfer from 12°C to 38°C. (See Figure 4.5).

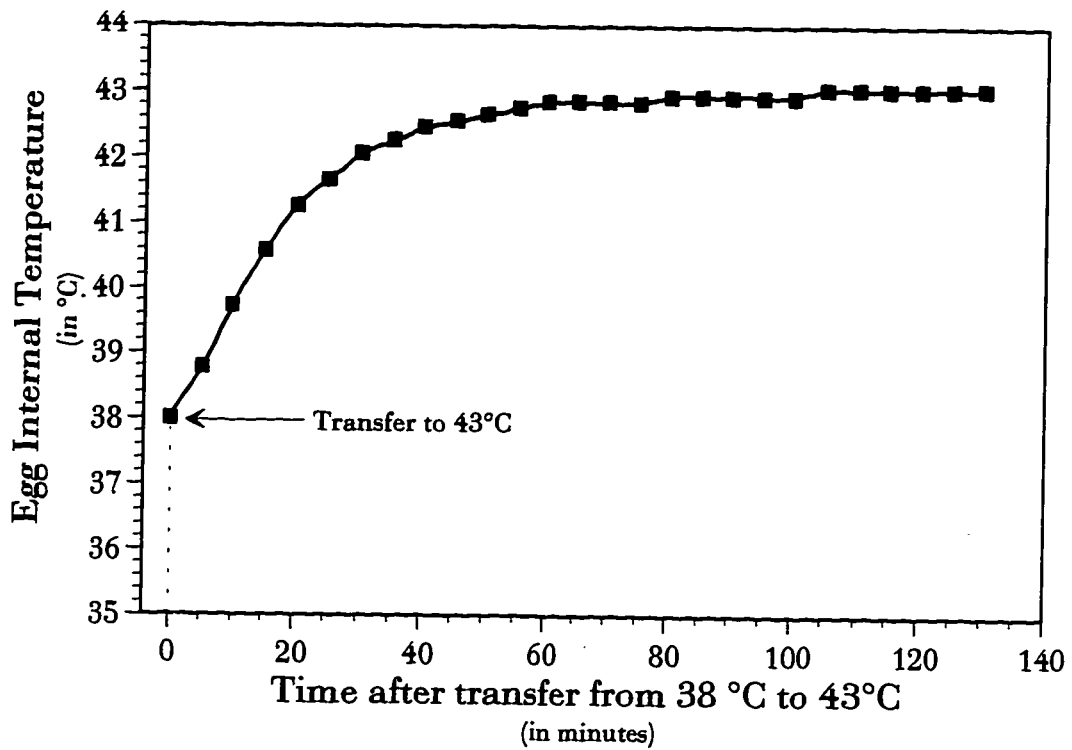


Figure 4.3 - Rate of temperature change in a chicken egg transferred from 38°C to 43°C. Temperature measurements were made using a thermocouple inserted into an unfertilized egg.

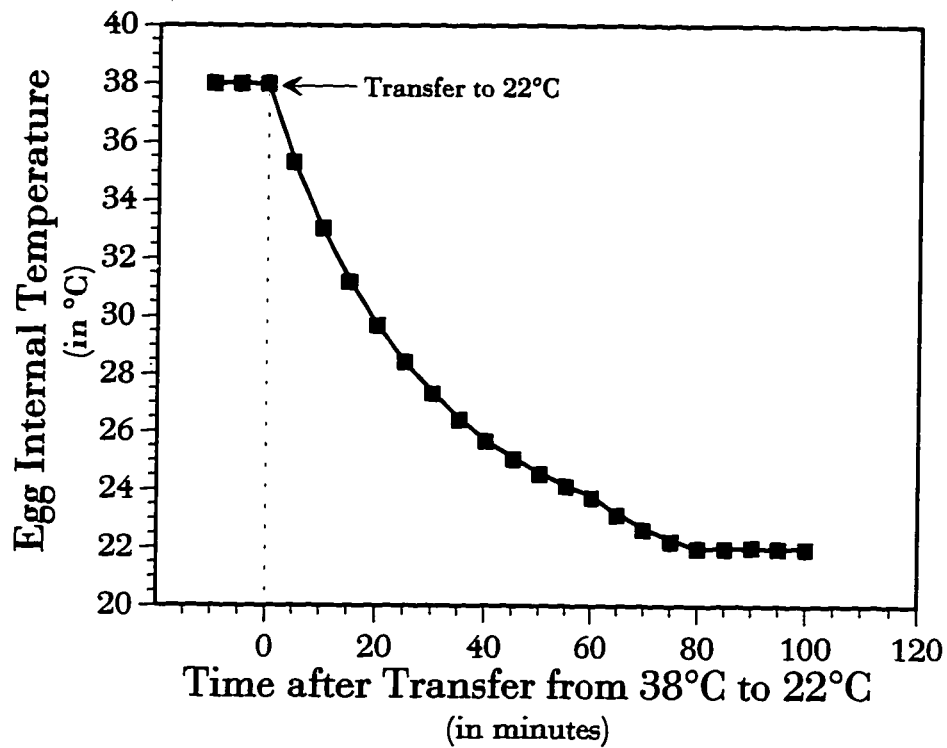


Figure 4.4 - Rate of temperature change in a chicken egg transferred from 38°C to 22°C. Temperature measurements were made using a thermocouple inserted into an unfertilized egg.

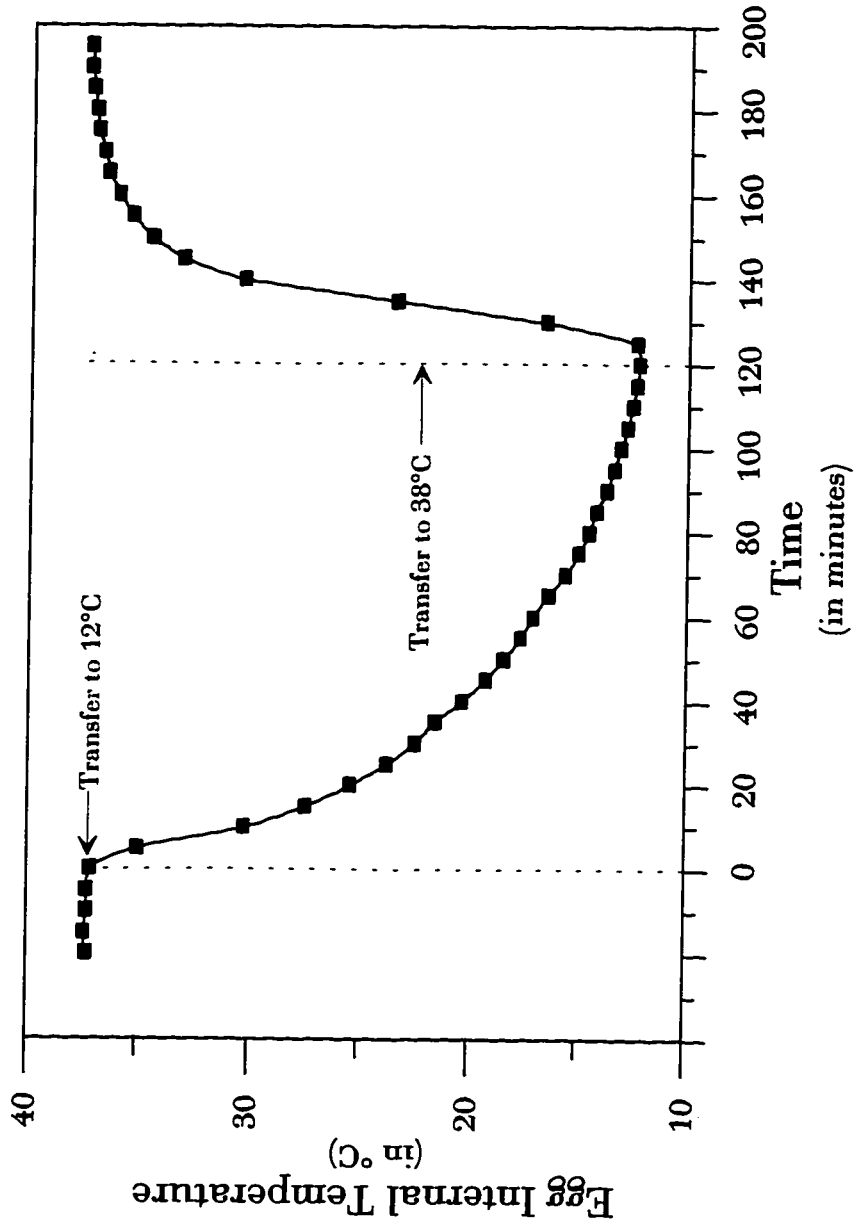


Figure 4.5 - Temperature change within an egg when transferred from 38°C to 12°C, then rewarmed to 38°C. Temperature measurements were made using a thermocouple inserted into an unfertilized egg. Cooling was done in a cold room at 12°C with limited air movement while reheating was done in a forced air incubator at 38°C.

Plasma Corticosterone Levels in response to cooling.

Cooling day 18 embryos from 38°C to 22°C did not produce a significant change in corticosterone levels during the experimental period (Figure 4.6; $F=0.289$, $df=5$ $P=0.916$). The untreated day 18 embryos (left at 38°C), also show no change in corticosterone levels over time (Figure 4.6; $H=7.229$, $df=5$, $P=0.204$)

The untreated day 19 embryos showed a significant decrease in corticosterone levels during the experimental period (Figure 4.7; $F=3.937$, $df=6$, $P=0.001$). This corresponds to the drop in basal corticosterone levels seen above during the 19th day of incubation. Cooling day 19 (See figure 4.7) embryos to either 22°C or 12°C produced no significant increase in plasma corticosterone levels ($F=0.206$, $df=6$, $P=0.973$ and $F=0.086$, $df=5$, $P=0.994$ respectively). Thus day 19 embryos did not show a TES response to cooling at either of these temperatures. Both the embryos cooled to 22°C and the embryos cooled to 12°C were significantly higher than the untreated embryos at 720 minutes of exposure ($T=3.316$, $df=15$, $P=0.004$, and $T=3.993$, $df=17$, $P=0.001$ respectively). When cooled, the day 19 embryos did *not* show the normal decrease in corticosterone levels.

Plasma corticosterone Levels in response to heating.

Overall Response to heating: Heating chicken embryos to 43°C caused a significant increase in plasma corticosterone levels on days 17, 18. and 20

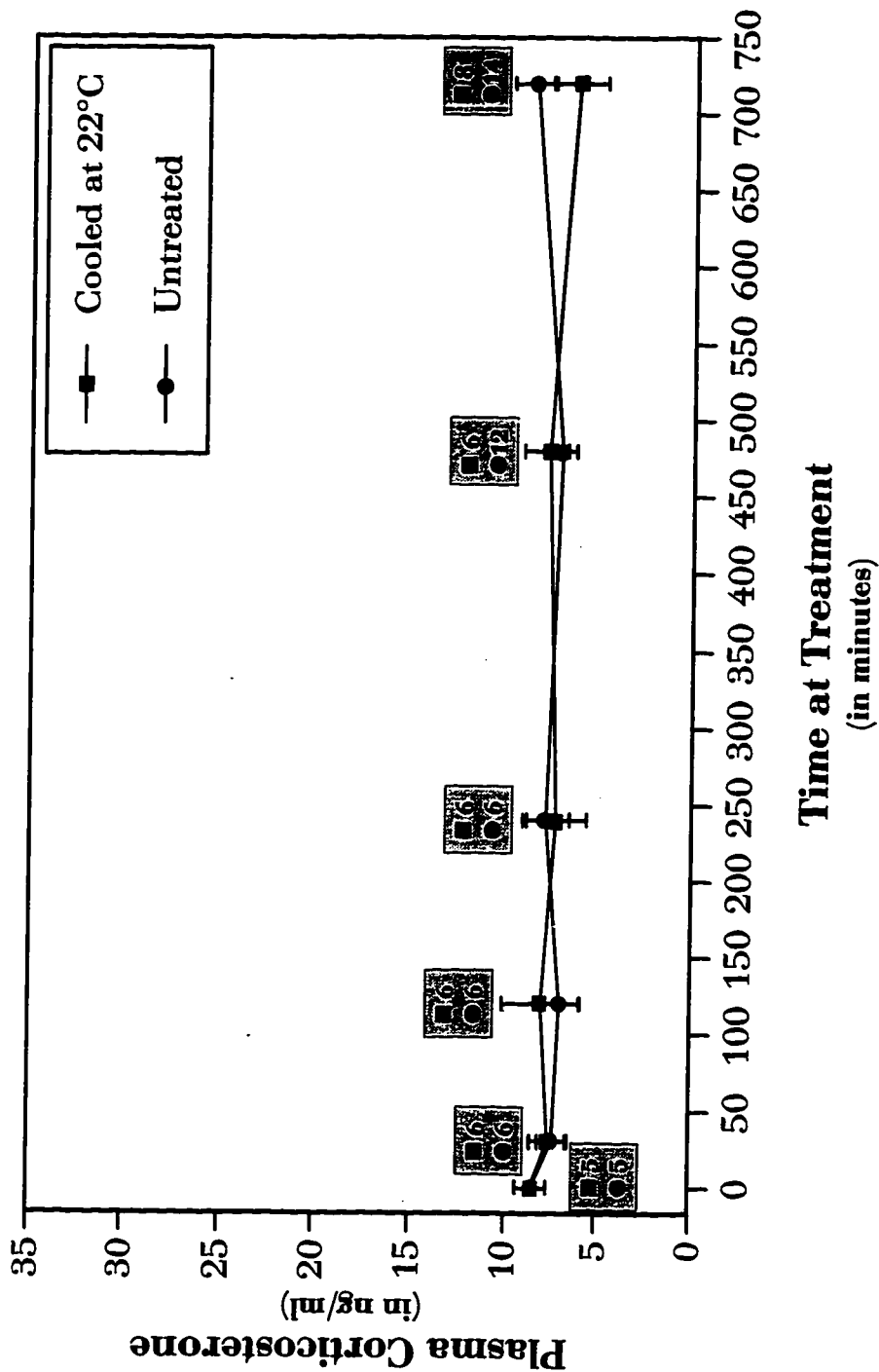


Figure 4.6 - Changes in plasma corticosterone levels of day 18 embryos in response to cooling to 22°C. Day 18 embryos were transferred from the 38°C to the counter top at 22°C (closed squares). Untreated embryos (closed circles) were left in the 38°C incubator throughout the experiment. Sample sizes are shown near the time points with their corresponding symbol. Error bars show +/- the standard error of the mean for each data point.

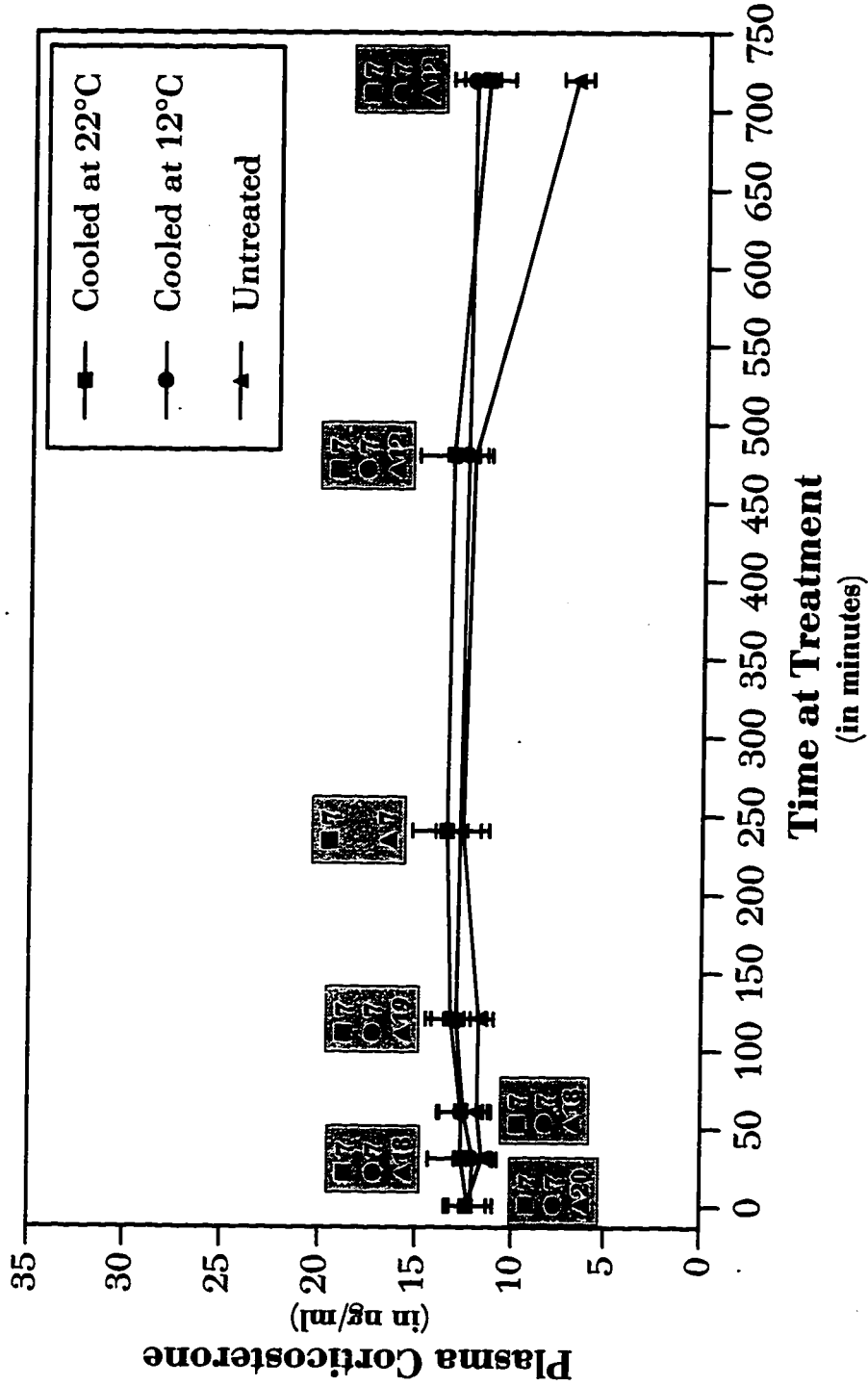


Figure 4.7 - Changes in plasma corticosterone levels of day 19 embryos in response to cooling. Day 19 embryos were transferred from the 38°C cold room (closed squares), or to the counter top at 22°C (closed circles). Untreated embryos (closed triangles) were left in the 38°C incubator throughout the experiment. Sample sizes are shown near the time points with their corresponding symbol. Error bars show +/- the standard error of the mean for each data point.

($F=5.707$, $df=5$, $P=0.001$; $F=5.295$, $df=7$, $P<0.001$; $F=11.671$, $df=10$, $P<0.001$ respectively), but not on day 14 or day 19 ($F=1.048$, $df=3$, $P=3.87$; $F=0.825$, $df=4$, $P=0.524$; see figure 4.8). Corticosterone levels in blood samples from day 14 embryos were so low as to be virtually undetectable: most of the samples were below the standard curve minimum detectable dose (<0.715 ng/ml).

Response during the first 2 hours of heating (See Figure 4.9): During the first 2 hours of heat exposure, day 17, 18, and 20 embryos exhibited significant increases in plasma corticosterone levels ($F=5.219$, $df=1$, $P=0.030$; $F=11.880$, $df=1$, $P=0.001$; and $F=21.691$, $df=1$, $P<0.001$, respectively). Neither day 14 nor day 19 embryos had significant changes in corticosterone during the first 2 hours ($F=2.031$, $df=1$, $P=0.165$ and $F=0.184$, $df=1$, $P=0.672$). The maximum values for day 17, 18, 19, and 20 were not statistically different ($F=0.129$, $df=3$, $P=0.942$), but the basal level for day 19 embryos was higher than day 17, 18, and 20 ($F=6.942$, $df=3$, $P>0.0004$). Basal values for day 17, 18, and 20 embryos were essentially identical ($F=0.614$, $df=2$, $P=0.544$). Both basal and maximal level for day 14 embryos were much lower than all other days ($F=205.654$, $df=1$, $P<0.001$ and $F=35.392$, $df=1$, $P<0.001$ respectively). The maximal levels of corticosterone on days 17, 18, 19, and 20 were not distinguishable from the basal level on day 19 ($F=0.231$, $df=4$, $P=0.919$).

Responses of males and females to heating: Both males and females showed a significant increase in response to heating to 43°C on day 20 ($F=24.822$,

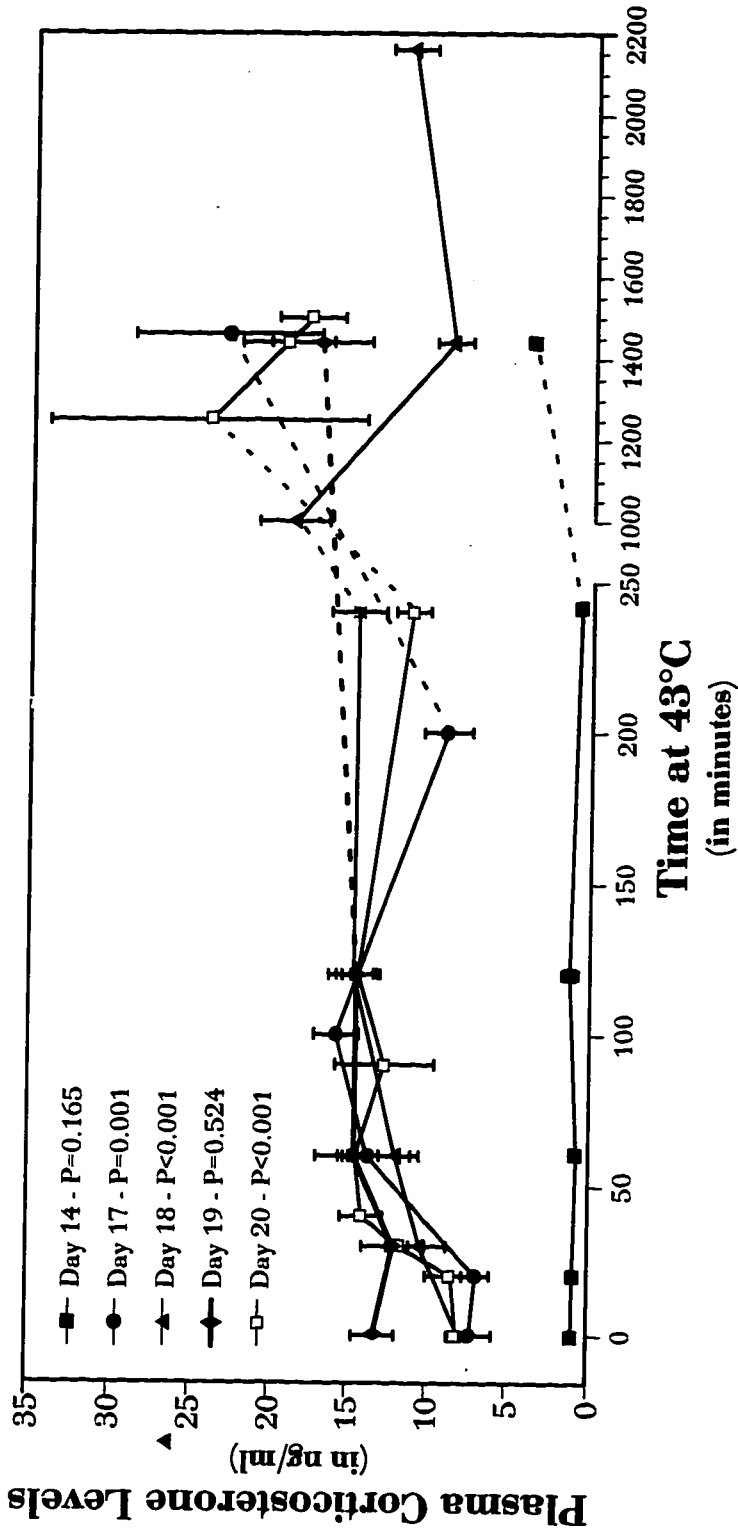


Figure 4.8 - Effect of heating to 43°C on plasma corticosterone levels in developing chick embryos. Embryos of the appropriate age were transferred to 43°C at time zero. Error bars show the +/- the standard error of the mean for each data point. Error bars show +/- the standard error of the mean for each data point.

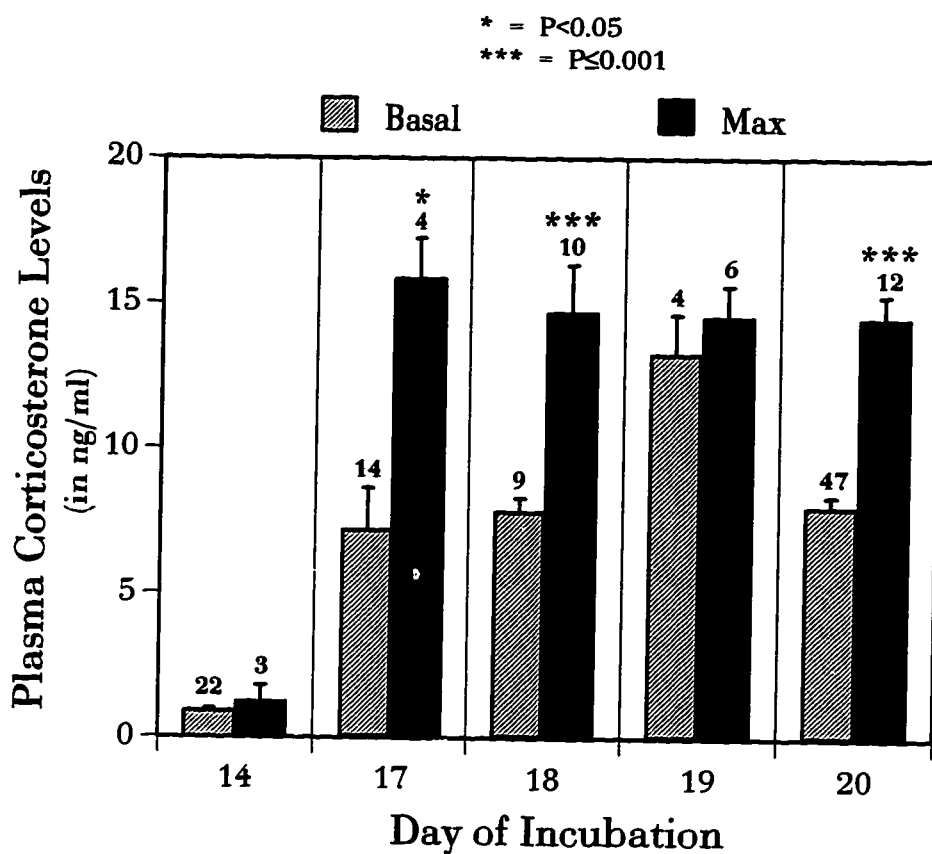


Figure 4.9 - Maximum plasma corticosterone levels in response to the first 2 hours of heating at 43°C. Hatched bars represent corticosterone levels at the beginning of the experiment. Solid bars represent the maximum corticosterone level reached in the first 2 hours of treatment at 43°C. All embryos had previously been incubated at 38°C. Error bars show the standard error of the mean for each bar.

$P < 0.0001$ and $F = 31.165$, $P = 0.001$ respectively), but there was no difference between the response of males and females ($T = 0.048$, $df = 13$, $P = 0.963$ for maximum corticosterone, and $T = 0.685$, $df = 10$, $P = 0.509$ for basal corticosterone levels) (see figure 4.10).

Plasma Corticosterone Levels in Response to Rewarming after Cooling.

Day 19 embryos returned to 38°C, after cooling to 12°C for 2 hours, showed a significant increase in plasma corticosterone levels at 30 minutes after returning to the 38°C incubator ($F = 7.562$, $df = 1$, $P = 0.011$; See Figure 4.11). This increase was very transitory, and the plasma corticosterone levels had returned to basal levels by 60 minutes. Neither embryos left at 12°C ($F = 0.114$, $df = 3$, $P = 0.951$) nor those left at 38°C ($F = 0.163$, $df = 3$, $P = 0.921$) showed any significant change during the 2 hour test period.

DISCUSSION

Basal corticosterone levels in the developing chicken embryo show a number of interesting changes. The capacity of the adrenal glands to secrete corticosterone is extremely limited in embryos before day 15, with plasma corticosterone levels virtually undetectable on days 12 (Wise and Frye, 1973) and 14. By day 17, basal levels of corticosterone are at levels identical to the average on days 18 and 20.

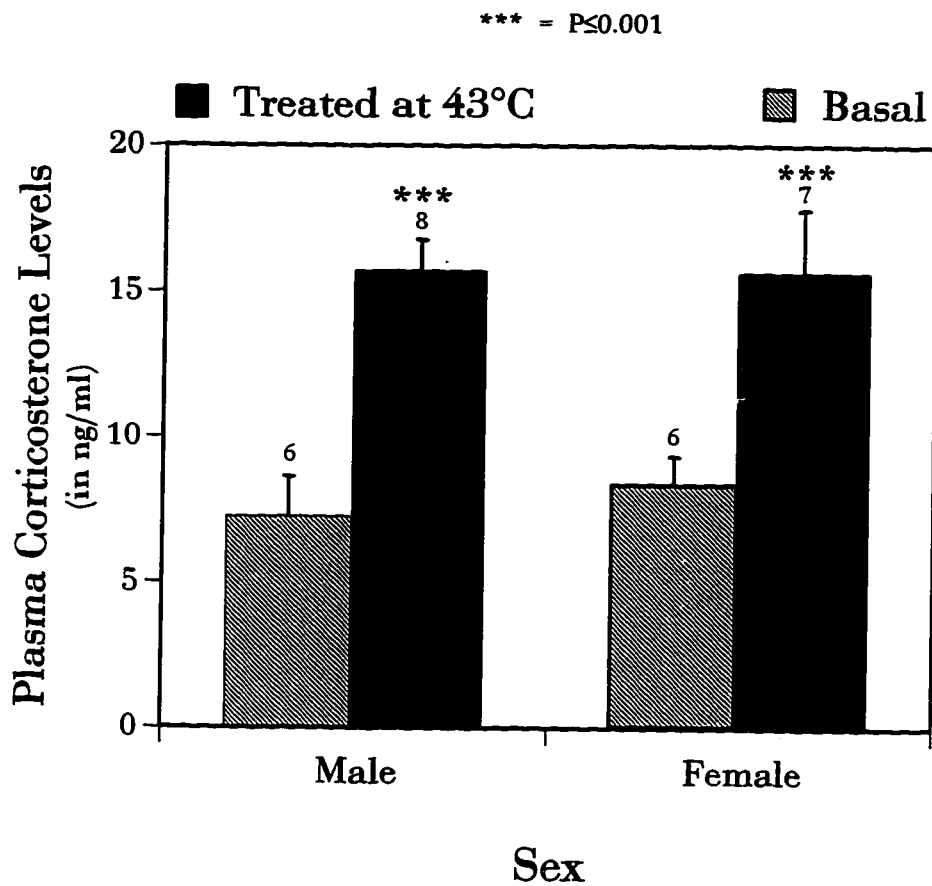


Figure 4.10 - Response of males and females of day 20 chick embryos treatment at 43°C. Hatched bars represent corticosterone levels at the beginning of the experiment. Solid bars represent the maximum corticosterone level reached in the first 2 hours of treatment at 43°C. All embryos had previously been incubated at 38°C. Error bars show the standard error of the mean for each bar.

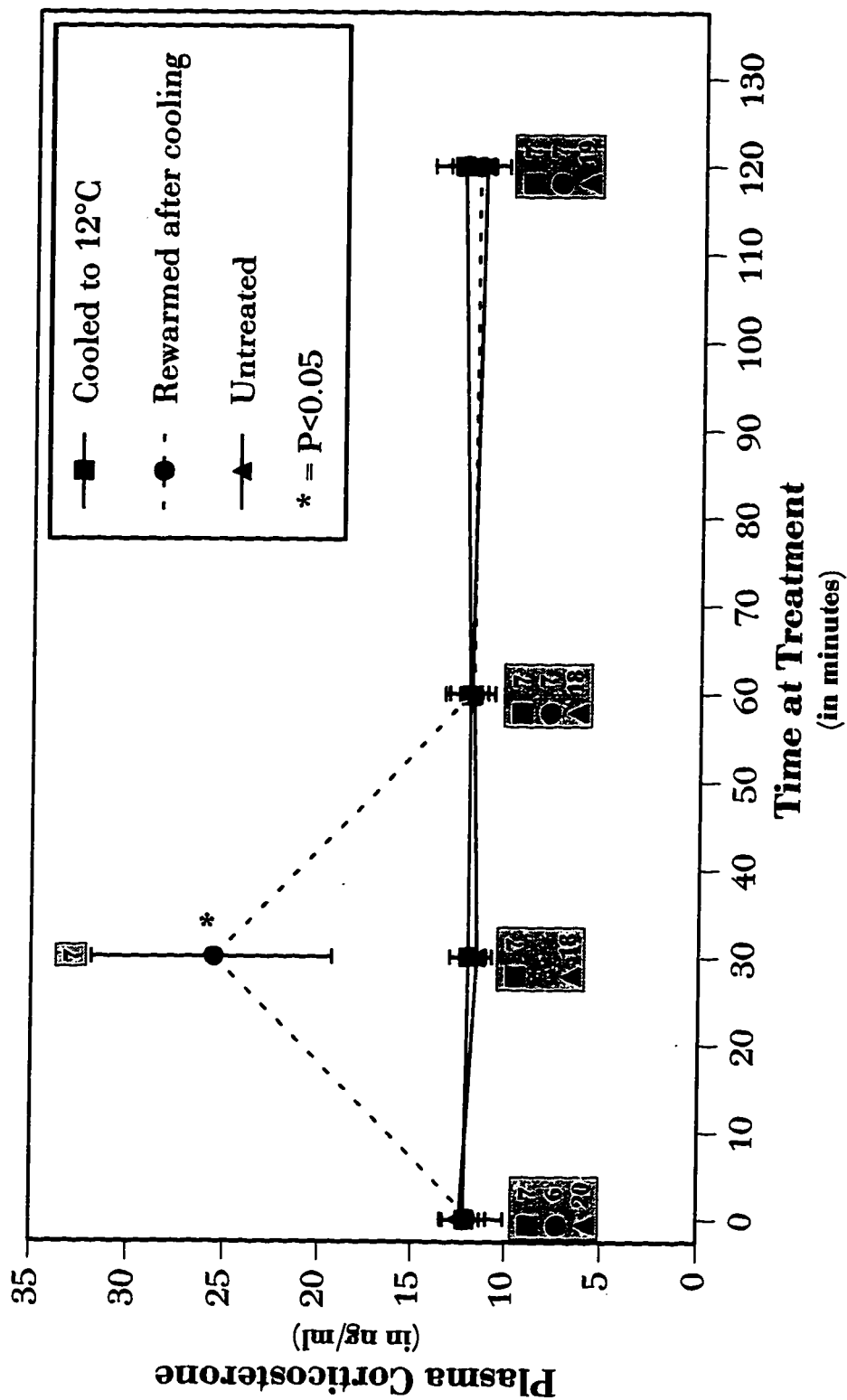


Figure 4.11 - Changes in plasma corticosterone levels of day 19 embryos in response to rewarming after cooling to 12°C. Rewarmed after cooling embryos (dotted line and closed circles) were transferred from a 38°C incubator to a 12°C cold room, then returned to 38°C after 2 hours. Embryos cooled at 12°C (solid squares) were transferred from the 38°C incubator to the 12°C incubator at time zero. Untreated embryos (closed triangles) were left in the 38°C throughout the experiment. Error bars show +/- the standard error of the mean for each data point.

There is major increase in the basal corticosterone levels beginning by mid-day 18 to early day 19 (see Fig. 4.2). Wise and Frye (1973) did not look at day 19 of incubation, so they did not see this peak in basal corticosterone levels. Neither Idelman and Marie (1985) nor Kalliecharan and Hall (1974) found this peak on day 19, but both studies took only one time point per day and could have missed this sharp peak all together. Internal pipping (puncturing of the egg air space by the beak) occurs in mid to late day 19 of incubation (Visschdijk, 1985). Once the embryo punctures the air space, it begins to breathe using its lungs. The rise in basal corticosterone levels just precedes internal pipping (and the use of the lungs) and may be analogous to the rise in glucocorticoids in mammals just before birth which stimulates final differentiation of the lung epithelia (Murphy, 1982).

The basal level also begins to climb just before hatching (day 21 of incubation; see Fig. 4.2). This rise begins just prior to the period when the embryo does the external pipping (the beak punctures the shell). After external pipping, respiration in the embryo increases dramatically (Visschdijk, 1985), and the embryo will soon hatch.

Cold appears not to induce a TES response in chicken embryos (see Figs. 4.6 and 4.7). Development at the reduced temperatures of 22°C and 12°C is retarded and inhibits normal developmental changes in basal corticosterone levels. (see figure 4.7).

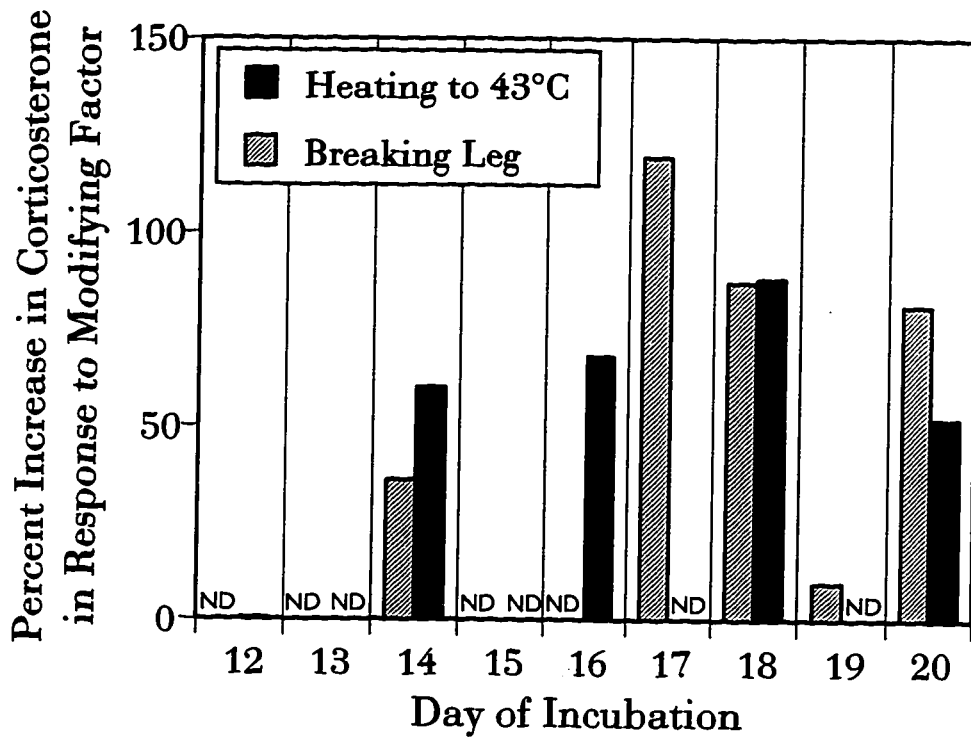


Figure 4.12: Comparison of 2 Label Modifying Factors (Stressors) on increases in plasma corticosterone levels. Cross-hatched bars represent data from Wise and Frye (1973) where the LMF was breaking of the femur. Solid bars represent data from embryos exposed to 43°C for 2 hours.

Heat induces a TES response in day 17, 18, 20, and 21 embryos. Though these embryos show a significant response to the heating, maximum corticosterone level is restricted and is identical to the normal basal level for day 19 embryos. Thus the glucocorticoid aspect of the TES response in chicken embryos seems to be limited within the normal range of basal corticosterone levels, and does not obtain the high, potentially damaging levels show in adult chicken TES responses.

Under the conditions of this experiment, it takes nearly 2 hours for an egg to cool from 38°C to 12°C (see figure 4.5). It is not clear whether a normally developing embryo would cool at a faster or slower rate, since cooling would be promoted by the circulation of blood, but reduced by the heat produced by the embryos at later stages of development (Romanoff, 1941). The embryos in the cooling-reheating experiment were probably down to 12°C by the time they were returned to 38°C or nearly so. During cooling to 12°C, embryos do not show a corticosterone response (see figure 4.7), but when they are returned to 38°C, there is a rapid peak in corticosterone levels by 30 minutes (see Fig. 4.11). This peak is very short lived and the embryos are back to basal levels by 60 minutes after transfer to 38°C. Normally, a TES response does not show this short time course (e.g., Wingfield *et al.*, 1992; Smith *et al.*, 1994). This short lived peak may be the result of changes in circulation in the animal on cooling. If blood flow to the adrenal glands was restricted, and the production of corticosterone continued, you would see an increase in plasma corticosterone levels when the blood flow to the

adrenals returned to normal (*i.e.*, on warming). It is doubtful that this short lived increase in circulating corticosterone is physiologically relevant, since it generally takes on the order of hours for genomic receptors to show a response (O'Malley and Tsai, 1992), and the embryo would already be back to its normal temperature by that time.

It seems that for chick embryos, cold does not act as a TPF as it does not elicit a corticosterone response. Moderate heating does elicit a response, but this response is within the normal range of developmental release of corticosterone in developing chickens. The modulation of the adrenocortical response to TPFs is exactly what would be expected in a constrained environment (see chapter three). This limiting of corticosterone levels when high levels might be detrimental is also seen in some birds during the breeding season (Wingfield *et al.* 1994a; Wingfield *et al.*, 1994b).

Chapter V:

Responses to Transient Perturbation Factors in Environments at the Extremes of Predictability: Fishes in the Intertidal Zone and in Caves.

Introduction

The bony fishes (Osteichthyes) comprise the most diverse group of vertebrates, and live in a wide variety of aquatic habitats and even some semi-terrestrial habitats (i.e. Mudskippers: Gobiidae). Some of these habitats, such as in caves, desert springs, Antarctic waters and the deep sea, experience long periods of very stable conditions. These stable environments have generally high levels of predictability, due to constancy.

Other aquatic habitats are not so predictable, and are far more at the whim of the weather. Some fishes, such as the lungfishes *Lepidosiren* (Lepidosirenidae) and *Protopterus* (Protopteridae) and many species of South American and African killifishes (Cyprinodontidae), live in areas that dry up for large portions of the year. Possibly the most chaotic and disturbed environment of all is the intertidal zone (Gibson, 1993). In the intertidal zone, large waves generated by storms can strip animals and plants from their hiding places and smash them on the rock or toss them on the beach. Boulders and logs can be dragged around the intertidal zone, scraping regions free of macro-life. In addition to unpredictable wave action, they are also subject to the rhythm of the tides. During the spring tides (as

opposed to the neap tides), intertidal animals are left behind by the receding waters, often for periods of several hours. Whether in pools or left high and dry, they are exposed to large changes in their environment. The sun can rapidly heat up small pools in the summer causing oxygen concentrations to plummet, and exposed soft-bodied animals are promptly desiccated. Heat and desiccation can trigger the release of noxious secondary metabolites from algae and sponges into tidepools. During the winter, freezing temperatures and snow can kill exposed animals and plants. Rain can also be a major problem, particularly in the Pacific Northwest as rain can dilute small pools, and run-off streams that flow into the intertidal zone can dilute even large pools. The intertidal zone is a harsh and unforgiving environment. (see Ambler and Chapman, 1950; Morris and Taylor, 1983; Daniel and Boyden, 1974; Truchot and Duhamel-Jouve, 1980)

While the rhythm of the tides is generally predictable, wave action and conditions on emersion can be quite unpredictable. A subtidal animal placed in the intertidal zone will normally succumb rapidly in the severe conditions (Gibson, 1993). Obviously, intertidal animals are adapted to these severe conditions, but even they can experience conditions which they are either quantitatively, or qualitatively not able to cope with, and would thus constitute *transient perturbation factors* (TPFs). Non-intertidal fishes, such as salmonids and pleuronectids show large glucocorticoid increases in response to TPFs (Horn and Gibson, 1988). If an intertidal fish were to express such large glucocorticoid responses to TPFs during

the spring tides, this could lead to greatly increased average glucocorticoid levels (see chapter three). Such chronically elevated levels of glucocorticoids could lead to serious problems for the animal.

In this study, I examined fishes from environments the extremes of the predictability range. *Ascelichthys rhodorus* (Rosy-lipped Sculpin: Cottidae) and *Xiphister atropurpureus* (Black Prickleback: Stichaeidae) live almost exclusively in the intertidal zone, and thus are adapted to a low predictability environment. In these fishes, I expected to find the stress response attenuated, to prevent chronically elevated cortisol levels due to frequent TPF events. These intertidal fishes were compared to three subtidal fishes: *Enophrys bison* (Buffalo Sculpin: Cottidae), *Lepidopsetta bilineata* (Rock Sole: Pleuronectidae), and *Pleuronichthys coenosus* (C-O Sole: Pleuronectidae). At the other end of the predictability range I studied *Astyanax mexicanus* (Blind Cave Tetra: Characidae) which is found in caves in Central America and Southern Mexico that have extremely small fluctuations in temperature and food availability throughout most of the year. *Astyanax* was used to test whether the glucocorticoid response to TPFs in a very predictable environment would be attenuated, since it may not be required in a predictable environment.

Methods

Study Animals:

Wild specimens of the intertidal fishes *Ascelichthys rhodorus* (Rosy-Lip Sculpin) and *Xiphister atropurpureus* (Black Prickleback) were captured on the Olympic Peninsula in the Straits of Juan de Fuca just west of the town of Joyce, Washington (long. 124°0'0" W; lat. 48°18'33" N; See Figure 5.1). Specimens were captured during the years 1986-1994. *Ascelichthys* was caught using bare hands, and *Xiphister* was caught using either bare hands or with the assistance of a net or nylon mesh bag.

Wild specimens of the subtidal fishes were caught using SCUBA and hand nets. *Enophrys bison* (Buffalo Sculpin) were caught at the Edmonds Oil Dock in Edmonds, Washington (long 122°23'29"W; lat 47°48'17"N), just off shore of Marina Beach, and *Lepidopsetta bilineata* (Rock Sole) was caught both at the Edmonds Oil Dock, and at Richmond Beach County Park (long 122°23'2"W; lat 47°45'53"N; See Figure 5.1).

Specimens of tank raised *Astyanax mexicanus* (Blind Cave Tetra) were purchased from a local retail aquarium store, and housed in bare-bottomed 38 or 57 liter aquaria. The aquaria were maintained in a constant temperature environmental chamber at 22°C, with constant dim light. The aquaria were filtered using air driven sponge filters.

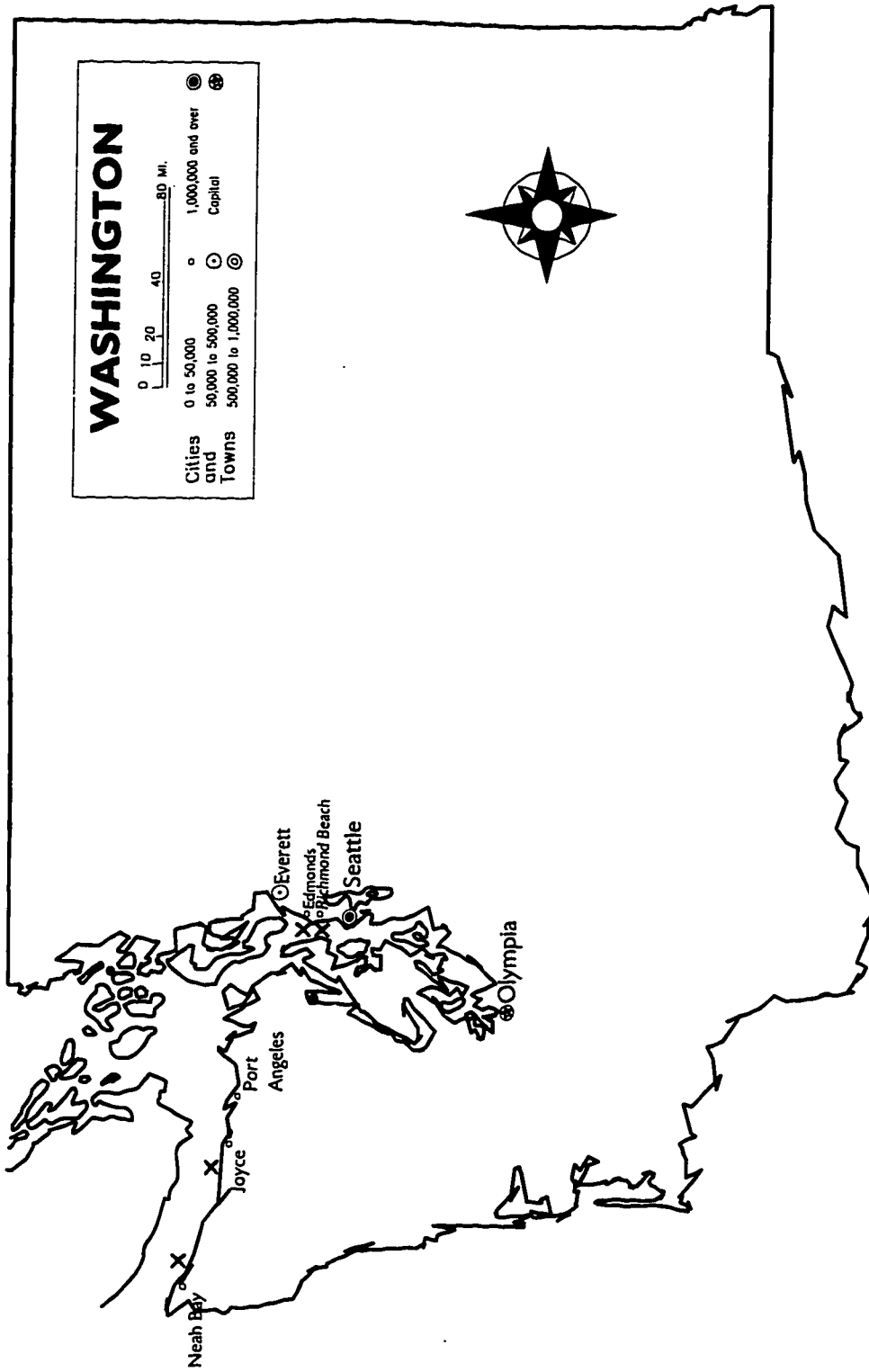


Figure 5.1 - Map of Washington showing study sites. Intertidal fishes were collected near Joyce and Neah Bay, and the subtidal fishes were collected at Edmonds and Richmond Beach.

Blood Sampling:

Bleeding Board: Conditions in the intertidal zone in Washington can be rather wet and windy, particularly during the winter and spring, so steps had to be taken to keep the bleeding equipment in place. A bleeding board was constructed (see figures 5.2, 5.3, and 5.4). The board (Figure 5.3) was constructed of coated 3/4" plywood, with 3 threaded inserts for connecting the elastic hold-down strap and the light arm to the board. The board was cut to fit inside of the backpack used on collecting trips. Cotton applicators were stored in a plastic box, and this box, along with the tubes of capillaries was placed under the elastic hold down strap. The elastic hold down strap also constrained the 27 gauge needles, and forceps to prevent them from blowing away or from being lost down holes between the rocks in the intertidal zone. The light arm (Figure 5.4) was used to illuminate the gill chamber, and greatly facilitated bleeding of the fish, particularly at night.

Mouth pipettor for blood: All blood samples from the fishes were taken with the aid of a mouth pipettor (see figure 5.5). This was necessary since the volume of blood in a sample was often low, and mucus from the gills tended to plug the capillary tubes.

Blood Sample Holder Box: As there is always the danger of falling when walking on slippery intertidal rocks, a method was devised to protect the blood samples from impact damage. A block of foam rubber was cut to shape to fit into a 1 pint freezer storage box, and a series of holes were made in it with a heated

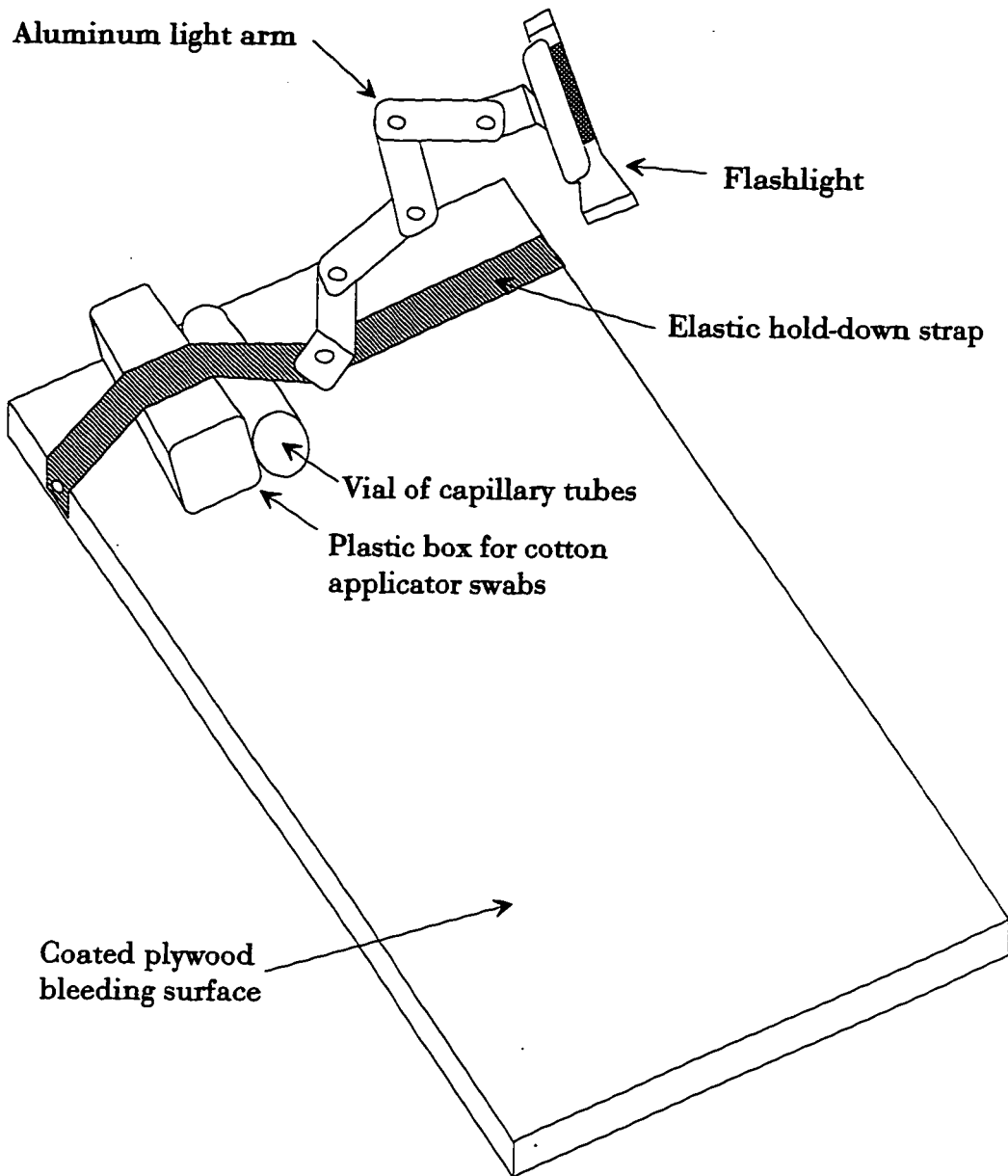


Figure 5.2 - Diagram of assembled fish bleeding board. The elastic hold-down strap keeps the swab box and hypodermic needles from blowing away during windy conditions.

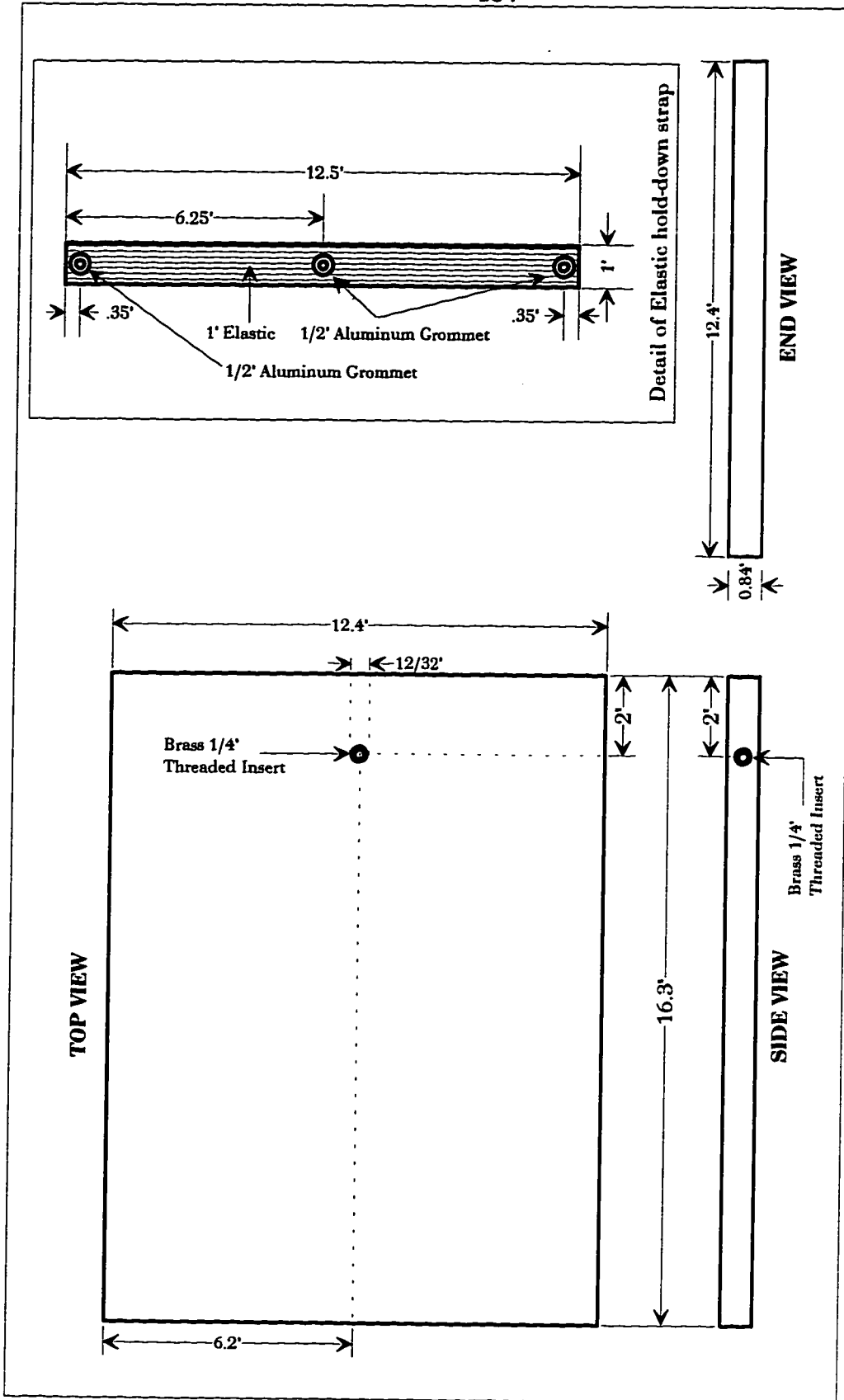


Figure 5.3 - Diagram of Fish bleeding board. The board was cut from a sheet of 3/4' veneered plywood, and had 3 1/4"x20 Brass threaded insert added to position the hold down strap. The hold-down strap, made from a strip of 1' wide elastic with 1/2' aluminum grommets, was held in place by 1/4"x20x3/4' stainless steel machine screws. The center screw was also used to hold the collapsable light holder. The elastic strap held down instruments which might be lost in the tide pools or blown away by the wind.

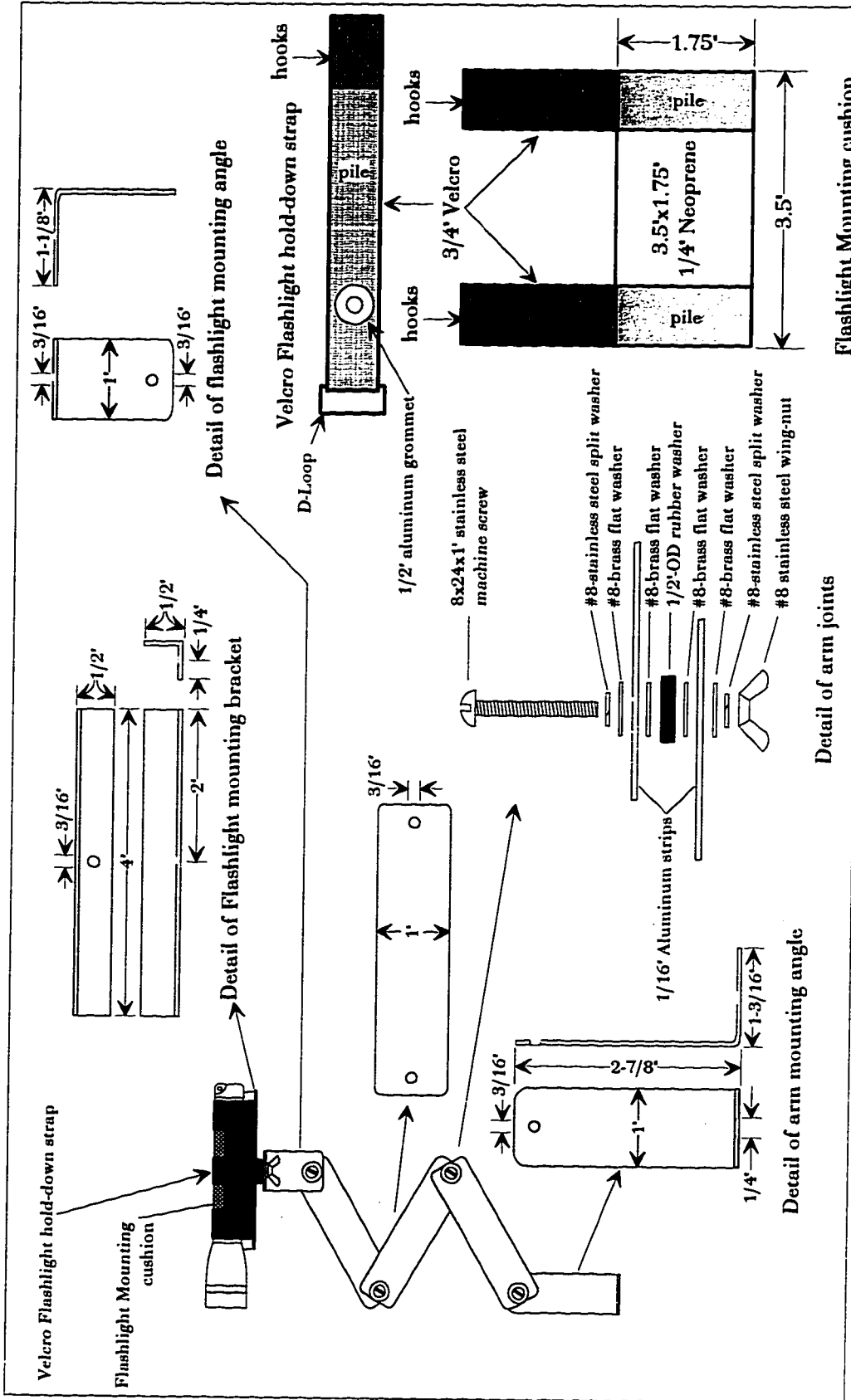


Figure 5.4 - Diagram of bleeding board light arm. The arm was constructed from $1/16'$ x $1'$ aluminum strips. The flashlight was surrounded by a cushion of neoprene, held in place by $3/4'$ velcro. The cushioned flashlight was held firmly to the mounting bracket by a velcro strap

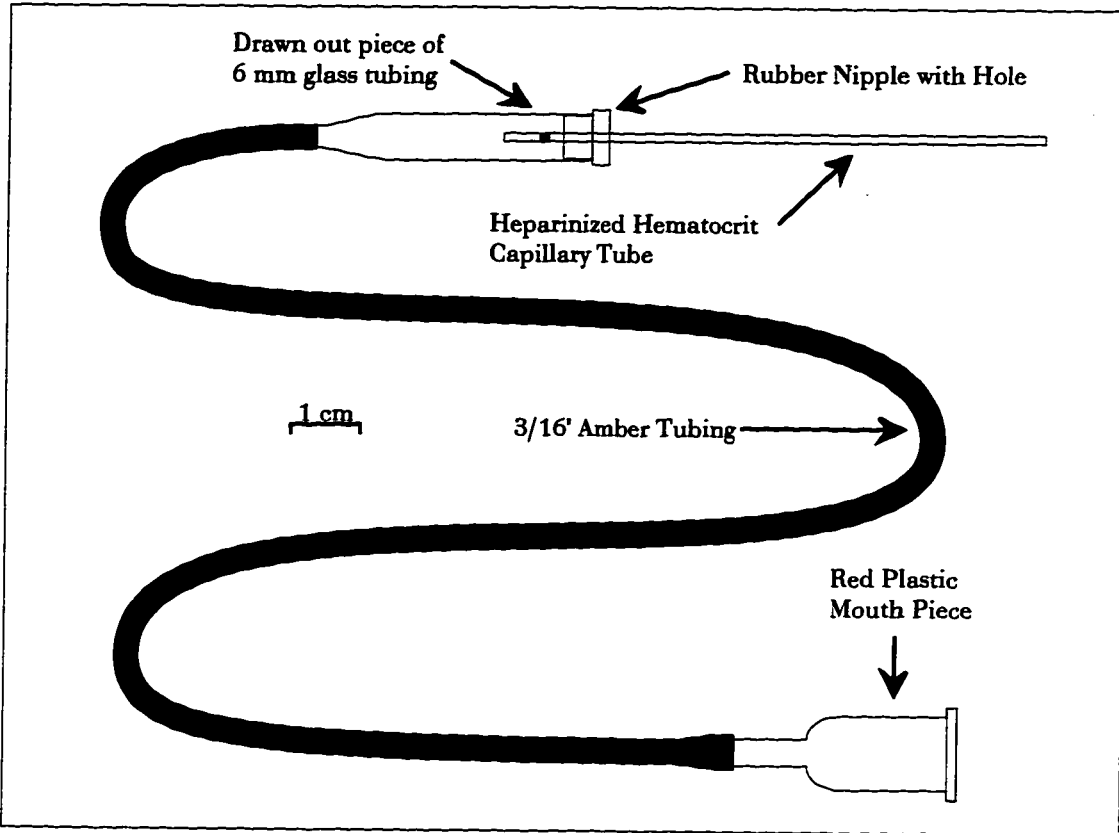


Figure 5.5 - Mouth pipetting apparatus for bleeding fish.

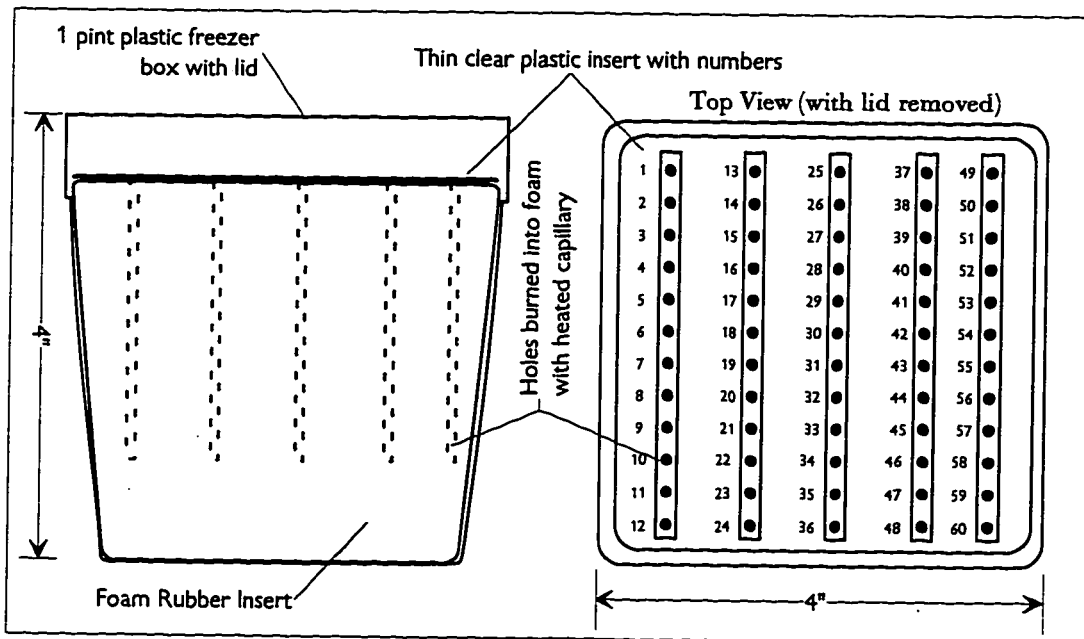


Figure 5.6 - Blood sample holder box. Constructed from a 1 pint white plastic freezer box, with a 3.5" foam insert. A clear plastic insert with numbers, and slots was placed on the foam rubber. A heated capillary burned holes in the foam, making slots for the blood samples.

capillary tube (Figure 5.6). A clear plastic sheet with numbers on it was used to keep track of the capillary tube numbers. Note that the insert and the holes in the foam were made in such a way that the plastic insert could only match the holes one way. Capillary tubes containing blood were sealed with Critoseal, and placed in the numbered holes in the storage box. The storage box was maintained in a small ice chest (with ice) until the samples could be centrifuged.

Ascelichthys rhodorus, *Astyanax mexicanus* and *Enophrys bison*: These fish were bled from either the efferent or afferent branchial arteries as they pass over the gill arches (Figure 5.7). The fish were held in a nylon mesh (1/4" mesh veil material) to prevent the animal from escaping. 6" square pieces of the mesh were wrapped around the body and mouth of the fish, leaving one of the opercular openings exposed. For *Ascelichthys* and *Astyanax*, one person held the fish, and opened the opercular cover with forceps, but for the larger *Enophrys*, this could be done by a single person. The gill chamber was cleaned of mucus and excess water with a cotton-tipped applicator (swab). The branchial artery was punctured with a 27 gauge needle, and the blood was sucked up into a heparinized capillary tube. Gentle mouth suction was applied to a rubber hose attached to the capillary to draw in the small quantities of blood (Figure 5.5).

Lepidopsetta bilineata: These fish remain fairly quiescent when removed from the water. These fish were simply placed on the bleeding board, and the

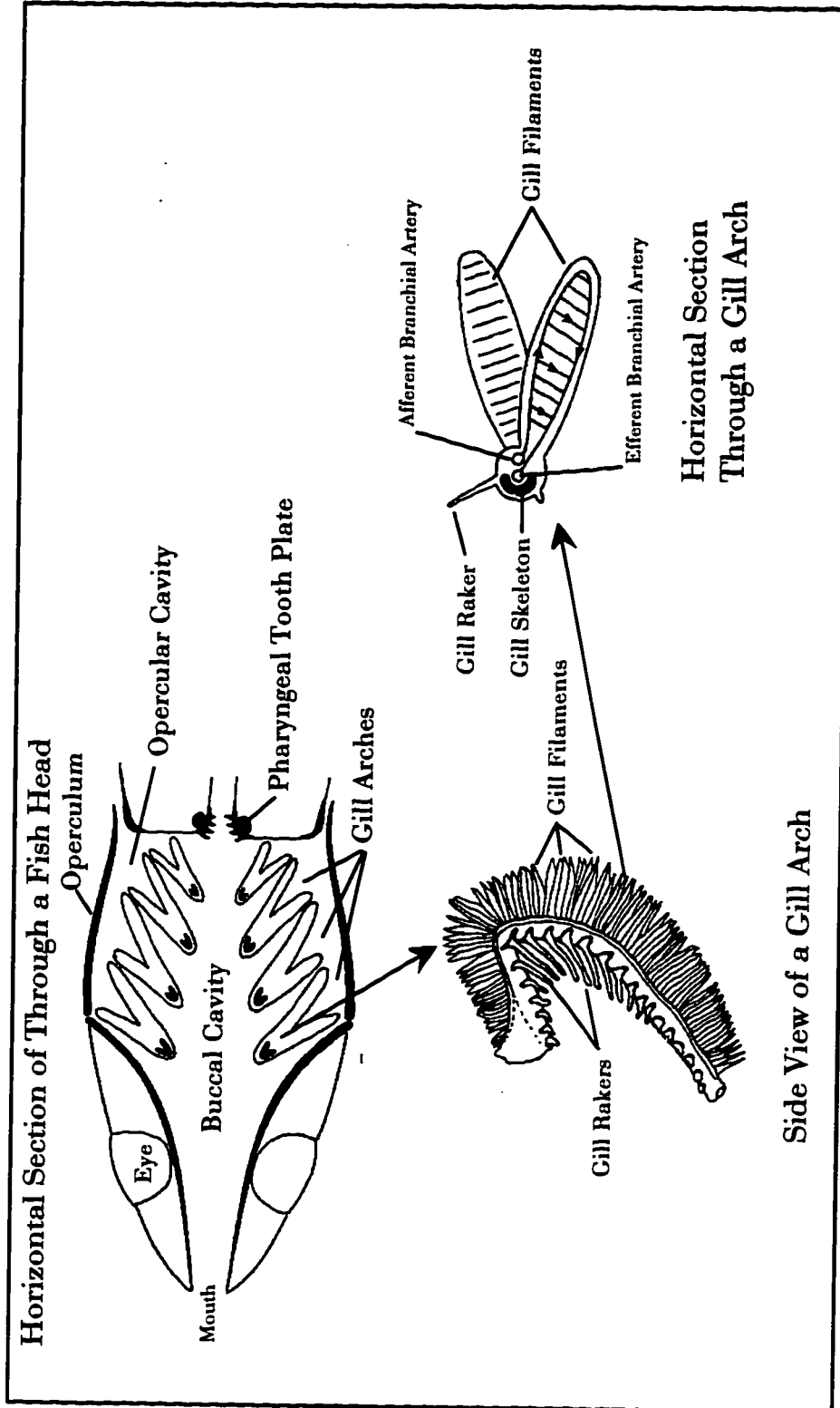


Figure 5.7 - Diagram of the positioning of gills within the head of a fish and details of the location of blood vessels used to take blood samples. Gills are oriented in a more or less vertical fashion within the buccal cavity. To take blood samples from the fish, the operculum is opened using a pair of forceps, and the Afferent or Efferent branchial arteries are punctured, and the blood is collected as it pools on the gill filaments.

opercular cover was lifted with a pair of forceps, and then the sample was taken as above for *Enophrys*.

Xiphister atropurpureus.: These pricklebacks are eel-like and extremely difficult to hold on to, so a holder was designed to make bleeding possible (Figure 5.8). The fish were coaxed into nylon mesh bags for storage, and when it was time to bleed the fish, the front few inches of the fish was allowed to escape the hole in the draw-string of the mesh bag, then the fish was clamped in the restraint device and the elastic straps were locked in place. The tension pressed the foam together with sufficient force to usually hold the fish in place, but not enough force to injure the fish. Once in the restraint device, the operculum could be lifted with forceps, and blood samples could be taken from the gills as above.

Capture Stress:

Fish were maintained in water filled white plastic buckets during the capture stress. The buckets were periodically agitated to prevent the fish from becoming acclimated to the bucket. *Xiphister* and *Ascellichthys* were maintained in nylon mesh bags within the buckets, and *Astyanax* was maintained in plastic bags in the buckets during the capture stress.

Radioimmunoassay of Cortisol

Tritiated-Cortisol Assay (used for *Ascellichthys*, *Astyanax*, *Lepidopsetta*, *Pleuronichthys*, and *Xiphister*): Plasma samples from 5-50 μ l were diluted to 400 μ l

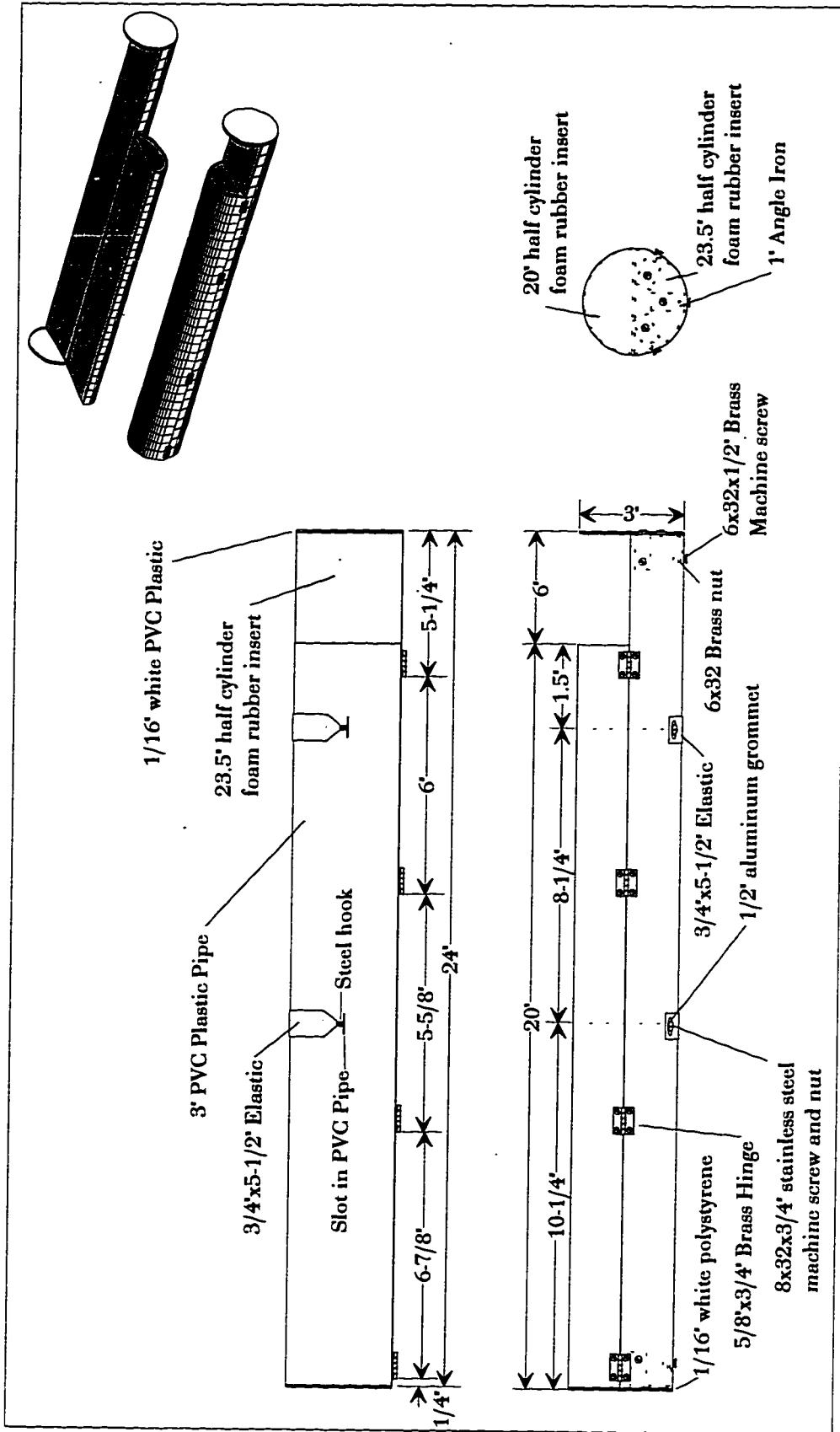


Figure 5.8 - Restraint device for bleeding pricklebacks. The device was constructed from a section of 3' PVC plastic pipe cut in half, and hinged. Two circular end pieces were affixed with glue and reinforced by angle irons to limit the possible motions of the fish. Two 1/2 cylindrical sections of firm foam rubber were fashioned to fit in the pipe, and 2 elastic straps added to apply pressure to the fish when in the holder.

with PBSG in a 15 ml conical centrifuge tube and 20 μ l of labeled corticosterone (~2000 cpm - New England Nuclear) solution was added (to determine recoveries) and the samples were incubated overnight at 4°C. The samples were extracted overnight at 4°C with 5 ml of redistilled dichloromethane. The extracted steroid was transferred in the dichloromethane to test tube, and the dichloromethane was evaporated using a stream of nitrogen while the tubes incubated in a water-bath at 40°C. The steroid was allowed to resuspend in 550 μ l of PBSG overnight at 4°C.

The following day, 100 μ l from each sample was transferred to a scintillation vial to determine recoveries and 4.5 ml of scintillation fluid was added. 200 μ l of each sample was transferred to each of 2 15x100mm disposable test tubes. Each tube was incubated with 100 μ l of anti-cortisol antibody (Cat # 440A - diluted 1:50, Endocrine Sciences, Tarzana, CA), and 100 μ l of tritiated cortisol solution (~10,000 cpm), and incubated overnight at 4°C. Separation of bound from free steroid was achieved by the addition of 0.5 ml of dextran-coated charcoal for 12 minutes at 4°C. All samples were then promptly centrifuged at 2,000 rpm for 10 minutes at 4°C in a Beckmann TJ-6 refrigerated centrifuge. Supernatants (containing bound cpm) were decanted into scintillation vials, 4.5 ml of scintillation cocktail added, and equilibrated overnight before counting in a Beckmann LS3801 system (for 10 min or 2% accuracy).

With each assay, 2 solvent blanks and a standard sample (containing 250 pg of cortisol) were taken through the entire assay procedure as a check on reliability criteria.

Data for the assays was collected from the scintillation counter by an IBM computer and plasma concentrations of cortisol were calculated using the program in Appendix B.

Iodinated-Cortisol Assay (used for *Enophrys*): This assay was done using a Clinical Assays™ GammaCoat™ Cortisol 125I RIA Kit (CA-1549, INCSTAR Corporation, Stillwater, MN) modified to increase sensitivity as follows:

- 1) The standard curve was diluted to the range of 2000 pg-7.8pg.
- 2) The volume used was increased to 25 µl, rather than 10 µl.
- 3) The volume of tracer solution added to each tube was decrease by 50% to 0.5 ml. This made the RIA curve more sensitive at the lower end.
- 4) Samples were briefly centrifuged after mixing to remove tracer/sample stuck to the walls of the tubes. This helped make the replicates more similar.
- 5) Incubation was done at 4°C overnight, rather than at 37°C for 45 minutes. This made a large increase in overall maximum binding (BMAX).
- 6) After incubation, the unbound tracer was removed using a vacuum aspirator and a drawn-out Pasteur pipette. The tubes were then briefly centrifuged to remove unbound tracer stuck to the walls of the tube, and the traces removed

with the vacuum aspirator. This removed most of the variability between replicates.

Statistics

All statistical analysis of the data was done using the computer program SYSTAT (Wilkinson, 1990). TPF responses over time for *Astyanax*, *Ascelichthys* and *Xiphister* were analyzed using one way ANOVAs. Responses to TPFs over time for *Pleuronichthys*, *Lepidopsetta*, and *Enophrys* were examined using one-way repeated measures ANOVAs. Post hoc comparisons between time points were made using routines for this purpose built into SYSTAT.

Results

Intertidal Fishes:

The intertidal sculpin *Ascelichthys rhodorus* showed a significant increase in cortisol in response to capture within 30 minutes (Figure 5.9: $F=19.955$, $df=1$, $P<0.001$) The peak level was reached around 50 minutes after capture, and began declining there after and was back to near basal by 140 minutes, even though the fish were periodically agitated within the bucket. Maximum cortisol levels were around 20 ng/ml, and basal cortisol levels were about 1 ng/ml.

The intertidal stichaeid *Xiphister atropurpureus* showed a similar trend to *A. rhodorus*, but there was no statistically significant effect of time after capture and

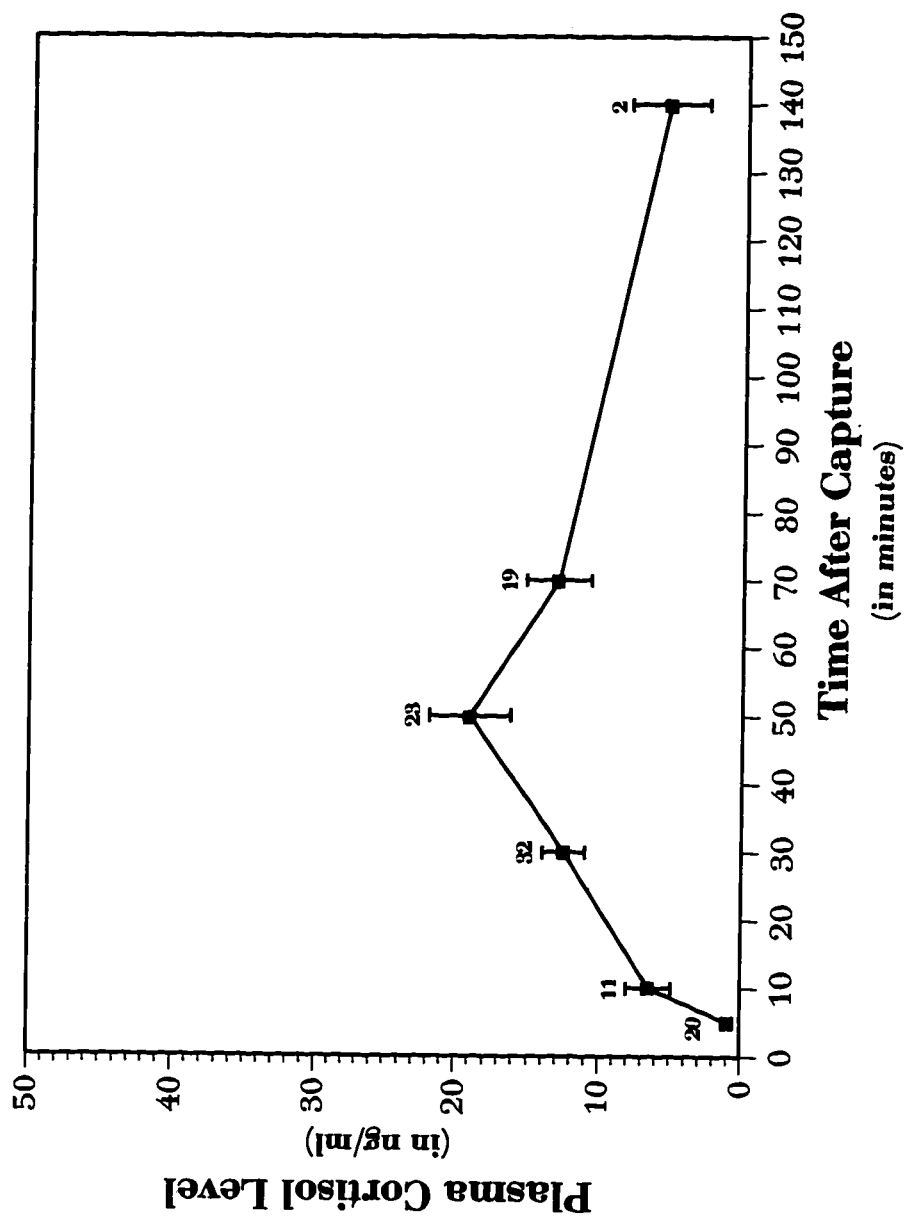


Figure 5.9 - Effect of capture and confinement on plasma cortisol in the intertidal cottid *Ascelichthys rhodorus*. Error bars represent \pm the standard error of the mean

cortisol levels (Figure 5.10: $F=1.806$, $df=4$, $P=0.180$). Maximum levels of cortisol were around 25 ng/ml.

Subtidal Fishes:

Enophrys bison did not show a glucocorticoid response to capture (Figure 5.11: $F=0.788$, $df=2$, $P=0.487$). In fact, there appears to be a trend toward decreasing cortisol levels, rather than increasing. The basal cortisol level was quite high, at around 80 ng/ml.

The pleuronectids *Lepidopsetta bilineata* and *Pleuronichthys coenosus* showed significant increases in plasma cortisol in response to capture (Figure 5.12: $F=26.027$, $df=1$, $P=0.007$ and $F=72.254$, $df=1$, $P=0.014$, respectively). Both had low (<10 ng/ml) basal cortisol concentrations.

Subterranean Fish:

Astyanax mexicanus showed a significant increase in cortisol levels in response to capture (Figure 5.13: $F=24.196$, $df=2$, $P=0.001$). Basal cortisol levels were under 10 ng/ml, and maximum level within the first 35 minutes of capture was >100 ng/ml. This much more similar to the pleuronectids than to that of the intertidal fishes.

Discussion

Both species of subtidal pleuronectids (*Pleuronichthys coenosus* and

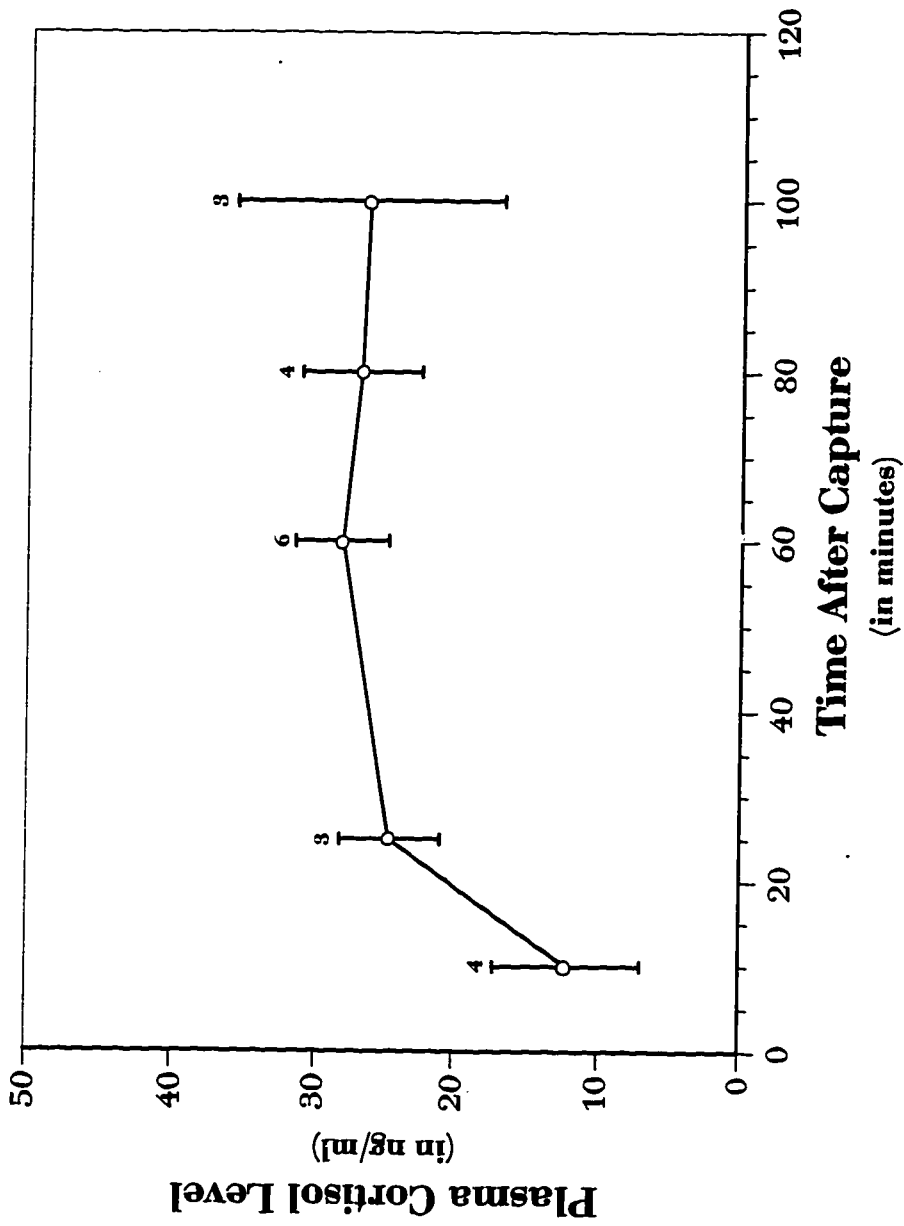


Figure 5.10 - Effect of capture and confinement on plasma cortisol in the intertidal stichaei *Xiphister atropurpureus*. Error bars represent +/- the standard error of the mean

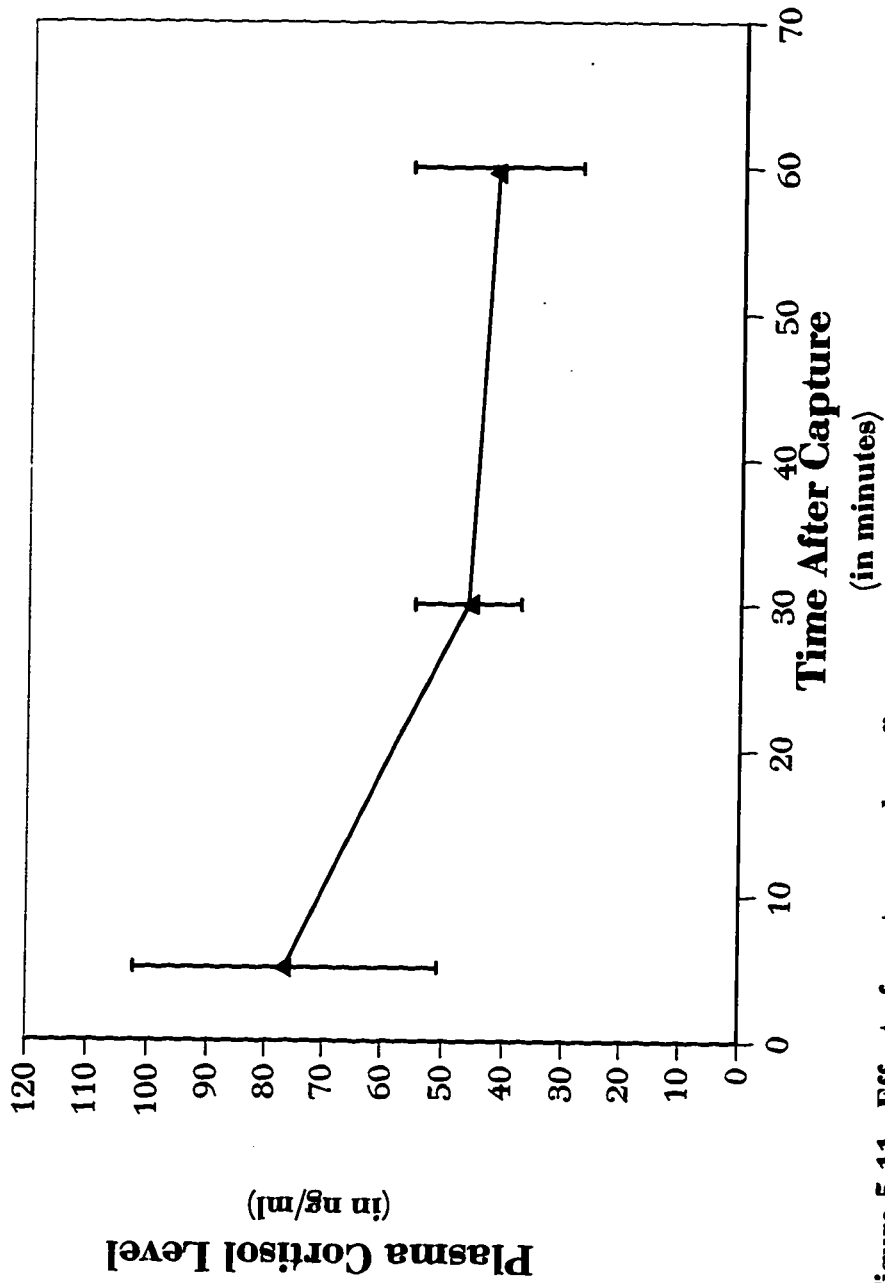


Figure 5.11 - Effect of capture and confinement on plasma cortisol in the subtidal cottid *Enophrys bison*. Each point represents 5 fish. Each fish was bled at each time point. Error bars represent +/- the standard error of the mean

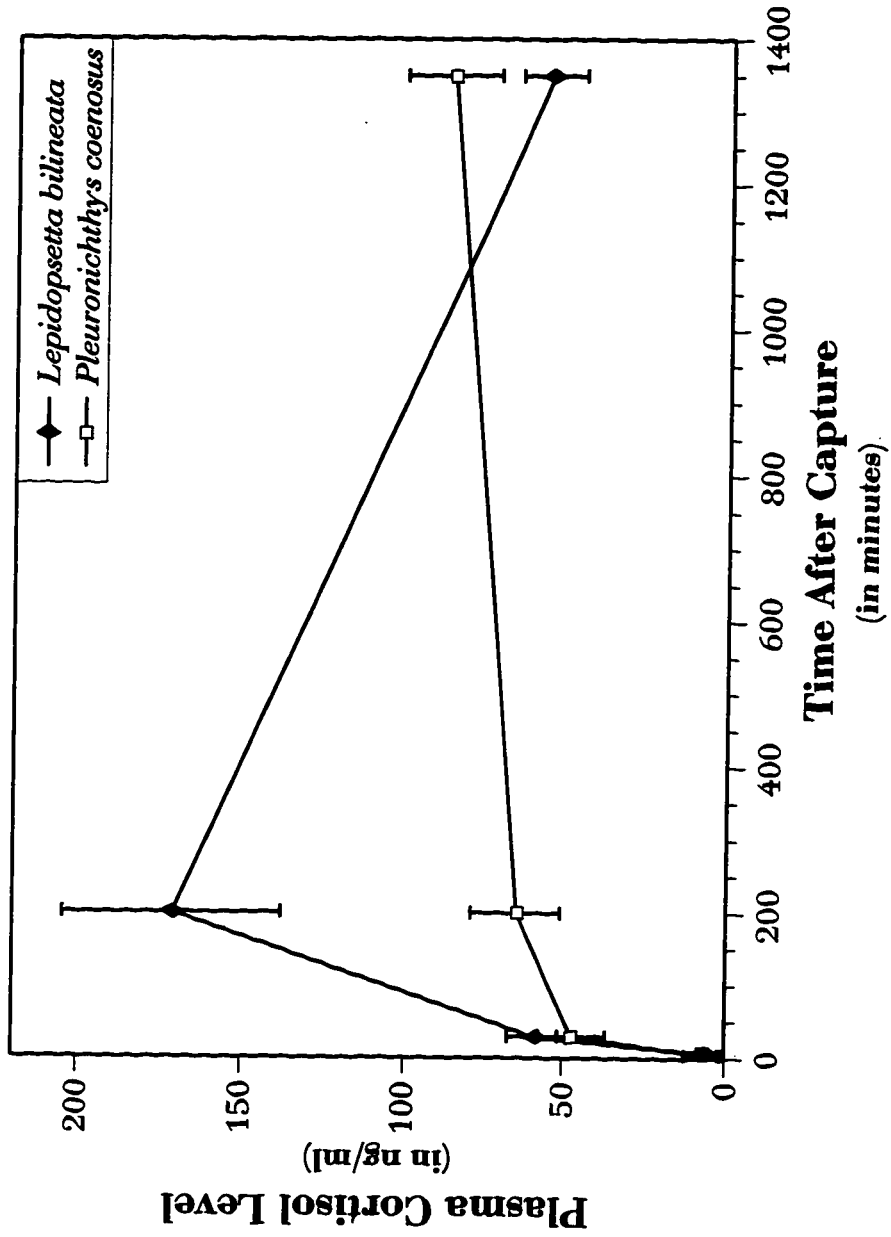


Figure 5.12 - Effect of capture and confinement on plasma cortisol in 2 subtidal pleuronectids. Each fish was bled at every time point. For *Pleuronichthys coenosus*, n=3 and for *Lepidopsetta bilineata*, n=5. Error bars represent +/- the standard error of the mean

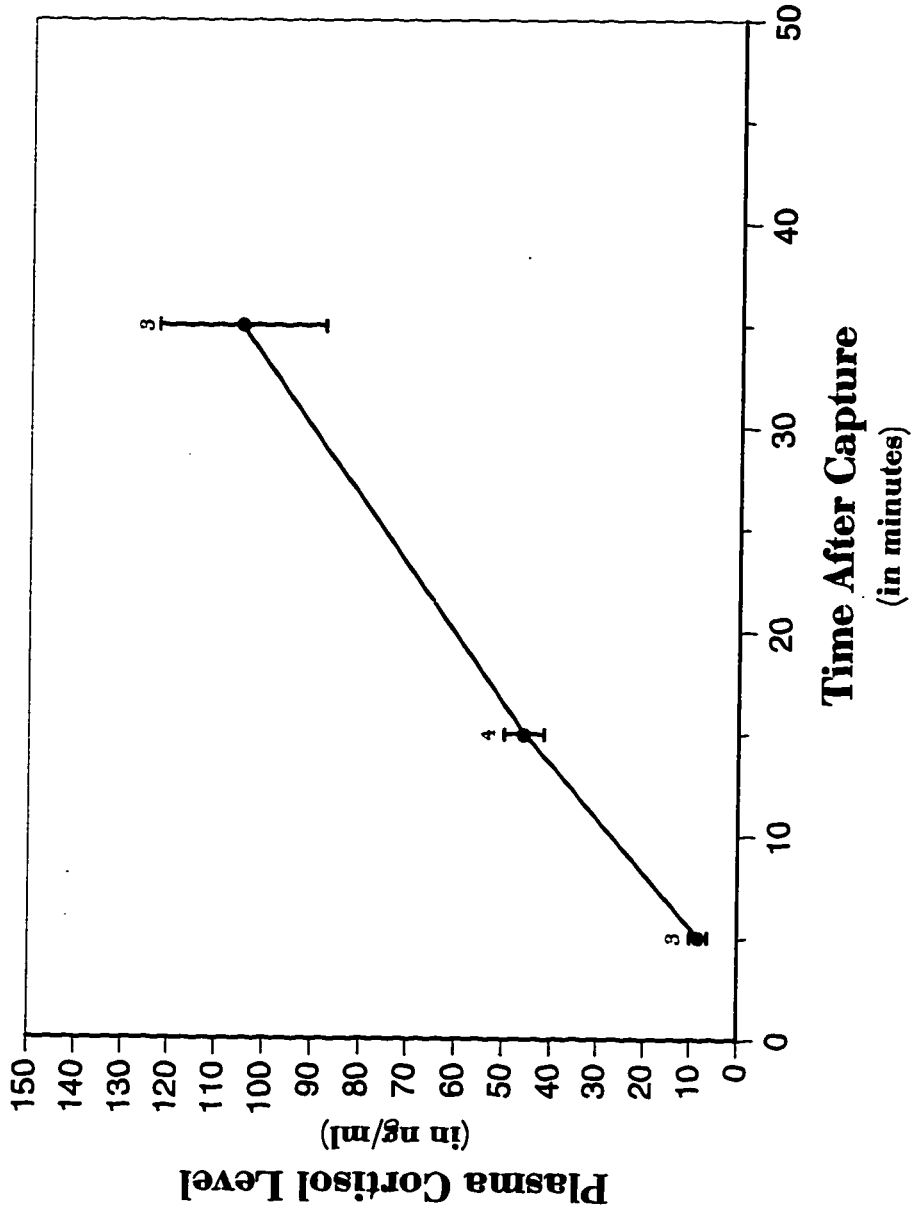


Figure 5.13 - Effect of capture and confinement on plasma cortisol in a cave living characid *Astyanax mexicanus*. Error bars represent \pm the standard error of the mean

Lepidopsetta bilineata) showed well developed adrenocortical responses in response to capture/confinement as have been seen in other pleuronectids (Wingfield, 1973). Basal cortisol levels for both pleuronectids was low as is normal in most animals. Unfortunately, there are apparently no intertidal pleuronectids, so it was not possible to compare this response to an intertidal form in the same family.

Both intertidal fishes examined (*Xiphister atropurpureus*: Stichaeidae and *Ascellichthys rhodorus*: Cottidae) showed significant responses to capture/confinement, but these levels were less than 50% of the maximal levels obtained in the pleuronectids (see figure 5.14). The response of *Ascellichthys* to the TPF decayed very rapidly, reaching its maximum at approximately 50 minutes, and reaching basal by 140 minutes after capture. In other fishes, elevated cortisol levels persist for hours after capture (Pickering and Pottinger, 1989), and if maintained in over-crowded or otherwise poor conditions, elevated cortisol levels can persist for at least 25 days (Pickering and Stewart, 1984) and possibly up to 6 months (Tam *et al.*, 1987). It is possible that since *Ascellichthys* normally is found in small pools under rocks in the intertidal zone that confinement in nylon bags within a bucket of water is not perceived as a TPF. Attempts were made to disturb the fish within the bucket by periodically hitting the bucket, and stirring the fish about, but this may not have been enough to maintain stimulation of the adrenocortical response. It should be noted that in the *Ascellichthys* that were serially bled (and thus handled far more), there was also only an attenuated

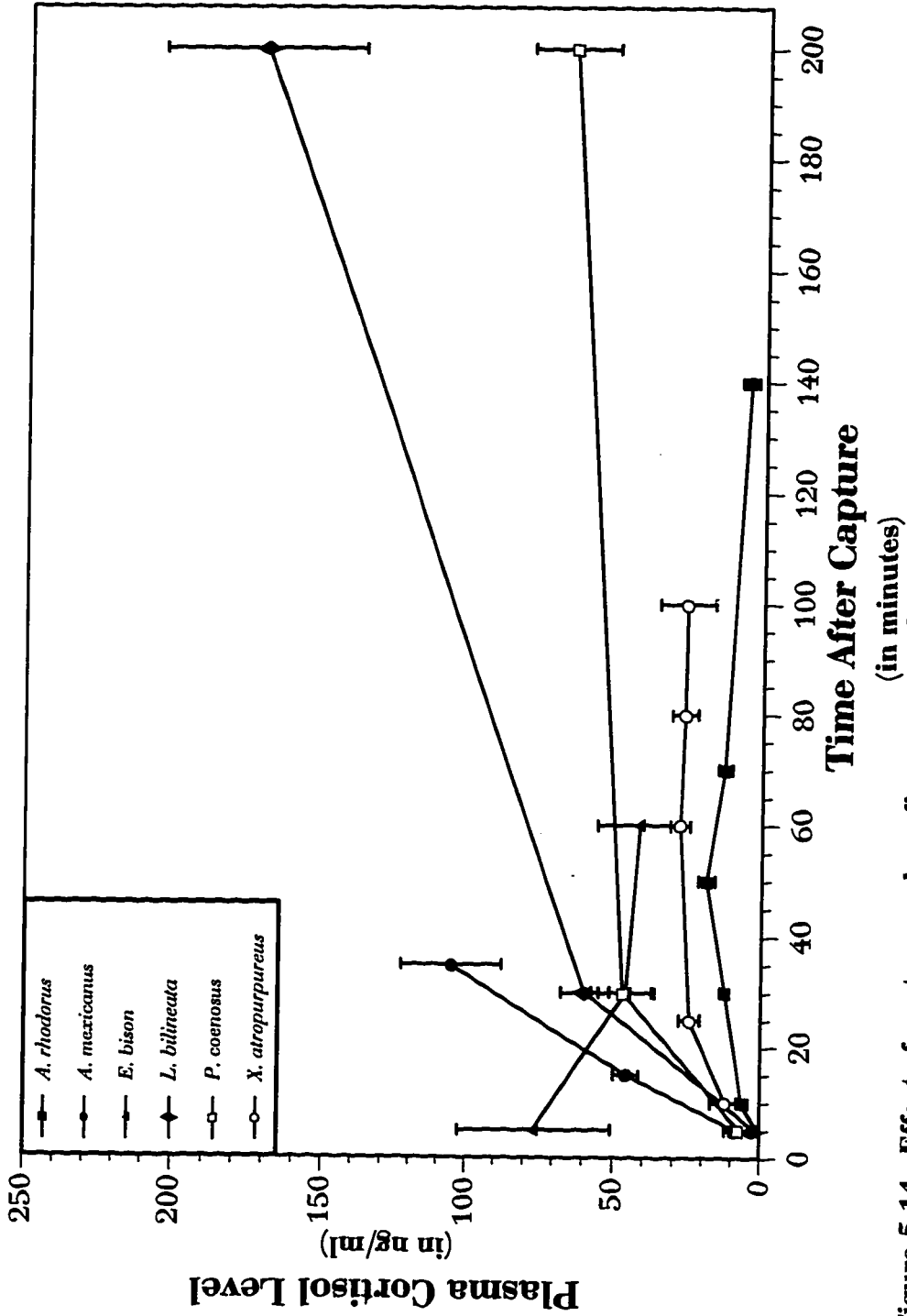


Figure 5.14 - Effect of capture and confinement on plasma cortisol in several fishes. Error bars represent +/- the standard error of the mean.

response to capture/confinement (personal observation), so additional handling does not increase the magnitude or duration of the response.

The adrenocortical response of the intertidal pricklyback *Xiphister atropurpureus*, while significant, was of a very low amplitude (approximately a 2 fold increase over the <10 minute sample). A similar attenuation of the response to TPFs is seen during the breeding in birds breeding in the Arctic (Wingfield *et al.*, 1994a, 1994b), and also birds breeding in the Sonoran Desert (Wingfield *et al.*, 1992). While the attenuation of the adrenocortical responses in these birds occurred while breeding, the attenuation in *Xiphister* was not due to breeding constraints, as samples were not collected from breeding fish (*Xiphister* breeds in the early spring, and males guard and "fan" a ball of eggs in the intertidal zone).

Surprisingly, the subtidal sculpin *Enophrys bison* did not show a response to capture/confinement. They also had remarkably high basal cortisol levels which were larger than the maximum levels for both intertidal fishes, and for *Pleuronichthys*. This elevated basal cortisol level may be in response to environmental pollutants, as the fish were collected near an abandoned commercial oil dock. While possible, this is not likely, as pleuronectids caught in the same area had low basal cortisol levels. Also the pilings of the oil dock have a health and diverse community of both invertebrates and fishes (personal observation). The lack of adrenocortical response to capture/confinement has been indicated by preliminary data from two other subtidal sculpins (*Myxocephalus*

polyacanthocephalus and *Hemilepidotus hemilepidotus*, unpublished data). While the lack of response of the subtidal sculpins was not expected, there may be an evolutionary explanation for this lack of response. Both the subtidal and intertidal sculpins show a number of adaptations useful for intertidal life (such as reduced numbers of scales to increase transdermal respiration) so it is possible that this group evolved in the unpredictable intertidal zone where the adrenocortical response became attenuated, and later expanded out into the subtidal zone, and never regained the responsiveness to TPFs. It is also possible that these fish are not secreting cortisol in response to TPFs. Many other vertebrates secrete corticosterone, and it may be possible that corticosterone or some other adrenal steroid is being released. Further investigations into the steroid biochemistry of Cottids need to be done to answer this question.

The cave fish *Astyanax mexicanus* show the most rapid increase in cortisol of any of the fishes examined. Part of this increase was undoubtedly due to the higher temperature of the fish (20°C for *Astyanax* vs. approximately 10°C for the marine fishes.) It may also be due to sensitization of the cave fishes to TPFs, as the caves they are adapted to may be very predictable with limited numbers of TPFs. There is ample evidence that exposure to noxious stimuli can lead to reduced overall response to TPFs, even to TPFs different than those they have become acclimated to (e.g., Camp and Robinson, 1988).

Both the intertidal fishes showed the expected limited adrenocortical responses for animals living in a relatively unpredictable environment (see chapter 2). Unfortunately, it was not possible to obtain samples from either a subtidal Stichaeid or an intertidal pleuronectid. The subtidal cottid did not respond as expected, but this may be due to this family having evolved in the intertidal zone.

The Cave fish *Astyanax* did not show the expected attenuation of the adrenocortical response for a fish evolving in a very predictable environment. As these fish have only recently entered caves and have an epigeal form in the same species, they may not have adapted to cave conditions enough to show an adrenocortical attenuation. Further investigations into more troglodytic fishes such as *Ambylopsis*, as well as getting samples from the epigeal form of *Astyanax mexicanus*. It would also be interesting to investigate the responses of some of the troglodytic salamanders found in caves of the south eastern United States.

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APPENDIX A. - PREDICT: A program to calculate the predictability of an environment, and the portions of that predictability due to constancy and contingency.

1. General Description

This program calculates the predictability for an environment and the two components of predictability, constancy and contingency, using the methods of Colwell (1974). It was designed to compile on Macintoshes (using Think-C), IBM-PCs (using Borland-C), and UNIX (using GNU-C) machines. Data sets can either be entered line-by-line from as the program prompts (this is the only way on the MAC), or a filename can be supplied on the command line. The data is entered with data separated by commas, with the time intervals in columns, and the classes in rows.

2. Source Listing

```
/*
 *-----*
 | This program determines the Predictability, Constancy and |
 | Contingency of Periodic Phenomena as described by Robert Colwell |
 | in Ecology 55:1148-1153(1974). |
 | |
 | This version is designed to compile under DOS, UNIX, and on a |
 | Macintosh using Think-C. The use of K & R procedure notation |
 | was required for the old version of the Think-C compiler. |
 *-----*
*/
#include <stdio.h>
#include <ctype.h>
#include <math.h>

#ifdef __MSDOS__
/* We are on a real computer. */
/* DOS lives!! */
# include <stdlib.h>
# include <process.h>
# include <alloc.h>
# include <string.h>
# else
# ifdef unix || __unix__
/* We are on a unix machine. */
# include <stdlib.h>
# include <string.h>
# else
# define __MAC__
/* Not a real computer, so */
/* assume it is a Macintosh. */
# include <storage.h>
# include <unix.h>
# include <strings.h>
# endif
#endif

/* *-----* */
/* | Define Constants | */
/* *-----* */
#define TRUE 1
#define FALSE 0
#define EOD 0x1A
```

```

/* ----- */
/* | Macros | */
/* ----- */
#define IS_NUMERIC(c) (((c)>'0' && (c)<'9' ? 1:0) + ((char)(c) == ':' ? 1:0))
#define IS_NOT_ZERO(c) (((c) > 1) || (int)( 100000.0 * (c))) ? TRUE : FALSE)

/* ----- */
/* | Subroutine Prototypes | */
/* ----- */
int    getdata( void );
int    finished( char * );
float * tokenize( char * );
void   calc_predictability( void );

/* +-----+ */
/* | Global variables | */
/* +-----+ */
float **N;
float X[100], Y[100];
int    States = 1;
int    Times_Per_Cycle;
int    Times = 0;
int    FLOAT_POINTER_SIZE = sizeof( float * );
char   *version="Version 2.0\n\n";
char   *copyright="Nudibranch Central Software 1993-1996 (Jerry Jacobs)";

#ifdef __MAC__
int
main() {
  #else
int
main( int argc, char*argv[] ) {
  #endif
    int done=FALSE;

    puts("Colwell Predictability Model");
    puts( version );
    puts("Nudibranch Central Software 1993-1996\n");

#ifdef __MAC__
    switch ( argc ) {
      case 1:
        #endif
          while ( !done ) {
            done = getdata();
            if ( !done ) {
              calc_predictability();
              puts("Press ENTER to continue.");
              (void)getc( stdin );
              puts("\n");
            }
          }
#ifdef __MAC__
        break;
      case 2:
        if ( read_data( argv[1] ) )
          calc_predictability();
        break;
    }
#endif
}

```

```

    default:
        puts( 'Too many command line arguments.' );
        puts( 'SYNTAX - predict' );
        puts( '      or predict filename' );
        puts( "\n\n" );
        return 1;
    }
#endif
puts( "Done.\n" );
return(0);
}

void
calc_predictability( ) {
    int i, j, k;
    float HX=0, HY=0;
    float HXY=0;
    float Z=0;
    float *_STATE_;
    float ConditionalUncertainty;
    float Predictability;
    float C, M, Gm, Gc, Gp;
    float S;
    char answer[10];
    char filename[80];
    char tofile=FALSE;
    FILE *output, *stream;
    char fileopen;

    S = States;
    /* ----- */
    /* | Initialize Arrays. | */
    /* ----- */
    for (j=0; j<Times; j++){
        X[j] = 0.0;
    }
    for (i=0; i<States; i++){
        Y[i] = 0.0;
    }
    /* ----- */
    /* | Determine Column Totals and Grand Total | */
    /* ----- */
    for (j=0; j<Times; j++){
        for (i=0; i<States; i++){
            _STATE_ = N[i];
            _STATE_++;
            X[j] = X[j] + _STATE_[j];
        }
        Z = Z + X[j];
    }
    /* ----- */
    /* | Determine Row Totals | */
    /* ----- */
    for (i=0; i<States; i++){
        _STATE_ = N[i];
        _STATE_++;
        for (j=0; j<Times; j++){
            Y[i] = Y[i] + _STATE_[j];
        }
    }
}

```

```

/* ----- */
/* | Determine Uncertainty with respect to time | */
/* ----- */
for (j=0; j<Times; j++){
    if ( IS_NOT_ZERO( X[j] ))
        HX - HX - ( X[j]/Z * log( X[j] / Z );
}
/* ----- */
/* | Determine Uncertainty with respect to state. | */
/* ----- */
for (i=0; i<States; i++){
    if ( IS_NOT_ZERO( Y[i] ))
        HY - HY - ( Y[i]/Z * log( Y[i] / Z );
}
/* ----- */
/* | Determine Uncertainty with respect to interaction of time | */
/* | and state. | */
/* ----- */
for (j=0; j<Times; j++){
    for (i=0; i<States; i++){
        _STATE_ = N[i];
        _STATE_++;
        if ( IS_NOT_ZERO( _STATE_[j] )){
            HXY - HXY - ( _STATE_[j]/Z * log( _STATE_[j] / Z );
        }
    }
}

ConditionalUncertainty = HXY - HX;
Predictability = 1 - ( ConditionalUncertainty / log( S );

/* ----- */
/* | Calculate Constancy | */
/* ----- */
C = 1 - ( HY / log( S );
/* ----- */
/* | Determine Contingency | */
/* ----- */
M = (HX + HY - HXY) / log( S );

printf( 'Send output to a file (as well as the screen)? ' );
gets( answer );
if ( strlen( answer ) != 0 )
    {
        if ( (*answer == 'Y') || (*answer == 'y') )
            {
                fileopen = FALSE;
                while ( !fileopen ) {
                    printf( 'Filename: ' );
                    gets( filename );
                    if ( (output=fopen( filename, 'r' )) != NULL ) {
                        fclose( output );
                        printf( 'File already exists. Overwrite? ' );
                        gets( answer );
                        if ( strlen( answer ) == 0 )
                            continue;
                        if ( (*answer == 'n') || (*answer == 'N') )
                            continue;
                    }
                    if ( (output=fopen( filename, 'w' )) == NULL ) {
                        printf( 'Error trying to open output file %s.\n', filename );
                    }
                }
            }
    }

```

```

        exit(1);
    }
    tofile = TRUE;
    fileopen = TRUE;
}
tofile = TRUE;
}
else
    tofile = FALSE;
}
else
    tofile = FALSE;
Gm = 2 * Z * (HX + HY + HXY);
Gc = 2 * Z * (log(S) - HY);
Gp = Gm + Gc;
for (k=0; k<1+tofile; k++) {
    if ( tofile && k==1 )
        stream = output;
    else
        stream = stdout;
    if (Times > 15)
        fprintf( stream, 'Data matrix too large to display. \n' );
    else
    {
        fprintf( stream, 'Input Data:\n' );
        fprintf( stream, '-----\n' );
        for (i=0; i<States; i++) {
            _STATE_ = N[i];
            for (j=0; j<Times; j++) {
                fprintf( stream, '%5.1f', _STATE_[j+1] );
            }
            fprintf( stream, '\n' );
        }
        fprintf( stream, '\n' );
        fprintf( stream, 'H(XY) = %11.5f\n', HXY);
        fprintf( stream, 'H(X) = %11.5f\n', HX);
        fprintf( stream, 'H(Y) = %11.5f\n', HY);
        fprintf( stream, 'Log(s) = %11.5f\n', log(S) );
        fprintf( stream, 'Grand Total (Z) = %7.1f\n', Z );
        fprintf( stream, 'Predictability = %9.5f\n', fabs( Predictability ) );
        fprintf( stream, 'Constancy (C) = %9.5f\n', fabs( C ) );
        fprintf( stream, 'Contingency (M) = %9.5f\n', fabs( M ) );
        fprintf( stream, '\n' );
        fprintf( stream, 'G statistics for:\n' );
        fprintf( stream, 'Contingency (Gm) = %11.5f\n', ( IS_NOT_ZERO(Gm)? Gm : 0.0 ) );
        fprintf( stream, 'Constancy (Gc) = %11.5f\n', ( IS_NOT_ZERO(Gc)? Gc : 0.0 ) );
        fprintf( stream, 'Predictability (Gp) = %11.5f\n', ( IS_NOT_ZERO(Gp)? Gp : 0.0 ) );
        fprintf( stream, '\n' );
        if (k == 1)
            fclose( output );
    }
}
return;
}

/* Read Data from a File */
int
read_data( filename )
char *filename;
{
    char input[255];

```

```

int T,Max_Times=0;
float *tokenized;
int first=TRUE;
FILE *dataFile;

States = 1;
Times = 0;
T = 0;
if ((dataFile = fopen( filename, 'r' )) == NULL) {
    printf( 'Unable to open data file: %s\n', filename );
    return 0;
}

while ( fgets(input, 254, dataFile) != NULL ) {
    tokenized = tokenize( input );
    if ( !finished( input ) ) {
        T = tokenized[0];
        if (((T>Max_Times) && !first) || T<Max_Times) {
            if ( T<Max_Times )
                printf( '\a--Error - This line has less times!\n' );
            else
                printf( '\a--Error - This line has more times!\n' );
        }
        else {
            if ( first ) {
                if ( ( N = (float **) malloc( FLOAT_POINTER_SIZE ) ) == NULL ) {
                    printf( 'Error allocating memory\n' );
                    exit (1);
                }
                Max_Times = T;
                first = FALSE;
            }
            else {
                if ( ( N = (float **) realloc( N, FLOAT_POINTER_SIZE * (States + 1) ) ) == NULL ) {
                    printf( 'Error reallocating memory\n' );
                    exit (1);
                }
            }
            N[States-1]=tokenized;
            States++;
        }
    }
} /* Done getting input */
States--;
Times = T;
if ( (States == 0) || (Times == 0) )
    return (0);
else
    return (1);
}

int
getdata() {
    char input[255];
    int done=FALSE;
    int T,Max_Times=0;
    float *tokenized;
    int first=TRUE;
    static char border[67];

```

```

printf( "\n" );
printf( "Enter the rows of the matrix with each datum separated by a ' ' );
printf( 'comma.\n' );
printf( "When done, leave a blank line.\n" );
memset( border, '-', 66 );
border[65] = 0;
printf( "%s\n", border );
printf( "\n" );

States = 1;
Times = 0;
T = 0;

while ( !done )
    tokenized = 0;
    while ( !tokenized )
        printf( "State %2.2u: ", States );
        gets( input );
        tokenized = tokenize( input );
    }
    if ( finished( input ) )
        done = TRUE;
    else
        {
            T = tokenized[0];
            if ( ( ( T > Max_Times ) && !first ) || T < Max_Times )
                {
                    if ( T < Max_Times )
                        printf( "\a—Error - This line has less times!\n" );
                    else
                        printf( "\a—Error - This line has more times!\n" );
                }
            else
                {
                    if ( first )
                        {
                            if ( ( N = (float **) malloc( FLOAT_POINTER_SIZE ) ) == NULL )
                                {
                                    printf( "Error allocating memory\n" );
                                    exit( 1 );
                                }
                            Max_Times = T;
                            first = FALSE;
                        }
                    else
                        {
                            if ( ( N = (float **) realloc( N, FLOAT_POINTER_SIZE * ( States + 1 ) ) ) ==
                                NULL )
                                {
                                    printf( "Error reallocating memory\n" );
                                    exit( 1 );
                                }
                        }
                    N[States-1] = tokenized;
                    States++;
                }
        }
}
/* Done getting input */
States--;
Times = T;

```

```

    if ( (States == 0) || (Times == 0) )
        return (TRUE);
    else
        return (FALSE);
}

int
finished( input )
    char *input;
{
    if ( *input == EOD )
        return ( TRUE );
    else
        return ( FALSE );
}

char drbottom[]={ 0x0A, 0x44, 0x72, 0x2E, 0x20, 0x47, 0x2E, 0x4A,
                  0x2E, 0x20, 0x4B, 0x65, 0x6E, 0x61, 0x67, 0x79,
                  0x20, 0x69, 0x73, 0x20, 0x61, 0x20, 0x68, 0x6F,
                  0x72, 0x73, 0x65, 0x27, 0x73, 0x20, 0x61, 0x73,
                  0x73, 0x21, 0x21, 0x21, 0x0A, 0x00 };

float *
tokenize( input )
    char *input;
{
    char *token, *next_token;
    float token_count=0;
    int t_count;
    char *begin;
    float *tokenized;
    int i;
    int length;
    char scratch[255];

    strcpy( scratch, input );
    if ( strlen( input ) == 0 ){
        *input=EOD;
        return( (float *)EOD );
    }
    begin = input;
    /* ----- */
    /* | Search for first digit | */
    /* ----- */
    for (i=0; i<strlen(input); i++){
        if ( IS_NUMERIC( *begin ) )
            break;
        begin++;
    }
    /* ----- */
    /* | If no digits found signal done | */
    /* ----- */
    if ( i == strlen( input ) ){
        *input=EOD;
        return( (float *)EOD );
    }
    token = begin;
    /* ----- */
    /* | Search for commas. | */
    /* ----- */
    while ((next_token = strchr(token, ',')) != NULL) {

```

```

token_count++;
/* ----- */
/* | Put a 0 at end of number. | */
/* ----- */
for (i=0; i<strlen(token); i++){
    if (!isdigit( token[i] ) && token[i] != '.'){
        token[i] = 0;
        break;
    }
}
token = next_token;
token++;
/* ----- */
/* | Move to the start of the next token | */
/* ----- */
length = strlen(token);
for (i=0; i<length; i++){
    if ( *token == 0 ){
        printf( " Error in input.\n" );
        printf( " [1]\n" );
        return ( NULL );
    }
    if ( IS_NUMERIC( *token ) )
        break;
    token++;
}
}
/* ----- */
/* | Search for beginning of last token | */
/* ----- */
for (i=0; i<strlen(token); i++){
    if ( !IS_NUMERIC( token[i] ) ) {
        token[i] = 0;
        break;
    }
}
token_count++;
t_count = token_count + 1;
if ((tokenized = (float *)malloc( ( t_count ) * sizeof( float ) )) == NULL ){
    printf( "\nMemory allocation error.\n" );
    exit (255);
}
tokenized[0] = token_count;
token_count=0;

begin = scratch ;
/* ----- */
/* | Search for first digit | */
/* ----- */
for (i=0; i<strlen(scratch); i++){
    if ( IS_NUMERIC( *begin ) )
        break;
    begin++;
}
token = begin;

/* ----- */
/* | Search for commas. | */
/* ----- */
while ((next_token = strchr(token, ',')) != NULL ) {

```


Data file used as input for PREDICT.EXE using the above data:

```

0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
0,0,0,0,0,0,1,0,3,2,0,0
6,5,4,6,1,4,4,10,7,6,7,3
4,5,6,4,9,6,5,0,0,2,3,7
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
    
```

4. Sample Output

Colwell Predictability Model
Version 2.0

Nudibranch Central Software 1993-1996

Send output to a file (as well as the screen)? n

Input Data:

| | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6.0 | 5.0 | 4.0 | 6.0 | 1.0 | 4.0 | 4.0 | 10.0 | 7.0 | 6.0 | 7.0 | 3.0 | 3.0 |
| 4.0 | 5.0 | 6.0 | 4.0 | 9.0 | 6.0 | 5.0 | 0.0 | 0.0 | 2.0 | 3.0 | 7.0 | 7.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

```

H(XY) - 3.10461
H(X) - 2.48491
H(Y) - 0.85173
Log(s) - 2.48491
Grand Total (Z) - 120.0
Predictability - 0.75061
Constancy (C) - 0.65724
Contingency (M) - 0.09337
    
```

```

G statistics for:
Contingency (Gm) - 1545.90063
Constancy (Gc) - 391.96188
Predictability (Gp) - 1937.86255
    
```

Done.

APPENDIX B. Radioimmunoassay Calculation Program

A. General Description

The RIA calculation program is used to capture data from a Beckman LC3801 and perform calculations on the data to determine hormone concentrations in blood samples. The scintillation counter calculates the actual raw dose, and the program integrates recoveries and plasma volumes to determine final plasma concentrations.

B. Shared Procedures (RIALIB.LIB)

1. Header Files

a. Header file for Lotus 1-2-3 reading procedures.

```
/*-----*/
/* | Contains constants for getting information from Lotus |
/* | 1-2-3 files. |
/*-----*/
#ifndef _123_
#define _123_

#define FILE_START 0
#define BLANK_CELL 12
#define INTEGER 13
#define REAL_NUMBER 14
#define LABEL 15
#define FORMULA 16
#define READERROR -1

extern char *datapath;
#endif
```

b. Header file for RIA Library.

```
/*-----*/
/* | FILE: RIALIB.H |
/* | |
/* | Header file for the RIA library |
/*-----*/
#ifndef RIALIB_H_
#define RIALIB_H_
#include <conio.h>
#include <stdio.h>
/*-----*/
/* | Global Constants |
/*-----*/
#define TRUE -1
#define FALSE 0
#define RIGHT 77
#define LEFT 75
#define DELETE 83
#define HOME 71
#define END 79
#define CR 13
#define LF 10
#define F2 60
#define RETURN 13
#define BACKSPACE 8
#define ESC 27
#define CNTRL_C 3
```

```
#define INPUT_OK 0
#define INPUT_BAD -1
#define IGNORE 0
#define RETRY 1
#define ABORT 2
/*-----*/
/* | Data from Prompt for Disk |
/*-----*/
#define RESET_DRIVE 0
#define VERIFY_SECTORS 4
#define HEAD 0
#define DISK 0
#define TRACK 0
#define SECTOR 1
#define NUMBER_SECTORS 1

/*-----*/
/* | Type definitions. |
/*-----*/
typedef struct Window_Style {
    int forecolor;
    int backcolor;
    int textcolor;
    int x1;
    int y1;
    int x2;
    int y2;
    int titlefore;
    int titleback;
    char title[81];
} WINSTYLE;

extern struct text_info TEXT;

/*-----*/
/* | Function Prototypes |
/*-----*/
char *allocate ( int );
void Beep ( void );
void Center_Text ( char * msg, struct Window_Style Window );
int Copy_File ( FILE *File, char *Filename, char *datapath, int
    *Copied_Files, WINSTYLE *File_Window, WINSTYLE
    *Records_Window, char Show_Data[[12]] );
int draw_box ( int, int, int, int );
void die ( char * );
extern int Display_Critical_Error ( char * );
FILE *Get_File ( char *Filename, char *Drive, int *start );
void Get_User_Name ( struct Window_Style *Name_Window,
    char *User_Name, char *Time_Stamp );
```

```

void far Hardware_Error( unsigned devert, unsigned errval,
    unsigned far *devhdr );
int Input_String( char *string, int width, int position, int
    describe );
int Overwrite( char *Filename );
void Prompt_New_Disk( char *Prompt_Disk, char *Drive );
void Read_Descriptions( char *datapath, int *fcount, char
    **file_desc );
void Save_Desc( char *datapath, char **file_desc, char
    **new_desc, int Copied_Files, int fcount );
void Screen_Setup( struct Window_Style **);

```

```

int strdup( char *, int );
int strlen( char *, char, int, int );
char *strstore( char *string );
void Window_Box( struct Window_Style * );
void Window_Restore( struct Window_Style *Window, char
    *savebuffer );
char *Window_Save( struct Window_Style *Window );

#endif

```

2. RIA Library Procedure Sources.

a. Lotus 1-2-3 Data File Title Reading Procedure

```

// *-----*
// | SOURCE FILE: 123.C
// *-----*
// | Routines to read title information from Lotus 1-2-3 data files.
// |
// | Contains procedures:   read_int   Reads an integer from
// |                       a 123 file.
// |                       get_format  Reads the format and
// |                       location for a cell.
// |                       get_123_title Reads the title from
// |                       an RIA 123 data file.
// *-----*
#include <stdio.h>
#include <alloc.h>
#include <process.h>
#include <mem.h>
#include <string.h>
#include '123.h'

// *-----*
// | PROCEDURE: read_int
// *-----*
// | PURPOSE: Reads a 2 byte integer from a 123 file.
// | ON CALL: file - Pointer to structure of opened 123
// |           data file.
// |           eof_code - Pointer to flag indicating if
// |           End-of-file reached.
// | RETURNS: 2 byte integer if OK.
// |           READERROR if premature EOF.
// *-----*
int
read_int( FILE *file, int *eof_code ) {
    int hi,low;

    *eof_code = 0;
    if( (low = fgetc( file )) == EOF ) {
        *eof_code = 1;
        exit(1);
        return (READERROR);
    }
    if( (hi = fgetc( file )) == EOF ) {
        *eof_code = 1;
        exit(1);
        return (READERROR);
    }
    return ((hi << 8) + low );
}
// *-----*

```

```

// | PROCEDURE: get_format
// *-----*
// | PURPOSE: Reads a cell format and location.
// | ON CALL: file - Pointer to structure of opened 123
// |           data file.
// |           format - Pointer to format to return.
// |           column - Pointer to cell column to return.
// |           row - Pointer to cell row to return.
// | RETURNS: Nothing
// *-----*
void
get_format( FILE *file, int *format, int *column, int *row ) {
    int i;

    if ((i=fgetc( file )) == EOF) {
        printf("Premature EOF\n");
        exit(99);
    }
    *format = i;
    *column = read_int( file, &i);
    *row = read_int( file, &i);
}

// *-----*
// | PROCEDURE: get_123_title
// *-----*
// | PURPOSE: Extracts the title from a 123 RIA worksheet.
// | ON CALL: filename - Pointer to the file name to get the
// |           title from.
// |           format - Pointer to format to return.
// |           column - Pointer to cell column to return.
// |           row - Pointer to cell row to return.
// |           End-of-file reached.
// | RETURNS: Pointer to the worksheet title.
// *-----*
char *
get_123_title( char *filename ) {
    FILE *file;
    int done;
    int OPCODE, LENGTH/*,misc */;
    int format, column, row,i,j,k;
    char *Label;
    char *TMP;
    double Double;
    char Tmpdbl[8];
    char *EOFError="premature end of file\n";

    if ((file = fopen( filename,'rb' )) == NULL ) {
        printf("Failed to open %s in module
123.c.\n",filename);
        return ( NULL );
    }
    while ((OPCODE = read_int( file, &done )) != 1 ) {

```

```

if (!done)
{
    LENGTH = read_int( file, &done );
    switch ( OPCODE ) {
        case FILE_START:
            read_int( file, &done );
            break;
        case BLANK_CELL:
            get_format( file, &format, &row,
                &column );
            if ( ( row | column ) == 0 ) {
                fclose( file );
                return( NULL );
            }
            break;
        case INTEGER:
            get_format( file, &format, &row,
                &column );
            read_int( file, &done );
            if ( ( row | column ) == 0 ) {
                fclose( file );
                return( NULL );
            }
            break;
        case REAL_NUMBER:
            TMP = (char *)&Double;
            get_format( file, &format, &row,
                &column );
            for (i=0; i<8; i++) {
                if ( (j = fgetc( file )) == EOF ) {
                    fclose( file );
                    return ( NULL );
                }
                Tmpdbl[i]=j;
            }
            memcpy( TMP, Tmpdbl,8);
            if ( (row | column) == 0 ) {
                fclose( file );
                return ( NULL );
            }
            break;
        case LABEL:
            get_format( file, &format, &row,
                &column );
            if ( ( Label = (char *) malloc(
                LENGTH - 6 + 2 )) == NULL ) {
                printf( "Malloc error.\n" );
                fclose( file );
                return ( NULL );
            }
            k=0;
            for (i=0; i<=(LENGTH - 6); i++) {
                if ( (j = fgetc( file )) == EOF )
                    exit(0);
                if ( (i != 0 && j != '\n' && j != '\r' &&
                    j != '~') ) {
                    Label[k++]=j;
                }
            }
            Label[i] = 0;
            if ( (row | column) == 0 ) {
                fclose( file );
                return ( Label );
            }
            break;
        case FORMULA:
        default:
            for (i=0; i<LENGTH; i++) {

```

```

                if ( (j = fgetc( file )) == EOF ) {
                    fclose( file );
                    printf( EOFError );
                    return ( NULL );
                }
                break;
            }
            else {
                printf( "Premature end of file.\n" );
                fclose( file );
                return( NULL );
            }
        }
        fclose( file );
        return ( NULL );
    }
}

```

b. Memory Allocation Procedure

```

// *-----*
// | SOURCE FILE: ALLOCAT.C |
// *-----*
// | Memory allocation routine used for RIA program. |
// *-----*
#include "rialib.h"
#include <alloc.h>

// *-----*
// | PROCEDURE: allocate |
// *-----*
// | PURPOSE: Dynamically allocates memory from the |
// | global heap |
// | ON CALL: block_size - Size of the desired memory block. |
// | RETURNS: Pointer to allocated block. |
// | If not enough memory, displays error, |
// | then exits to system |
// *-----*
char *
allocate ( int block_size ) {
    char *temp;

    if ( ( temp = (char *) malloc( block_size ) ) == NULL )
        die( "Ran out of memory during '\malloc' call.\n" );
    return ( temp );
}

```

c. Alarm Procedure

```

// *-----*
// | SOURCE FILE: BEEP.C |
// *-----*
// | Alarm noise for RIA program. |
// *-----*
#include <dos.h>

// *-----*
// | PROCEDURE: Beep |
// *-----*
// | PURPOSE: Sounds a warning beep at 110 Hertz. |
// | RETURNS: Nothing. |
// *-----*
void
Beep() {
    sound(110);
}

```



```

fclose( File );
fclose( Output_File );
if ( Records == 0 )
    return (-1);
else
    return ( Records );
}

```

f. Error Exit Procedure

```

// |-----|
// | SOURCE FILE: DIE.C |
// |-----|
// | Routine to display error and Exit. |
// |-----|
#include <conio.h>
#include <stdio.h>
#include <process.h>
#include <ctype.h>

extern struct text_info TEXT=(0,0,0,0,0,0,0,0,0);

// *-----*
// | PROCEDURE: die |
// *-----*
// | PURPOSE: Display an error message and exit to system |
// | ON CALL: Message - points to the message to print. |
// | RETURNS: Nothing. |
// *-----*

void
die (char *Message ) {
    textattr( TEXT.attribute );
    clrscr();
    printf(stderr, Message);
    exit(1);
}

```

g. Window Drawing Procedure

```

// *-----*
// | SOURCE FILE: DRWBOX.C |
// *-----*
// | Routine to draw box on the screen. |
// *-----*
#include <string.h>
#include <conio.h>
#include 'rialib.h'

// *-----*
// | PROCEDURE: draw_box |
// *-----*
// | PURPOSE: Draws a box on the screen. |
// | ON CALL: ul_X - Upper left corner X coordinate. |
// |           ul_Y - Upper left corner Y coordinate. |
// |           lr_X - Lower right corner X coordinate. |
// |           lr_Y - Lower right corner Y coordinate. |
// | RETURNS: Always returns zero. |
// *-----*

int
draw_box( int ul_X, int ul_Y, int lr_X, int lr_Y ) {
    int width, height, x, y, i;
    char Line[81];

    _wscroll = 0;
    width = lr_X - ul_X - 1;
    height = lr_Y - ul_Y - 1;
    memset( Line, UPPERLEFT, 1 );
    memset( Line+1, HORIZ_SIDE, width );

```

```

memset( Line+1+width, UPPERRIGHT, 1 );
memset( Line+2+width, 0, 1 );
x = wherex();
y = wherex();
gotoxy( ul_X, ul_Y );
cputs( Line );
memset( Line, VERT_SIDE, 1 );
memset( Line+1, BLANK, width );
memset( Line+1+width, VERT_SIDE, 1 );
for ( i = 1; i <= height; i++ ) {
    gotoxy( ul_X, ul_Y + i );
    cputs( Line );
}
memset( Line, LOWERLEFT, 1 );
memset( Line+1, HORIZ_SIDE, width );
memset( Line+1+width, LOWERRIGHT, 1 );
gotoxy( ul_X, ul_Y + height + 1 );
cputs( Line );
gotoxy( x, y );
_wscroll = 1;
return (0);
}

```

h. File Opening Procedure

```

// *-----*
// | SOURCE FILE: GETFILE.C |
// *-----*
// | Routine to open next file on floppy. |
// *-----*
#include <string.h>
#include <alloc.h>
#include <dos.h>
#include <dir.h>
#include <stdio.h>
#include 'rialib.h'

// *-----*
// | PROCEDURE: Get_File |
// *-----*
// | PURPOSE: Opens the next file on the floppy drive. |
// | ON CALL: Filename - Buffer for filename. |
// |           Drive - Drive to read from. |
// |           Start - Flag for findfirst/findnext |
// | RETURNS: Pointer to open file structure |
// |           Returns NULL when no more files. |
// *-----*

FILE *
Get_File( char *Filename, char *Drive, int *start ) {
    FILE *Opened_File;
    char File[20];
    int done;
    static struct fblk fblk;
    char Error_Msg[100];

    strcpy( File, Drive );
    strcat( File, ".*" );
    if ( *start ) {
        done = findfirst( File, &fblk, 0 );
        *start = 0;
    }
    else
        done = findnext( &fblk );
    if ( done )
        return( NULL );
    strcpy( File, Drive );
    strcat( File, fblk.ff_name );
    if ( ( Opened_File = fopen( File, 'r' ) ) == NULL ) {
        sprintf( Error_Msg, 'Error: Can\'t open %s\n', File );

```

```

        die( Error_Msg );
    }
    strcpy( Filename, fblk_ff_name );
    return ( Opened_File );
}

```

i. Get User Name Procedure

```

// *-----*
// | SOURCE FILE: GETUNAM.C |
// *-----*
// | Routine to get user name. |
// *-----*
#include <conio.h>
#include <stdio.h>
#include <string.h>
#include 'rialib.h'
// *-----*
// | PROCEDURE: Get_User_Name |
// *-----*
// | PURPOSE: Have the user input their name for use in file |
// | descriptions. |
// | ON CALL: Name_Window - Description for input name |
// | window. |
// | User_Name - Points to buffer for user name. |
// | Time_Stamp - File name for time stamp file. |
// | RETURNS: Nothing |
// *-----*

```

```

void
Get_User_Name( struct Window_Style *Name_Window,
char *User_Name, char *Time_Stamp ) {
    char *savebuffer;
    FILE *Time;
    struct text_info ti;

    gettextinfo( &ti );
    savebuffer = Window_Save( Name_Window );
    Window_Box( Name_Window );
    gotoxy( Name_Window->x1+1, Name_Window->y1+1 );
    textcolor( Name_Window->textcolor );
    textbackground( Name_Window->backcolor );
    while( strlen( User_Name ) == 0 ) {
        Input_String( User_Name, 12, 0, 0 );
        if( strlen( User_Name ) == 0 )
            Beep();
    }
    if( (Time=fopen( Time_Stamp, 'w' )) == NULL )
        die( 'Can\'t open timestamp file.\n' );
    sprintf( Time, '%s', User_Name );
    fclose( Time );
    Window_Restore( Name_Window, savebuffer );
    textattr( ti.attribute );
}

```

j. High Level Hardware Error Handling Procedure

```

// *-----*
// | SOURCE FILE: HARDERR.C |
// *-----*
// | High level routine to process hardware errors. |
// *-----*
#include <dos.h>
#include <string.h>
#include <stdio.h>
#include 'rialib.h'

static char *error_message[] = {

```

```

    'write protect',
    'unknown unit',
    'drive not ready',
    'unknown command',
    'data CRC error',
    'bad request',
    'seek error',
    'unknown media type',
    'sector not found',
    'printer out of paper',
    'write fault',
    'read fault',
    'general failure',
    'reserved',
    'reserved',
    'invalid disk change'
}
;

```

```

// *-----*
// | PROCEDURE: Hardware_Error |
// *-----*
// | PURPOSE: Process hardware errors that occur. |
// | ON CALL: Called by system - |
// | devert - Device error code |
// | errval - Error code |
// | deviceHeader - Pointer to device driver header. |
// | RETURNS: Nothing |
// *-----*

```

```

#pragma warn -par
void far Hardware_Error( unsigned devert, unsigned errval,
unsigned far *deviceHeader ) {
    static char message[80];
    int drive, errorno, request;

    drive = devert & 0x00FF;
    errorno = errval & 0x00FF;
    if( devert & 0x8000 ) {
        sprintf( message, 'Non-disk related device error.(%s)',
            error_message[errorno] );
        request = Display_Critical_Error( message );
        _hardretn( request );
    }
    sprintf( message, 'When trying drive %c, %s error
occurred.',
        'A'+drive, error_message[errorno] );
    _hardresume( Display_Critical_Error( message ) );
}
#pragma warn +par

```

k. String Input Procedure

```

// *-----*
// | SOURCE FILE: INPUTSTR.C |
// *-----*
// | Get String Input from the user. |
// *-----*
#include <conio.h>
#include <string.h>
#include <process.h>
#include 'rialib.h'

// *-----*
// | PROCEDURE: Input_String |
// *-----*
// | PURPOSE: Get a string from the user. |
// | ON CALL: string - buffer to store string. |
// | width - Size of string buffer |
// | position - Beginning position in buffer |

```

```

// | describe - Enables F2 key if TRUE |
// | RETURNS: INPUT_OK if string was entered. |
// | INPUT_BAD if string input was aborted. |
// *-----*
int
Input_String( char *string, int width, int position, int describe ) {
    int x,y, xhome;
    int done=0;
    int i = 0,j;
    char blank[80], ch;

    i = position;
    xhome = x = wherex();
    xhome = xhome - position;
    y = wherey();

    while ( !done ) {
        ch = getch();
        if ( ch == 0 ) {
            ch = getch();
            switch (ch) {
                case F2:
                    if (!describe) {
                        Beep();
                        break;
                    }
                    return ( INPUT_BAD );
                case RIGHT:
                    if ( i >= strlen(string) ) {
                        Beep();
                        break;
                    }
                    ++x;
                    ++i;
                    break;
                case LEFT:
                    if ( i <= 0 ) {
                        Beep();
                        break;
                    }
                    --x;
                    --i;
                    break;
                case DELETE:
                    if (string[i] == 0) {
                        Beep();
                        break;
                    }
                    strdel( string, i );
                    break;
                case HOME:
                    x = xhome;
                    i = 0;
                    break;
                case END:
                    x = xhome + ( strlen( string ) <
                        width ? strlen(string) :
                        strlen(string - 1) );
                    i = x - xhome;
                    break;
                default:
                    Beep();
                    break;
            }
        }
        else
        {
            switch (ch) {

```

```

                case RETURN:
                    done = 1;
                    break;
                case CNTRL_C:
                    exit(1);
                case BACKSPACE:
                    if (i==0) {
                        Beep();
                        break;
                    }
                    --i;
                    --x;
                    strdel( string, i );
                    break;
                case ESC:
                    i = -1;
                    done = 1;
                    break;
                default:
                    if ( i >= width || ch < 32 ||
                        strlen(string) >= width ) {
                        Beep();
                        break;
                    }
                    strins(string, ch, i, width);
                    ++i;
                    ++x;
                    break;
            }
        }
        gotoxy(xhome,y);
        strcpy(blank, " ");
        for (j = 0; j < width-1; j++)
            strcat(blank, " ");
        cputs(blank);
        gotoxy(xhome,y);
        cputs(string);
        gotoxy(x,y);
    }
    if ( i < 0 )
        return ( INPUT_BAD );
    return ( INPUT_OK );
}

```

I. Prompt to Overwrite File Procedure

```

// *-----*
// | SOURCE FILE: OVERWRIT.C |
// *-----*
// | Routine to ask permission to overwrite files. |
// *-----*
#include <conio.h>
#include <ctype.h>
#include 'rialib.h'

struct Window_Style Overwrite_Window = {WHITE, CYAN,
    YELLOW, 2, 10, 79, 13, WHITE, CYAN, ""};

#define NOT_DETERMINED 5

// *-----*
// | PROCEDURE: Overwrite |
// *-----*
// | PURPOSE: Asks user if it is OK to overwrite a file. |
// | ON CALL: Filename - Name of file to overwrite. |
// | RETURNS: TRUE if OK to overwrite. |
// | FALSE if not OK to overwrite. |
// *-----*
int

```

```

Overwrite( char *Filename ) {
    char *savebuffer, ch;
    struct text_info ti;
    int overwrite = NOT_DETERMINED;
    char msg[80];

    gettextinfo( &ti );
    savebuffer = Window_Save( &Overwrite_Window );
    Window_Box( &Overwrite_Window );
    gotoxy( Overwrite_Window.x1+2, Overwrite_Window.y1+1 );
    textcolor( Overwrite_Window.textcolor );
    textbackground( Overwrite_Window.backcolor );
    sprintf( msg, "File %s already exists.", Filename );
    Center_Text( msg, Overwrite_Window );
    gotoxy( Overwrite_Window.x1+2, Overwrite_Window.y1+2 );
    Center_Text( "Do you want to overwrite it (y/n)?",
                Overwrite_Window );
    while ( overwrite == NOT_DETERMINED ) {
        ch = getch();
        tolower(ch);
        switch (ch) {
            case 'y':
                overwrite = TRUE;
                break;
            case 'n':
                overwrite = FALSE;
                break;
            default:
                Beep();
                break;
        }
    }
    Window_Restore( &Overwrite_Window, savebuffer );
    textattr( ti.attribute );
    return( overwrite );
}

```

m. Prompt for New Disk Procedure

```

/* _____ */
/* | SOURCE FILE: PNEWDISK.C |
/* | _____ |
/* | Prompts the user to insert a new disk. |
/* | _____ |
#include <dos.h>
#include <dir.h>
#include <string.h>
#include <stdio.h>
#include <bioe.h>
#include 'rialib.h'

#define BOX_UL_X 6
#define BOX_UL_Y 11
#define BOX_LR_X 73
#define BOX_LR_Y 13

/* _____ */
/* | PROCEDURE: Prompt_New_Disk |
/* | _____ |
/* | PURPOSE: Prompt the user to insert a new floppy disk. |
/* | ON CALL: Prompt_Disk - Flag to indicate if Prompting. |
/* | Drive - Disk Drive in use. |
/* | RETURNS: Nothing |
/* | _____ |
void
Prompt_New_Disk( char *Prompt_Disk, char *Drive ) {

```

```

    struct fblk File_Block;
    struct text_info ti;
    int done;
    FILE *dummy;
    char box_save( ( BOX_LR_X-BOX_UL_X+2 ) * ( BOX_LR_Y
        - BOX_UL_Y+2 ) * 2 );
    char Search_String[20];
    char titleLeft[2]= [LEFTCROSS,0],
        titleRight[2]= [RIGHTCROSS,0];

    *Prompt_Disk = 1;
    gettextinfo( &ti );
    gotoxy( BOX_UL_X, BOX_UL_Y, BOX_LR_X, BOX_LR_Y,
        box_save );
    textattr( RED*16+WHITE );
    draw_box( BOX_UL_X, BOX_UL_Y, BOX_LR_X,
        BOX_LR_Y );
    gotoxy( BOX_UL_X+3, BOX_UL_Y+1 );
    gotoxy( BOX_UL_X+3, BOX_LR_Y );
    cputs( titleRight );
    textattr( RED*16+YELLOW );
    cputs( "Press the ENTER key when ready." );
    textattr( RED*16+WHITE );
    cputs( titleLeft );
    _setcursortype( _NOCURSOR );
    gotoxy( BOX_UL_X+3, BOX_UL_Y+1 );
    cputs( "Please place the RIA data disk into the 5-1"
        "EXTERNAL drive." );
    while ( getch() != 13 ) {
        sound(110);
        delay(200);
        nosound();
    }
    puttext( BOX_UL_X, BOX_UL_Y, BOX_LR_X, BOX_LR_Y,
        box_save );
    _setcursortype( _NORMALCURSOR );
    bioedisk( RESET_DRIVE, 0, 0, 0, 0, 0, NULL );
    lowvideo();
    strcpy( Search_String, Drive );
    strcat( Search_String, "acdskt.r");
    /* Loop until we found disk */
    while ( (dummy=fopen( Search_String, "w" )) == NULL );
    fclose( dummy );
    unlink( Search_String );
    strcpy( Search_String, Drive );
    strcat( Search_String, ".unk" );
    done = 0;
    while ( !done ) {
        done = findfirst( Search_String, &File_Block, 0 ) ^ 1;
        if ( !done ) {
            strcpy( Search_String, Drive );
            strcat( Search_String, ".rec" );
            done = findfirst( Search_String, &File_Block,
                0 ) ^ 1;
            if ( !done ) {
                gotoxy( BOX_UL_X, BOX_UL_Y,
                    BOX_LR_X, BOX_LR_Y+1,
                    box_save );
                textattr( RED*16+WHITE );
                draw_box( BOX_UL_X, BOX_UL_Y,
                    BOX_LR_X, BOX_LR_Y+1 );
                gotoxy( BOX_UL_X+3, BOX_UL_Y+1 );
                _setcursortype( _NOCURSOR );
                cputs( " This disk does not contain
                    RIA data files. " );
                gotoxy( BOX_UL_X+3, BOX_UL_Y+2 );
                cputs( " Please insert a an RIA data disk,
                    then push the ENTER key." );

```



```

extra = new_desc[i];
for (j=0; j<strlen(new_desc[i]); j++){
    if (extra[j] == ''){
        len = j;
        j = 5000;
    }
}
strcpy( tmp_desc, new_desc[i] );
for ( j=0; j < fcount; j++ ) {
    if (strcmp(new_desc[i], file_desc[j], len) == 0)
    {
        extra = strchr( file_desc[j], 4 );
        if (extra != NULL)
            strcat( tmp_desc, extra );
        j = 5000;
        file_desc[j] = NULL;
    }
}
fprintf( Desc_File, "%s\n", tmp_desc );
}
for ( i = 0; i < fcount+1; i++ ) {
    if ( file_desc[i] != NULL )
        fprintf( Desc_File, "%s\n", file_desc[i] );
}
fclose( Desc_File );
strcpy( tmp_desc, datapath );
strcat( tmp_desc, 'description' );
_dos_setfileattr( tmp_desc, _A_HIDDEN );
free ( tmp_desc );
}

```

p. Screen Set-up Procedure

```

// *-----*
// | SOURCE FILE: SCRNSU.C |
// |-----*
// | Draws screen windows. |
// |-----*
#include <stdio.h>
#include 'rialib.h'

// *-----*
// | PROCEDURE: Screen_Setup |
// |-----*
// | PURPOSE: Draw a series of Windows on the screen. |
// | ON CALL: windowList - Pointer to a list of window |
// | definition structs. |
// | RETURNS: Nothing |
// *-----*
void
Screen_Setup( struct Window_Style **windowList ) {
    while ( *windowList != NULL ) {
        Window_Box( *windowList );
        windowList++;
    }
}

```

q. Character Deletion Procedure

```

// *-----*
// | SOURCE FILE: STRDEL.C |
// |-----*
// | Procedure to delete a character from string. |
// |-----*
#include <string.h>

// *-----*
// | PROCEDURE: strdel |
// |-----*

```

```

// | PURPOSE: Deletes a character from the middle of a string. |
// | ON CALL: string - Pointer to string Buffer |
// | posit - Position of character to delete. |
// | RETURNS: New position for cursor |
// | -1 if can't do anything |
// *-----*
int
strdel( char *string, int posit ) {
    int length, i;

    length = strlen(string);
    if ( posit >= length || length == 0 )
        /* The string is empty, or we are beyond end of string.
        */
        return (-1);
    if ( posit == length-1 ) { /* End of the string */
        string[posit]=0;
        return (posit);
    }
    for ( i = posit; i < length; i++ )
        string[i] = string[i+1];
    return ( posit );
}

```

r. Character Insertion Procedure

```

// *-----*
// | SOURCE FILE: STRINS.C |
// |-----*
// | Procedure to insert character in a string. |
// |-----*
#include <string.h>

// *-----*
// | PROCEDURE: strins |
// |-----*
// | PURPOSE: Inserts a character within a string. |
// | ON CALL: string - Pointer to string buffer. |
// | ch - Character to insert |
// | posit - Position to insert character. |
// | size - Length of string. |
// | RETURNS: Next position for cursor. |
// *-----*
int
strins( char *string, char ch, int posit, int size ) {
    int length, i;

    length = strlen(string);
    if ( posit > size - 1 || posit > length )
        /* The string is already full, so we can't add any more
        */
        return (-1);
    if ( posit == length ) { /* End of the string */
        string[posit]=ch;
        string[posit+1]=0;
        return (posit+1);
    }
    for ( i = length; i >= posit; i- )
        string[i+1] = string[i];
    string[posit] = ch;
    return ( posit + 1 );
}

```

s. String Storage Procedure

```

// *-----*
// | SOURCE FILE: STRSTORE.C |
// |-----*
// | Routine to store a string in the far Heap |

```

```

// *-----*
#include <string.h>
#include <alloc.h>
#include 'rialib.h'

// *-----*
// | PROCEDURE: strstore |
// |-----|
// | PURPOSE: Allocates space for a string on the heap |
// |           and copies it. |
// | ON CALL: string - pointer to the string to store |
// | RETURNS: pointer to stored string. |
// *-----*
char *
strstore ( char *string ) {
    char *temp;

    temp = allocate( strlen( string ) + 1 );
    strcpy( temp, string );
    return ( temp );
}

```

t. Window Drawing Procedure

```

// *-----*
// | SOURCE FILE: WINBOX.C |
// |-----|
// | Procedure to draw windows. |
// *-----*
#include <conio.h>
#include <string.h>
#include 'rialib.h'

// *-----*
// | PROCEDURE: Window_Box |
// |-----|
// | PURPOSE: Draw a window on the screen |
// | ON CALL: Window - Pointer to a window definition |
// |           structure. |
// | RETURNS: Nothing |
// *-----*
void
Window_Box( struct Window_Style *Window ) {
    struct text_info ti;
    int offset;
    char titleLeft[2]={LEFTCROSS,0},
        titleRight[2]={RIGHTCROSS,0};

    gettextinfo( &ti );
    textcolor( Window->forecolor );
    textbackground( Window->backcolor );
    draw_box( Window->x1, Window->y1, Window->x2,
              Window->y2 );
    offset = (( Window->x2 - Window->x1 + 1 ) - strlen( Window->
        title ) - 2 ) / 2;
    if ( offset < 0 ) offset = 0;
    if ( strlen( Window->title ) != 0 ) {
        gotoxy( Window->x1+offset, Window->y1 );
        cputs( titleRight );
        textcolor( Window->titlefore );
        textbackground( Window->titleback );
        cputs( Window->title );
        textcolor( Window->forecolor );
        textbackground( Window->backcolor );
        cputs( titleLeft );
    }
    textattr( ti.attribute );
}

```

u. Screen Background Restoring Procedure

```

// *-----*
// | SOURCE FILE: WINREST.C |
// |-----|
// | Restore a saved portion of the screen. |
// *-----*
#include <conio.h>
#include <alloc.h>
#include 'rialib.h'

// *-----*
// | PROCEDURE: Window_Restore |
// |-----|
// | PURPOSE: Restores a saved portion of the screen. |
// | ON CALL: Window - Pointer to window definition |
// |           for the window we are about |
// |           to close. |
// |           savebuffer - Pointer to saved data. |
// | RETURNS: Nothing |
// *-----*
void
Window_Restore( struct Window_Style *Window, char
                *savebuffer ) {

    puttext( Window->x1, Window->y1, Window->x2, Window->
        y2, savebuffer );
    free( savebuffer );
}

```

v. Screen Background Saving Procedure

```

// *-----*
// | SOURCE FILE: WINSAV.C |
// |-----|
// | Procedure to save a portion of the screen. |
// *-----*
#include <alloc.h>
#include <conio.h>
#include <stdio.h>
#include <process.h>
#include 'rialib.h'

// *-----*
// | PROCEDURE: Window_Save |
// |-----|
// | PURPOSE: To save a portion of the screen for restore |
// |           later. |
// | ON CALL: Window - Pointer to window definition for |
// |           window we are about to open |
// | RETURNS: Pointer to the buffer with the saved data. |
// *-----*
char *
Window_Save( struct Window_Style *Window ) {
    int buffersize;
    char *savebuffer;
    buffersize = ( Window->x2 - Window->x1 + 1 ) * ( Window->
        y2 - Window->y1 + 1 ) * 2;
    if ( ( savebuffer = (char *) malloc( buffersize ) ) == NULL ) {
        clrscr();
        fprintf( stderr, "Error allocating temporary buffer.\n" );
        exit ( 1 );
    }
    gettext( Window->x1, Window->y1, Window->x2, Window->
        y2, savebuffer );
    return ( savebuffer );
}

```

w. Critical Error Handling Procedure

```

TITLE CRITICAL.ASM Critical Error Handler
PAGE 55,192
;-----*
; | Critical Error routines for use with RIA Program. |
;-----*
MODEL small

BUFFER_SIZE EQU 10240
UPPERLEFT EQU 0DAH
UPPERRIGHT EQU 0BFH
LOWERLEFT EQU 0C0H
LOWERRIGHT EQU 0D9H
VERT_SIDE EQU 0B3H
HORIZ_SIDE EQU 0C4H
SPACE EQU 020H
GET_VIDEO_MODE EQU 0FH
GET_CURSOR EQU 03H
GET_KEY_STATUS EQU 1
GET_KEY EQU 0
IGNORE EQU 0
RETRY EQU 1
ABORT EQU 2
FAIL EQU 3

.DATA
EXTRN _Prompt_Disk:BYTE
Init_Msg DB 'Please place the RIA data disk into the 5-1/2" EXTERNAL Drive.'
Len_Init_Msg EQU $ - Init_Msg
Init_Msg_2 DB 'Press any key to continue.'
Len_Init_Msg_2 EQU $ - Init_Msg_2
Abort_Msg DB 'Correct error then press a key: A)abort, R)etry, I)gnore, or F)ail.'
Len_Abort_Msg EQU $ - Abort_Msg
Video_Segment DW 0
Vid_Offset DW 0
Cursor_Type DW 0
Cursor_Position DW 0
Active_Page DB 0
Save_Buffer DB 64 DUP (SCREENBUFF)

.CODE
ASSUME CS:@code, DS:@data

;-----*
; | PROCEDURE: Sound |
;-----*
; | PURPOSE: Sound the speaker for a specific duration and frequency. |
; | ON CALL: BX has the duration in msec |
; | DI has the frequency in Hertz |
; | RETURNS: Nothing |
;-----*
Sound PROC NEAR
;-----*
; | Actual duration is in multiples of 55 msec as we are using |
; | timer ticks. |
;-----*
PUSH AX ; Save registers.
PUSH BX
PUSH CX
PUSH DX
PUSH DI

MOV AL,0B6H ; Load Timer notification code into AL.
OUT 43H,AL ; Notify Timer that count is coming.
;-----*

```

```

; | Timer frequency is 1.19318 MHz ( 144F38H )
; | Timer count is (Timer Frequency) / (Sound Frequency)
; *
MOV     DX,14H           ; Load DX:AX with Timer frequency
MOV     AX,4F38H        ;
DIV     DI              ; Divide by sound frequency.
OUT     42H,AL         ; Send low order byte of count to timer.
MOV     AL,AH          ; Move high order byte of count into AL.
OUT     42H,AL         ; Send high order byte of count to timer.
IN      AL,61H         ; Read byte from Programmable Peripheral Interface Chip (PPI).
MOV     AH,AL          ; Save it in AH.
OR      AL,3           ; Set Bits 1 and 2.
OUT     61H,AL        ; Tell PPI to turn the speaker on.
PUSH    AX             ; Save AX
MOV     AX,BX          ; Get the number of msec in AX.
MOV     CX,55          ; Load CX with the number of msec/tick.
DIV     CL             ; Get number of timer ticks into AL.
CMP     AH,27          ; Do we need to round up (remainder>27)?
JLE     Sound_1        ; If no, jump.
INC     AL             ; Round up.
Sound_1:
XOR     AH,AH          ; Clear high order byte.
MOV     BX,AX          ; Save number of ticks.
CALL    Get_Ticks      ; Get current ticks
MOV     DI,AX
MOV     CX,DX          ; CX:DI has starting timer count.
Sound_2:
CALL    Get_Ticks      ; Get the current Tick Count.
SUB     AX,DI          ; Determine Ticks elapsed.
SBB     DX,CX
CMP     AX,BX          ; Has the time expired?
JLE     Sound_2        ; If no, loop until done.

POP     AX             ; Restore PPI port value in AH.
MOV     AL,AH          ; Move original PPI port into AL.
OUT     61H,AL        ; Tell PPI to turn off the speaker.

POP     DI             ; Restore Registers.
POP     DX
POP     CX
POP     BX
POP     AX
RET

Sound   ENDP

```

```

; *
; | PROCEDURE: Get_Ticks
; *
; | PURPOSE: Gets the current timer tick count in DX:AX
; | ON CALL: Nothing
; | RETURNS: DX:AX has the timer tick count.
; *
Get_Ticks PROC NEAR
PUSH    DI
PUSH    DS
PUSH    ES
MOV     AX,ROMBIOS    ; Load the ROM BIOS segment.
MOV     ES,AX
MOV     DI,06CH        ; Point to the timer ticks.
MOV     AX,ES:DI      ; Get timer ticks into DS:AX
MOV     DX,ES:DI[2]   ; Timer Count now in DX:AX
POP     ES
POP     DS
POP     DI
RET
Get_Ticks ENDP

```

```

// *-----*
// | PROCEDURE: _Display_Critical_Error
// *-----*
// | PURPOSE: Display critical error message, or prompt for new disk if
// |           the drive was empty when we tried to read the RIA data.
// | ON CALL: Error_Message - Points to the NULL terminated message.
// |           _Prompt_Disk - Global flag set in the calling program.
// |           Determines if we prompt for a new disk.
// | RETURNS: Returns control to hardware error handler.
// *-----*

_Display_Critical_Error PUBLIC _Display_Critical_Error
PROC NEAR
ARG Error_Message:WORD

    PUSH BP
    MOV BP,SP

    PUSH BX
    PUSH CX
    PUSH DX
    PUSH DI
    PUSH SI
    PUSH ES
    MOV AH,GET_VIDEO_MODE
    INT 10H
    MOV Active_Page,BH
    MOV Video_Segment,0B800H
    CMP AL,07H
    JNE Display_Critical_Error_10
    MOV Video_Segment,0B000H

Display_Critical_Error_10:
    MOV AL,AH
    XOR AH,AH
    PUSH BX
    MUL BH
    MOV BH,25
    MUL BH
    MOV Vid_Offset,AX
    MOV AH,GET_CURSOR
    POP BX
    INT 10H
    MOV Cursor_Type,CX
    MOV Cursor_Position,DX
    PUSH DS
    POP ES
    CMP _Prompt_Disk,0
    JE Display_Critical_Error_20
    MOV AX,Len_Init_Msg
    MOV DI,Len_Init_Msg+4
    JMP Display_Critical_Error_50

Display_Critical_Error_20:
    MOV DI,[Error_Message]
    PUSH DI
    MOV CX,100
    MOV AL,0
    REPNE SCASB
    CMP CX,0
    JNE Display_Critical_Error_30
    POP AX
    MOV AX,-1
    JMP Display_Critical_Error_190

Display_Critical_Error_30:
    DEC DI
    POP AX
    SUB DI,AX
    MOV AX,DI

```

; Load BIOS function 'Get Video Mode'
; Get the video mode.
; Save the active video page.
; Assume for now that this is not a mono card.
; Are we on a mono card?
; If no, jump.
; Set video segment for monochrome adapter.

; Load the page width into AL.
; Zero out the high order byte.
; Save the active display page.
; Multiply by the page number.
; Load the number of lines per page.
; Multiply by the number of lines.
; Save the Video offset.
; Load BIOS function 'Get Cursor Position'
; Get active page in BX.
; Get the cursor position.
; Save the cursor type.
; Save the cursor position.
; Make our data segment the extra segment.

; Are we prompting for a new disk?
; If no, jump.
; Load the length of the disk prompt.
; Load size of message with 2 spaces at each end.
; Jump over other error processing stuff.

; Load offset to error message.
; Save it on the stack.
; Load maximum line length.
; Load a zero to search for.
; Search for 0 termination of message.
; Was the string terminated?
; If yes, jump.
; Clear the offset from the stack.
; Signal error.
; Error no end of string found, Exit.

; Points to zero at end of line.
; Get the starting point of the string.
; DI has string length.
; Save the message length in DI.


```

                                STOSW                                ; Display it on the screen.
                                LOOP    Display_Critical_Error_110 ; Loop until done.

Display_Critical_Error_120:
                                PUSH    DI                        ; Save DI.
                                PUSH    BX                        ; Save BX.
                                MOV     DI,300                    ; Set frequency to 300 Hertz.
                                MOV     BX,150                    ; Set Duration to 150 MS.
                                CALL    Sound                    ; Sound a beep.
                                MOV     DI,110                    ; Set frequency to 110 Hertz.
                                MOV     BX,200                    ; Set Duration to 200 MS.
                                CALL    Sound                    ; Sound a beep.
                                POP     BX                        ; Restore BX, and DI
                                POP     DI                        ;

Display_Critical_Error_130:
                                MOV     AH,GET_KEY_STATUS        ; Load BIOS function get keyboard status.
                                INT     16H                      ; See if a key is waiting.
                                JZ      Display_Critical_Error_130 ; If no, loop until one is there.
                                MOV     AH,GET_KEY                ; Load BIOS function get key press.
                                INT     16H                      ; Get the key.
                                CMP     Prompt_Disk, 0           ; Are we prompting for a new disk?
                                JNE     Display_Critical_Error_170 ; If yes, jump.
                                ;-----*
                                ; | Processing disk critical errors. |
                                ;-----*
                                CMP     AH,23                    ; Was I (Ignore) Pressed?
                                JNE     Display_Critical_Error_140 ; If no, then jump.
                                MOV     AX,IGNORE                 ; Load IGNORE into AX.
                                JMP     SHORT Display_Critical_Error_170 ; Jump to process return.

Display_Critical_Error_140:
                                CMP     AH,30                    ; Was A (Abort) pressed?
                                JNE     Display_Critical_Error_150 ; If no then jump.
                                MOV     AX,ABORT ; Load ABORT into AX
                                JMP     SHORT Display_Critical_Error_170 ; Jump to process return.

Display_Critical_Error_150:
                                CMP     AH,19                    ; Was R (Retry) pressed?
                                JNE     Display_Critical_Error_160 ; If no, then jump.
                                MOV     AX,RETRY                 ; Load RETRY into AX.
                                JMP     SHORT Display_Critical_Error_170 ; Jump to process return.

Display_Critical_Error_160:
                                CMP     AH,33 ; Was F (Fail) pressed?
                                JNE     Display_Critical_Error_120 ; If no, jump to sound beep
                                                                ; and get another key.
                                MOV     AX,FAIL ; Load FAIL into AX.

Display_Critical_Error_170:
                                ;-----*
                                ; | Restore the screen |
                                ;-----*
                                PUSH    AX                        ; Save the critical error processing code.
                                MOV     AX,Video_Segment          ; Make the video segment our Data Segment.
                                MOV     ES,AX
                                                                ;
                                MOV     DI,BX                      ; Point to the upper left box corner.
                                MOV     SI,OFFSET Save_Buffer     ; Point to our storage buffer.
                                MOV     CX,4                      ; Load number of lines to save.

Display_Critical_Error_180:
                                PUSH    CX                        ; Save line count.
                                MOV     CL,DL                    ; Load Box width into CL.
                                XOR     CH,CH                    ; Clear the high order byte.
                                PUSH    DI                        ; Save pointer to beginning of line.
                                REP     MOVSW                     ; Save the line in the buffer.
                                POP     DI                        ; Restore pointer to beginning of line.
                                ADD     DI,160                    ; Point to the next line.
                                POP     CX                        ; Restore the line count.
                                LOOP    Display_Critical_Error_180 ; Continue until done.
                                POP     AX                        ; Restore the critical error processing code.
                                CMP     _Prompt_Disk,0           ; Are we prompting for a new disk?
                                JE      Display_Critical_Error_190 ; If no, jump.

```

```

MOV      AX,RETRY                ; Signal retry.
Display_Critical_Error_190:
POP      ES                      ; Restore Registers.
POP      SI
POP      DI
POP      DX
POP      CX
POP      BX

POP      BP
RET
_Display_Critical_Error  ENDP

END

```

C. MAKE file for RIA Program

```

*-----*
* | Make file for the RIA Program Modules.
*-----*
* | Works using Borland's Make program.
* | To Compile RIA.EXE shell, you must have the
* | Spontaneous Assembly Library Version 2.0
* | or greater (Base Two Development, 11 East 200 North,
* | OREM, Utah 84057 - 801-222-9500)
* |
*-----*
.autodepend
# Path to C libraries.
LPCPATH=d:\c\lib
#
# Defines for Assembly Modules.
# RIA.EXE uses Spontaneous Assembly Library, version 2.0
#
ASM = D:\C\BIN\TASM
ASM_INCLUDE_PATH = D:\MASM\INCLUDE
ASM_LPCPATH = .\LIB
ASM_LIB = SAS.LIB
ASM_START = .\LIB\START.S.OBJ
ASM_SWITCHES = /mx /D_model=small
/$(ASM_INCLUDE_PATH)

RIA_OBJ = RIA.OBJ
RIADESC_OBJ = RIADESC.OBJ
RIALIB_OBJ = 123.obj allocat.obj beep.obj die.obj drwbox.obj
getunam.obj \
    harderr.obj inputstr.obj pnewdisk.obj readdesc.obj \
    savedesc.obj scrnsu.obj strdel.obj strins.obj \
    winbox.obj winrest.obj winsav.obj \
    ststore.obj getfile.obj centext.obj overwrit.obj \
    copyfile.obj critical.obj

#
# C modules compile using Borland C/C++ Version 3.1
#
SWITCHES= -ms -w -N -c
# Add -v for source level debugging
CC = BCC

#
# Compiles using PowerBasic Compiler, version 3
#
BASCOMP=C:\POWERBAS\PBC
# Directory for BASIC include files.
BASINC=

default: rialib.lib ria.exe fixit.exe riacopy.exe riadesc.exe \

```

```

riadir.exe riakill.exe

#
# Implicit Rules
#
.c.obj:
$(CC) $(SWITCHES) $*.c

.asm.obj:
$(ASM) $(ASM_SWITCHES) $*.asm

#
# Explicit Rules
#
riacopy.exe: riacopy.obj rialib.lib
tlink @&&|
$(LPCPATH)\c0s+
$**
$*

$(LPCPATH)\cs+
rialib
|

riadir.exe: riadir.obj rialib.lib
tlink @&&|
$(LPCPATH)\c0s+
$**
$*

$(LPCPATH)\cs+
rialib
|

riakill.exe: riakill.obj rialib.lib
tlink @&&|
$(LPCPATH)\c0s+
$**
$*

$(LPCPATH)\cs+
rialib
|

fixit.exe: fixit.bas
$(BASCOMP) -DSS$(BASINC) -ce fixit.bas

ria.exe: $(RIA_OBJ)
tlink @&&|
$(ASM_LPCPATH)\STARTS.OBJ+
$**
$*

```

```

$(ASM_LPATH)\$(ASM_LIB)
|

riadesc.exe: $(RIADDESC_OBJS) rialib.lib
tlink @&&|
$(LPATH)\c0s+
$**
$*

$(LPATH)\cs+
rialib
|

lacdata.exe: lacdata.obj combndlr.obj rialib.lib
tlink @&&|
$(LPATH)\c0s+
$**
$*

rialib+
$(LPATH)\cs+
$(LPATH)\EMU+
$(LPATH)\MATHS
|

rialib.lib: $(RIALIB_OBJS)
&tlib rialib -+ $?
#
# End of Make File : RIA.MAK
#

```

D. Batch files to run RIA Program

1. RIA.BAT - Main Batch File to Run RIA Program

```

@ECHO OFF
REM *-----*
REM | RIA.BAT - RIA Program version 1.1 |
REM *-----*
REM | This batch file runs the RIA program. This 'shell' is |
REM | required because our machine needs to reboot to |
REM | activate the external 5-1/4' 360K floppy drive. |
REM | The internal 5-1/4' drive make 360K disks |
REM | unreadable. |
REM | This file should not be in the RIA Programs |
REM | directory and on the PATH. |
REM | |
REM | This Batch file requires the +DOS Command Shell |
REM | which is a shareware command shell that can be |
REM | found at: |
REM | ftp://simtel.net/simtelnet/mados/+dos |
REM *-----*
c:\lotus\lac\programs\ria.exe
IF ERRORLEVEL=15 GOTO Start
REM *-----*
REM | If RIA.EXE did not return 15, then we don't need |
REM | to reboot, so we are done. |
REM *-----*
ECHO RIA data processing completed.
QUIT
:Start
REM *-----*
REM | Draw the main window (Full Screen) |
REM *-----*

```

```

DRAWBOX 0 0 24 79 1 bright cyan on blue fill blue
REM *-----*
REM | Put the title on the window. |
REM *-----*
SCREEN 0 30
COLOR bright cyan on blue
ECHO %@CHAR[180]
SCREEN 0 31
COLOR bright yellow on blue
ECHO Steroid RIA Program
COLOR bright cyan on blue
SCREEN 0 50
ECHO %@CHAR[195]
REM *-----*
REM | Draw the reboot window, and display message |
REM *-----*
DRAWBOX 4 8 6 72 1 bright white on red fill red
SCREEN 5 10
COLOR bright white on red
ECHO The machine needs to reboot to use the 360K external
drive.
REM *-----*
REM | Draw the instructions window, and fill it in |
REM *-----*
DRAWBOX 9 2 13 77 2 red on white fill white
SCREEN 10 5
COLOR Blue on white
ECHO 1) Please turn on the external drive. (The switch is on the
left rear).
SCREEN 12 5
ECHO 2) Push 'Y' to run RIA program. (Push 'N' to abort.)
REM SCREEN 14 5
:Get_Key
REM *-----*
REM | Get the user's input (y' or 'n') |
REM *-----*
SCREEN 12 75
INKEY %temp
IF %temp%=='n' GOTO Abort
IF %temp%=='Y' GOTO Set_360
REM *-----*
REM | Invalid Key pressed. Beep then wait for |
REM | another key |
REM *-----*
BEEP
GOTO Get_Key
:Set_360
REM *-----*
REM | Set up system to reboot to activate |
REM | the external 360K drive |
REM *-----*
C:
CD \System
REM *-----*
REM | Copy 360K CMOS system configuration into |
REM | file used by ROM.COM. |
REM *-----*
COPY CMOS_360.DAT CMOSROM.DAT >NUL
REM *-----*
REM | Copy the new AUTOEXEC.BAT to run the RIA |
REM | program after rebooting. A copy of the normal |
REM | AUTOEXEC.BAT file must be in the C:\SYSTEM |
REM | directory and it will be overwritten and lost |
REM | otherwise. |
REM *-----*
COPY AUTORIA.BAT \AUTOEXEC.BAT >NUL
REM *-----*
REM | Tell ROM.COM to load the 360K system configuration |

```

```

REM | in CMOSROM.DAT in CMOS, then reboot.
REM | switches: -r Read data from CMOSROM.DAT
REM | and store in CMOS.
REM | -b Reboot computer when done.
REM | ROM.COM can be obtained from the SIMTEL archive:
REM | ftp://simtel.net/simtelnet/mdos/sysuul/rom2.zip
REM *-----*
ROM -r -b
REM *-----*
REM | Should never get to this point. |
REM *-----*
COLOR bright cyan on blue
ECHO Program C:\SYSTEM\ROM.COM is apparently not
present:
ECHO Find another copy or obtain via ftp from
ECHO ftp://simtel.net/simtelnet/mdos/sysuul/rom2.zip
COLOR bright white on blue
BEEP
QUIT
:abort
REM *-----*
REM | Abort program. Do not reboot. |
REM *-----*
COLOR bright white on blue
CLS
COLOR blink bright red on blue
ECHO RIA program aborted by user.
COLOR bright white on blue
BEEP
QUIT

```

2. AutoRIA.BAT - Autoexec.Bat File Used When Rebooting to Initialize 360K Drive

```

@ECHO OFF
REM *-----*
REM | AUTORIA.BAT - RIA Program version 1.1
REM *-----*
REM | This batch file is renamed to AUTOEXEC.BAT after by
REM | RIA.BAT and is run after the machine reboots.
REM |
REM | This program should be located in C:\SYSTEM
REM |
REM | This Batch file requires the 4DOS Command Shell
REM | which is a shareware command shell that can be
REM | found at:
REM | ftp://simtel.net/simtelnet/mdos/4dos
REM *-----*
REM *-----*
REM | Clear the screen and set the border color.
REM *-----*
C:\DOS\Video Clear Border
C:\DOS\COLORReg 00 00 43 00
REM *-----*
REM | Set the keyboard repeat rate.
REM *-----*
C:\DOS\FastKey /C /1
ECHO System Setup:
ECHO Keyboard Speed Set.
REM *-----*
REM | Turn the number lock off.
REM *-----*
C:\DOS\NUMOFF
ECHO Number Lock turned off.
REM *-----*
REM | Load the system aliases.

```

```

REM *-----*
ALIAS /R C:\System\Alias.Dat
ECHO System aliases loaded.
REM *-----*
REM | Set up the ANSI keyboard remapping.
REM *-----*
C:\DOS\ANSI -{0;134;C:\Batch\Menu.Bar;13p
C:\DOS\ANSI -{0;133;C:\Batch\tools FFIND *p
ECHO ANSI Keyboard assignments loaded.
C:\DOS\Video on
REM *-----*
REM | Switch to the C: disk and Users directory.
REM *-----*
C:
CD C:\USERS
C:\DOS\Video clear border
REM *-----*
REM | Load type ahead buffer.
REM *-----*
C:\SYSTEM\VISITYPE.COM
REM *-----*
REM | Load the screen saver with a RAM wedge.
REM *-----*
C:\DOS\RamLoad ScrnSave.Com
C:\DOS\ScrnSave /D 3 -63
C:\DOS\video Clear Border
REM *-----*
REM | Set the environment variables, path and prompt.
REM *-----*
PROMPT SE[0;30;46m$P$C$E[1;37;44m
SET PATH=C:\4dos;C:\DOS;C:\BATCH;C:\NORTON;C:\Win3;
C:\Lotus;C:\NC;C:\WORD;
SET TEMP=C:\WIN3\TEMP
C:\4DOS\Palette North
REM *-----*
REM | Run the menu program.
REM *-----*
C:
CD \Users
\system\runria.bat

```

3. RUNRIA.BAT - Runs RIA Program after Rebooting to use 360K Drive.

```

@ECHO OFF
REM *-----*
REM | RUNRIA.BAT - RIA Program version 1.1
REM *-----*
REM | This batch file is launched from AUTOEXEC.BAT after
REM | rebooting to use the 360K external drive.
REM |
REM | This program should be located in C:\SYSTEM
REM |
REM | This Batch file requires the 4DOS Command Shell
REM | which is a shareware command shell that can be
REM | found at:
REM | ftp://simtel.net/simtelnet/mdos/4dos
REM *-----*
CD c:\lotus
REM *-----*
REM | Run the RIA program.
REM *-----*
c:\lotus\lsc\programs\ria.exe
REM *-----*
REM | Prompt the user to turn off the External Drive
REM | before we reboot.

```

```
REM *-----*
DRAWBOX 0 0 24 79 1 magenta on cyan fill cyan
DRAWBOX 3 5 5 75 1 yellow on blue fill blue
COLOR BRI WHITE ON BLUE
SCREEN 4 7
ECHO The machine needs to reboot to return to its normal
      configuration.
DRAWBOX 9 2 13 77 2 white on red fill red
SCREEN 10 5
COLOR BRI WHITE ON RED
ECHO 1) Please turn off the external drive. (The switch is on the
      left rear).
SCREEN 12 5
ECHO 2) Push 'Y' to continue.
:Get_Key2
INKEY %%temp
IF %temp%=='y' GOTO Set_12M
BEEP
GOTO Get_Key2

REM *-----*
REM | Load the 1.2M ROM BIOS Data and reboot. |
REM *-----*
:Set_12M
C:
CD \System
COPY CMOS_12M.DAT CMOSROM.DAT >NUL
COPY autoexec.bat \autoexec.bat >NUL
ROM -r -b
```

E. RIA.EXE - Shell Program run from RIA.BAT.

```

;-----*
;| FILE: RIA.ASM |
;-----*
;| DESC: Shell to run the various RIA program modules.
;|
;| NOTES: Version 1.1
;|
;| To Compile RIA.EXE shell, you must have the
;| Spontaneous Assembly Library Version 2.0
;| or greater (Base Two Development, 11 East 200
;| North, Orem, Utah 84057 - 801-222-9500)
;-----*

```

```

include MODEL.INC
include CONSOLE.INC
include WINDOW.INC
include ECODES.INC
include FILEIO.INC

```

```
.codeseg lib
```

```

;-----*
;| External Procedures. |
;-----*
.extrn start:auto, exit:auto, exit_ok:auto
.extrn cget_chr:auto, console_init:auto, cput_str:auto
.extrn near_init:auto, near_malloc:auto
.extrn restore_screen:auto
.extrn save_screen:auto, screen_bufsize:auto
.extrn set_80cols:auto, set_backdropbuf:auto
.extrn win_bufsize:auto, win_create:auto
.extrn win_init:auto, win_move:auto
.extrn goto_xy:auto
.extrn get_chr:auto
.extrn exec_prog:auto
.extrn win_open:auto
.extrn shrink_prog:auto
.extrn sound_beep:auto
.extrn win_close:auto
.extrn win_select:auto
.extrn clr_region:auto
.extrn ch_dir:auto
.extrn set_drive:auto
.extrn set_directvideo:auto
.extrn open_h:auto
.extrn close_h:auto
.extrn Remove_F:auto
.extrn read_h:auto
.extrn write_h:auto
.ends

```

```
.dataseg
```

```

;-----*
;| Local Data |
;-----*
Option1 DB '1) Run a complete RIA (Dump data, do
DB 'calculations and',0
Option1a DB 'delete files',0
len1 EQU $ - Option1 - 1
Option2 DB '2) Just transfer data from floppy.',0
len2 EQU $ - Option2 - 1
Option3 DB '3) Transfer data from floppy and do some
DB 'calculations',0
len3 EQU $ - Option3 - 1
Option3a DB '(No files deleted)',0

```

```

len3a      EQU $ - Option3a - 1
Option4    DB '4) Do calculations only (No data from
            DB 'data transferred)',0
len4       EQU $ - Option4 - 1
Option4a   DB ' (No files deleted)',0
len4a      EQU $ - Option4a - 1
Option5    DB '5) Do calculations and delete files.',0
Option5a   DB ' (No data from data transporter)',0
len5       EQU $ - Option5 - 1
Option6    DB '6) Unload the data transporter.'
            DB '[Obsolete option]',0
Num_Selections EQU 6
Option_Table DW Option1, Option1a, Option2, Option3
            DW Option3a, Option4, Option4a
            DW Option5, Option5a, Option6
Num_Options EQU ($ - Option_Table) / 2
edit_prompt DB 'Do you want to edit any of the data files'
            DB '(y/n)?',0
len_edit    EQU $ - edit_prompt - 1
Prompt     DB 'Press the number (1-6) for you desired'
            DB 'options:',0
lenp       EQU $ - Prompt
Prompt_Handle DW ?
RIAVersion DB 'RIA.exe - Version 1.1'

scrbuf_addr DW ?
Menu_Handle DW ?
Edit_Handle DW ?
Memory_Error DB 0DH,0AH,'Error while allocating'
            DB 'memory.',0DH,0AH,0
Selection    DB ?
Lotus_Dir    DB 'C:\LOTUS',0
Time        DB 'C:\LOTUS\RIADATA\Timestamp.p',0
Return_Code  DB 0
Lotus_Keys   DB '/', ' ', 'r', 'a', 'u', 'r', 'o', 't', '2', '3', ':', 'y', 't', 'a', 0
edit_window  window < (80-len_edit-4)/2, (25-3)/2, len_edit+4, 3, BLUE*16+WHITE, >
win          window < 32, 8, 18, 6, 47h, 2, 2, 1, 2, WCURSOR_OFF >
menu_window  window < (80-len+4)/2, (25-Num_Options)/2, len+4+4, Num_Options + 2, RED*16+WHITE, >
Prompt_Window window < (80-lenp-4)/2, 2, lenp+4, 3, LTGRAY*16+RED, >

Command_Line    DB 128 DUP(0)
Command_Unload  DB 'c:\lotus\lsc\programs\unload.com',0
Command_Convert DB 'c:\lotus\lsc\programs\nounload.exe',0
Empty_Command_Line DB 0
Command_RIAdesc DB 'c:\lotus\lsc\programs\riadesc.exe',0
Command_RIAcopy DB 'c:\lotus\lsc\programs\riacopy.exe',0
RIACopy_Command_Line DB 0
Command_123     DB 'c:\lotus\123.exe',0
Command_Line_123 DB 0
Command_RIAfix  DB 'c:\lotus\lsc\programs\fixit.exe',0
RIAFix_Command_Line DB 0
Command_RIAsort DB 'c:\lotus\lsc\programs\riadir.exe',0
RIAsort_Command_Line DB 0
Command_RIAkill DB 'c:\lotus\lsc\programs\riakill.exe',0
RIAKill_Command_Line DB 0
RIA_Run_Code    DB 'C:\lotus\lsc\programs\Run_Code.RIA',0
.ends

```

```

IF NOT __TINY__
.stackseg
db 1024 dup(?) ; define a 1024 byte stack
.ends
ENDIF

```

```

;-----
:| PROCEDURE: MAIN
|

```

```

:|-----|
:|  DESC:   Main body of program.
:|
:|  IN:    DX segment address of PSP
:|
:|  ASSUMES: DS,ES @DATASEG (same as @CODESEG in TINY model)
:|          SS @STACKSEG (same as @CODESEG in TINY model)
:|-----|

```

```
.codeseg
```

```
IF __TINY__
```

```
assume cs:@codeseg, ds:@dataseg, es:@dataseg, ss:@dataseg
```

```
ELSE
```

```
assume cs:@codeseg, ds:@dataseg, es:@dataseg, ss:@stackseg
```

```
ENDIF
```

```
.public main
```

```
.proc main auto
```

```
MOV AX,@dataseg
MOV ES,AX
MOV DS,AX
```

```
CALL Initialize
CALL shrink_prog
CALL Time_Stamp
MOV SI,OFFSET RIA_Run_Code ; Point to the file which contains reboot info.
MOV AX,O_RDONLY ; Set attribute to read only.
.call open_h ; Open the file.
JC main_10 ; If can't open the file, then doesn't exist.
MOV CX,1 ; Ready 1 byte to read.
MOV SI,OFFSET Selection ; Point to the selection code.
MOV Return_Code,OFFH ; Load Return code to signal reboot.
.call read_h ; Read the selection.
JE main_5 ; If just rebooted, then jump.
MOV Return_Code,0 ; Set Return code for no need to reboot.
```

```
main_5:
```

```
.call close_h ; Close the code file.
MOV SI,OFFSET RIA_Run_Code ; Point to the code file name.
.call remove_f ; Delete the code file.
CMP Return_Code,OFFH ; If we just rebooted, then jump over rest.
JE main_25
```

```
main_10:
```

```
CALL Draw_Menu
CALL Get_Selection
MOV BX,Menu_Handle
CALL win_close
CMP Selection,6
JNE main_20
CALL Dump_DTP
MOV DI,scrbuf_addr ;ES:DI->screen buffer address
CALL restore_Screen
JMP SHORT main_10
```

```
main_20:
```

```
CALL Test_Reboot
```

```
main_25:
```

```
;
; If requested, copy files from floppy.
;
```

```
CALL RIACopy
MOV DI,scrbuf_addr ;ES:DI->screen buffer address
CALL restore_screen
```

```
;
; Sort the Data files.
;
```

```
CALL RIAsort
MOV DI,scrbuf_addr ;ES:DI->screen buffer address
```

```

CALL    restore_screen
;
; If required, run the Fixit program.
;
CALL    RIAfix
MOV     DI,scrbuf_addr      ;ES:DI->screen buffer address
CALL    restore_screen
;
; Sort the Data files.
;
CALL    RIAsort
MOV     DI,scrbuf_addr      ;ES:DI->screen buffer address
CALL    restore_screen
;
; Run Lotus
;
CALL    Run_Lotus
MOV     DI,scrbuf_addr      ;ES:DI->screen buffer address
CALL    restore_screen
;
; Describe created worksheets
;
CALL    RIAdesc
MOV     DI,scrbuf_addr      ;ES:DI->screen buffer address
CALL    restore_screen
;
; Kill data files if requested.
;
CALL    RIAkill
MOV     DI,scrbuf_addr      ;ES:DI->screen buffer address
CALL    restore_screen      ;restore original screen
;
; Kill time stamp file.
;
CALL    kill_stamp
MOV     AL, Return_Code
MOV     AH,4CH
INT     21H
.endp main

.proc    Test_Reboot auto
CMP     Selection,3
JC     Test_Reboot_10
MOV     AX, O_WRONLY OR O_CREAT OR O_TRUNC ; Set file open attributes.
MOV     SI, OFFSET RIA_Run_Code           ; Point to the RIA run code file name.
.call   open_h                            ; Create/Truncate it.
MOV     CX,1                             ; Load one byte to write.
MOV     SI,OFFSET Selection               ; Point to our selection.
.call   write_h                            ; Write the byte.
.call   close_h                            ; Close the code file.
MOV     AH,4CH                            ; Load DOS function to exit with Return code.
MOV     AL,0FH                            ; Load Return code.
INT     21H                                ; Exit
Test_Reboot_10:
RET                                         ; Don't need to reboot.
.endp    Test_Reboot

.proc    RIAcopy    auto
CMP     Selection,3
JC     RIAcopy_10

MOV     DI,OFFSET Command_RIAcopy
MOV     SI,OFFSET RIAcopy_Command_Line
CALL    exec_prog
RIAcopy_10:
RET
.endp    RIAcopy

```

```

*-----*
;| Procedure: RIAdesc |
;|-----|
;| This program runs the RIA worksheet description program. |
*-----*
.proc    RIAdesc auto
        CMP     Selection,2           ; Are we just unloading the data?
        JE      RIAdesc_1           ; If yes, then nothing to describe?
        CMP     Selection,6           ; Are we doing an old data dump?
        JE      RIAdesc_1           ; If yes, then nothing to describe.
        MOV     DI,OFFSET Command_RIAdesc ; Point to the RIA Description command line.
        MOV     SI,OFFSET Empty_Command_Line ; Give it an empty command line.
        CALL    exec_prog            ; Run the program.
RIAdesc_1:
        RET
.endp    RIAdesc

.proc    Time_Stamp auto
        MOV     SI,OFFSET Time
        MOV     AX,O_RDWR OR O_TRUNC OR O_CREAT
        CALL    Open_H
        CALL    Close_H
        RET
.endp    Time_Stamp

.proc    Kill_Stamp auto
        MOV     SI,OFFSET Time
        CALL    Remove_F
        RET
.endp    Kill_Stamp

.proc    Dump_DTP auto
        MOV     DI,OFFSET Command_Unload
        MOV     SI,OFFSET Empty_Command_Line
        CALL    exec_prog
        MOV     DI,OFFSET Command_Convert
        MOV     SI,OFFSET Empty_Command_Line
        CALL    exec_prog
Dump_DTP_10:
        RET
.endp    Dump_DTP

.proc    RIAsort auto
        MOV     DI,OFFSET Command_RIAsort
        MOV     SI,OFFSET RIAsort_Command_Line
        CALL    exec_prog
RIAsort_10:
        RET
.endp    RIAsort

.proc    RIAkill auto
        CMP     Selection,1
        JE      RIAkill_5
        CMP     Selection,5
        JNE     RIAkill_10
RIAsort_5:
        MOV     DI,OFFSET Command_RIAkill
        MOV     SI,OFFSET RIAkill_Command_Line
        CALL    exec_prog
RIAsort_10:
        RET

```

```

.endp   RIAkill

.proc   Run_Lotus auto
        CMP     Selection,2
        JE      Run_Lotus_10
        CMP     Selection,6
        JE      Run_Lotus_10
        MOV     SI,OFFSET @dataseg:Lotus_Dir
        MOV     AL,BYTE PTR [SI]
        CALL    set_drive
        CALL    ch_dir
        MOV     DI,OFFSET Command_123
        MOV     SI,OFFSET Command_Line_123
        CALL    exec_prog
Run_Lotus_10:
        RET
.endp   Run_Lotus

.proc   RIAfix auto
        CMP     Selection,2
        JE      RIAfix_10
        CMP     Selection,6
        JE      RIAfix_10
        MOV     BX,@dataseg:Edit_Handle
        CALL    win_open
        CALL    win_select
        XOR     AX,AX
        INC     AL
        CALL    goto_xy
        MOV     SI,OFFSET @dataseg:Edit_Prompt
        CALL    cput_str
RIAfix_5:
        CALL    get_chr
        CMP     AL,'y'
        JE      RIAfix_8
        CMP     AL,'Y'
        JE      RIAfix_8
        CMP     AL,'n'
        JE      RIAfix_10
        CMP     AL,'N'
        JE      RIAfix_10
        MOV     AX,110
        MOV     DX,10
        CALL    sound_beep
        JMP     RIAfix_5
RIAfix_8:
        MOV     BX,@dataseg:Edit_Handle
        CALL    win_close
        MOV     DI,OFFSET Command_RIAfix
        MOV     SI,OFFSET RIAfix_Command_Line
        CALL    exec_prog
RIAfix_10:
        MOV     BX,@dataseg:Edit_Handle
        CALL    win_close
        RET
.endp   RIAfix

.proc   Get_Selection auto
Get_Selection_10:
        CALL    get_chr
        JC      Get_Selection_20
        CMP     AL,'1'
        JL     Get_Selection_20
        CMP     AL,'1'+ Num_Selections

```

```

    JGE     Get_Selection_20
    SUB     AL,0
    MOV     Selection,AL
    RET

Get_Selection_20:
    MOV     AX,110
    MOV     DX,10
    CALL    sound_beep
    JMP     SHORT Get_Selection_10
.endp     Get_Selection

.proc     Draw_Menu auto
    MOV     BX,@dataseg:Prompt_Handle
    CALL    win_open
    CALL    win_select
    XOR     AX,AX
    INC     AL
    MOV     SI,OFFSET @dataseg:Prompt
    CALL    Goto_XY
    CALL    CPUT_STR
    MOV     BX,@dataseg:Menu_Handle
    CALL    win_open
    CALL    win_select
    MOV     AH,0
    MOV     AL,1
    MOV     CX,Num_Options
    XOR     BX,BX
Draw_Menu_10:
    MOV     SI,Option_Table[BX]
    PUSH    AX
    PUSH    BX
    CALL    Goto_XY
    CALL    CPUT_STR
    POP     BX
    ADD     BX,2
    POP     AX
    INC     AH
    LOOP   Draw_Menu_10
    RET
.endp     Draw_Menu

;-----*
;| Procedure: Initialize |
;-----*
;| Initializes Console, windowing system, |
;-----*

.proc     Initialize auto
    MOV     AX,8000 ; ask for all available heap space
    CALL    near_init ; init the near heap
    CALL    console_init ; init the console i/o system
    CALL    set_directvideo
    CALL    screen_bufsize ; get size of screen buffer
    CALL    near_malloc ; error allocating screen buffer?
    JC     Initialize_70 ; y: exit without screen restore
    MOV     scrbuf_addr,di ; n: save screen buffer address
    CALL    save_screen ; and save the screen
    CALL    set_80cols ; force 80 column mode if not already
    MOV     AX,WORKBUF_SIZE ; window system work buffer size
    CALL    near_malloc ; error allocating work buffer?
    JC     Initialize_70 ; y: exit
    CALL    win_init ; n: init windowing system
    MOV     DI,scrbuf_addr ; ES:DI->screen buffer address
    CALL    set_backdropbuf ; use screen as backdrop
    MOV     SI,offset @dataseg:Menu_Window
    CALL    win_bufsize ; get buffer size for WIN
    CALL    near_malloc ; error allocating WIN buffer?
    JC     Initialize_70 ; y: exit

```

```

CALL    win_create          ; n: create WIN
MOV     @dataseg:Menu_Handle, BX
MOV     SI, offset @dataseg:Prompt_Window
CALL    win_bufsize        ; get buffer size for WIN
CALL    near_malloc        ; error allocating WIN buffer?
JC      Initialize_70      ; y: exit
CALL    win_create          ; n: create WIN
MOV     @dataseg:Prompt_Handle, BX
MOV     SI, offset @dataseg:edit_window
CALL    win_bufsize        ; get buffer size for WIN
CALL    near_malloc        ; error allocating WIN buffer?
JC      Initialize_70      ; y: exit
CALL    win_create          ; n: create WIN
MOV     @dataseg>Edit_Handle, BX
CALL    win_close          ; Make sure window is closed.

Initialize_60:
RET
Initialize_70:
MOV     SI, OFFSET @dataseg:Memory_Error
CALL    CPUT_STR
MOV     AX, 1
CALL    EXIT
.endp   Initialize

.ends

```

```

; *-----*
; | Stack normalization and memory management initialization labels
; |
; | NOTE: These declarations must remain after the declaration of the stack
; | and anything in the stack segment. These labels define the end of the
; | stack and the program, which is where the near and far heaps are placed
; | by default. These declarations do not affect the size of the program and
; | may be left here even if the stack is not normalized and the heaps are
; | not used.
; *-----*

```

```

.public  nheap_default, fheap_default
IF NOT __TINY__
.stackseg
IF __SMALL__ OR __MEDIUM__
.public  stack_end          ;used by START to normalize stack
.label  stack_end  word ;must be defined past entire stack
ENDIF
.label  nheap_default  word ;used by the near heap
.label  fheap_default  word ;used by the far heap
.ends
ELSE
_BSEND  segment  byte public STACK
.label  nheap_default  word ;used by the near heap
.label  fheap_default  word ;used by the far heap
_BSEND  ends
% @codeseggroup_BSEND
ENDIF

```

end start ;specify START as starting address

F. LSCDATA.EXE - Data Collection Program.

1. Header File for LSCDATA.C

```

#ifndef _LSCDATA_H_
#define _LSCDATA_H_
/*-----*
// | SOURCE FILE: LSCDATA.H
// *-----*

```

```

// | Header file for LSCDATA.C - Data Collection
// |
// | RIA program version 1.1
// *-----*
// |
// | Prototypes for com/hndlr.asm functions.
// *-----*
extern void Disable_INT_Handler( void );

```

```

extern void Enable_INT_Handler( void );
extern int Display_Critical_Error( char * );
// *-----*
// |           Function prototypes           |
// *-----*
void Initialize_Video( void );
void Capture_Interrupt( void );
void Allocate_Buffers( void );
void Clear_Screen ( void );
void Move_To_Output_Buffer( void );
unsigned int Length_Data( unsigned int , unsigned int );
unsigned int Scan_for_NULL( int );
void Display_Waiting ( void );
int Done( void );
void Signal_OK( void );
void Error_Exit( char *, char * );
void Write_Data( void );
int Get_Code( unsigned int );
void Move_Data_Up ( unsigned int );
void Reset_Timer( void );
time_t Elapsed_Time( void );
void Open_File( int , unsigned int );
void No_NULL_Found ( unsigned int );
void Display_Bytes_Received( void );
int draw_box( int, int, int, int );
void Display_Free( void );
void promptForNewDisk( void );
// *-----*
// |           Global Constants           |
// *-----*
#define TRUE -1
#define FALSE 0
#define INPUT_UL_X 4
#define INPUT_UL_Y 4
#define INPUT_LR_X 22
#define INPUT_LR_Y 22
#define IGNORE 0
#define RETRY 1
#define ABORT 2
// *-----*
// | Data from Prompt for Disk           |
// *-----*
#define RESET_DRIVE 0
#define VERIFY_SECTORS 4
#define HEAD 0
#define DISK 0
#define TRACK 0
#define SECTOR 1
#define NUMBER_SECTORS 1
#define BOX_UL_X 6
#define BOX_UL_Y 11
#define BOX_LR_X 73
#define BOX_LR_Y 13
// *-----*
// | Data for Waiting for data           |
// *-----*
#define Wait_X1 9
#define Wait_X2 9+64
#define Wait_Y1 9
#define Wait_Y2 9+4
#endif

2. LSC Raw Data Collection
   Program - LSCDATA.
// *-----*
// | SOURCE FILE: LSCDATA.C           |
// *-----*
// | Collects data from the Beckman LSC |
// *-----*
#include <stdlib.h>
#include <stdio.h>
#include <dos.h>
#include <dir.h>
#include <conio.h>
#include <time.h>
#include <string.h>
#include <bios.h>
#include <ctype.h>
#include 'lscdata.h'
#include 'rialib.h'
// *-----*
// |           Program - LSCdata.Exe (Version 1.2)
// *-----*
// | Gets data from the Beckman LSC, extracts the needed info,
// | and stores
// | it to disk. To try to minimize data loss due to power
// | outages, the data is stored after 15 second waits, and the
// | file is closed until the next data is ready.
// |
// | Needs lscdata.h and comhndlr.asm.
// |
// | Compiled using Borland C/C++ 3.1.
// |
// | Version 1.1 - Fixed bug during wrapping of COM buffer.
// | Version 1.2 - Try to fix mysterious bug where file start
// | code (200,BE) gets repeated causing a user
// | number of 20.
// *-----*
#ifdef DEBUG
#include <fcntl.h>
#include <sys/stat.h>
int Input_Handle;
FILE *debug_file;
#endif
#ifdef DEBUG
#define WAIT 0
#else
#define WAIT 15
#endif
#endif
#define BUFFER_SIZE 10240

char *COM_Buffer,
      *Ourput_Buffer,
      Output_Path[80]="",
      COM_Data_Pending=0,
      Output_Data_Waiting=0,
      Output_File_Name[13]="",
      Prompt_Disk=0,
      Assay_Name[13];

int Page,
    Columna,
    Mode,
    User_Number=0,
    Use_Code[14]={ 0,0,0,0,0,0,0,0,0,0,0,0,0,0 },
    Window_Line=1;

unsigned char Normal_Attrib;
unsigned int COM_Head=0,
            COM_Tail=0,
            COM_Errors=0,
            REC_Code=0,
            REC_Sub_Code=0,
            Cluster_Size,

```

```

Records,
UNK_Code=0,
REC_Records=0,
Check_Sum_Error,
Output_Tail=0;
unsigned long Bytes_Received=0,
Bytes_Free;
struct diskfree_t Disk_Data;
#ifdef DEBUG
int Drive=1;
#else
int Drive=0;
#endif
char Disk_Drive[3]='a:';
char far *Screen;
time_t Last_Time=0;
FILE *Output_File;

/* ----- */
/* | PROCEDURE: main |
/* ----- */
/* | PURPOSE: Main module of RIA data collection program |
/* | ON CALL: Nothing. |
/* | RETURNS: Nothing |
/* ----- */
void
main() {
    unsigned defaultdrive;

    _dos_getdrive( &defaultdrive );
    if ( Drive == 0 ) Drive = defaultdrive;
    if ( Drive != 1 )
        Disk_Drive[0] = 'a' + Drive - 1;
    Output_File = NULL;
    Allocate_Buffers();
    Capture_Interrupts();
    /* ----- */
    /* | Shouldn't have to exit, except on error | */
    /* ----- */
    while ( TRUE ) {
        promptForNewDisk();
        Reset_Timer();
        /* ----- */
        /* | Continue until the F10 key is pressed | */
        /* ----- */
        while ( !Done() ) {
            if ( COM_Data_Pending )
                Move_To_Output_Buffer();
            else
                if ( Output_Data_Waiting )
                    if ( Elapsed_Time() > WAIT )
                        Write_Data();
                        Reset_Timer();
                Write_Data();
                /* | Waits until the F10 key is pressed again |
                Display_Waiting();
            }
        }
    }
}
/* End Main */
/* ----- */

```

```

/* | PROCEDURE: Display_Status |
/* ----- |
/* | PURPOSE: Displays current status of current function on |
/* | screen |
/* | ON CALL: code - code for current action |
/* | RETURNS: Nothing |
/* ----- |
void
Display_Status( int Code ) {
    char Scratch[80];
    int x,y, Center;

    x = wherex();
    y = wherey();
    memset(Scratch, '\0', 45);
    memset(Scratch+45, 0, 1);
    gotoxy( 33, 16 );
    cputs( Scratch );
    switch (Code) {
        case 0:
            strcpy( Scratch, 'Initializing' );
            break;
        case 200:
            strcpy( Scratch, 'Beginning new ' );
            if ( User_Number == 6 )
                strcat( Scratch, 'RECOVERY file.' );
            else
                strcat( Scratch, Assay_Name );
            strcat( Scratch, ' file.' );
            break;
        case 100:
            strcpy( Scratch, 'Extracting RECOVERY
            record.' );
            break;
        case 52:
            strcpy( Scratch, 'Extracting ' );
            strcat( Scratch, Assay_Name );
            strcat( Scratch, ' replicate average.' );
            break;
        case 999:
            strcpy( Scratch, 'Data file closed. Waiting for
            data.' );
            break;
        default:
            strcpy( Scratch, 'Skipping unneeded data.' );
            break;
    }
    Center = 33 + (45 - strlen(Scratch)) / 2;
    gotoxy( Center, 16 );
    cputs( Scratch );
    gotoxy( x, y );
}

/* ----- */
/* | PROCEDURE: Display_Free |
/* ----- |
/* | PURPOSE: Displays free space on the data disk. |
/* | ON CALL: Nothing |
/* | RETURNS: Nothing |
/* ----- |
void
Display_Free() {
    int x,y,i,j,l,p=19,s;
    char Scratch[21];
    x = wherex();
    y = wherey();
    gotoxy( 63, 23 );
}

```

```

sprintf( Scratch, "%10lu", Bytes_Free );
l = strlen( Scratch );
s = l-1;
Scratch[20] = 0;
for ( i=l-1; i>=0; i-3 ) {
    for ( j = 0; j<3; j++ ) {
        if ( Scratch[s] == '' )
            {
                i -= 1;
                j = 4;
            }
        else
            Scratch[p-] = Scratch[s-];
    }
    if ( i>=0 && Scratch[s] != '' )
        Scratch[p-] = '';
}
for ( i = 0; i <= p; i++ )
    Scratch[i] = '';
cputs( Scratch + 10 );
gotoxy( x, y );
}

```

```

// *-----*
// | PROCEDURE: Display_Filename |
// |-----|
// | PURPOSE: Display the filename for file created. |
// | ON CALL: filename - name of file created. |
// | RETURNS: Nothing |
// *-----*

```

```

void
Display_Filename( char *filename ) {
int x, y;
x = wherex();
y = wherey();
window( INPUT_UL_X, INPUT_UL_Y, INPUT_LR_X,
INPUT_LR_Y );
gotoxy( 2, Window_Line );
cprintf( "%s\n", filename );
gotoxy( x, y );
window( 1, 1, 80, 25 );
if ( Window_Line != INPUT_LR_Y - INPUT_UL_Y + 1 )
    ++Window_Line;
Display_Free();
}

```

```

// *-----*
// | PROCEDURE: Display_Num_Records |
// |-----|
// | PURPOSE: Display the number of records in created file. |
// | ON CALL: Num_Records - Number of records in file. |
// | RETURNS: Nothing |
// *-----*

```

```

void
Display_Num_Records( int Num_Records ) {
int x, y;
x = wherex();
y = wherey();
window( INPUT_UL_X, INPUT_UL_Y, INPUT_LR_X,
INPUT_LR_Y );
gotoxy( 16, Window_Line - 1 );
cprintf( "%3d", Num_Records );
gotoxy( x, y );
window( 1, 1, 80, 25 );
Display_Free();
}

```

```

/*
-----
| Procedure: Move_To_Output_Buffer |
|-----|
| Moves data from the COM buffer to the Output buffer as a |
| series of NULL terminated strings. |
|-----|
| Parameters: (none) |
| Returned: (none) |
| Variables: unsigned int Head COM buffer head on call. |
|             unsigned int Tail COM buffer tail on call. |
|             unsigned int i Loop counter. |
|             unsigned int Pointer Pointer into COM buffer. |
|             unsigned char ch Current character. |
-----
*/

```

```

void
Move_To_Output_Buffer() {
unsigned int Head, Tail, i, Pointer;
unsigned char ch;
// *-----*
// | Disable interrupts while we copy current COM buffer |
// | head and tail, and clear the data pending flag. |
// *-----*
disable();
Head = COM_Head;
Tail = COM_Tail;
COM_Head = COM_Tail;
COM_Data_Pending = 0;
enable();
for ( i=0; i < Length_Data( Head, Tail ); i++ ) {
    if ( (Head + i) == BUFFER_SIZE )
        Pointer = Head + i - BUFFER_SIZE;
    else
        Pointer = Head + i;
    ch = COM_Buffer[Pointer];
    Bytes_Received++;
    if ( ( ch < 32 && ch != 0x0A ) || ch > 126 )
        /* If not normal character or linefeed */
        /* set ch to 0xFF so we won't move it */
        /* to the output buffer. */
        ch = 0xFF;
    else
        switch ( ch ) {
            case 0x0A:
                /* Set End of line to NULL so we
                // can easily access them later.
                ch = 0x00;
                break;
            default:
                break;
        } /* End switch */
        /* If it's a normal character, store it, else skip.
        if ( ch != 0xFF )
            Output_Buffer[Output_Tail++] = ch;
} /* End for loop */
Display_Bytes_Received();
++Output_Data_Waiting;
}

```

```

// *-----*
// | PROCEDURE: Display_Bytes_Received |
// |-----|
// | PURPOSE: Display bytes recieved in current file so far. |
// | ON CALL: NOTHING |
// | RETURNS: NOTHING |
// *-----*

```

```

// *-----*
void
Display_Bytes_Received() {
  int x,y;
  x = wherex();
  y = wherey();
  gotoxy( 68,2 );
  printf( "%10lu", Bytes_Received );
  gotoxy( x, y );
}

// *-----*
// | PROCEDURE: Length_Data |
// |-----|
// | PURPOSE: Returns the amount of data in the COM buffer. |
// | ON CALL: Head - Position of start of ring buffer |
// |           Tail - Position of end of ring buffer. |
// | RETURNS: Number of bytes waiting in the COM buffer. |
// *-----*
unsigned int
Length_Data( unsigned int Head, unsigned int Tail ) {
  if ( Head < Tail )
    return ( Tail - Head );
  else
    return ( BUFFER_SIZE + Tail - Head );
}

// *-----*
// | PROCEDURE: Scan_for_NULL |
// |-----|
// | PURPOSE: Find start of next line in COM buffer. |
// | ON CALL: Offset - Current position in buffer. |
// | RETURNS: Position of next line in COM buffer |
// |           zero if line end not found. |
// *-----*
unsigned int
Scan_for_NULL( int Offset ) {
  int i;

  for ( i=Offset; i<Output_Tail; i++ ) {
    if ( Output_Buffer[i] == 0 )
      return ( i + 1 );
  }
  return ( 0 );
}

// *-----*
// | PROCEDURE: Display_Waiting |
// |-----|
// | PURPOSE: Display message when disk is to be removed to |
// |           dump data. |
// | ON CALL: Nothing |
// | RETURNS: Nothing |
// *-----*
void
Display_Waiting() {
  char box_save [(Wait_X2 - Wait_X1 + 1) * (Wait_Y2 -
    Wait_Y1 + 1) * 2];

  gettext( Wait_X1, Wait_Y1, Wait_X2, Wait_Y2, box_save );
  highvideo();
  draw_box( Wait_X1, Wait_Y1, Wait_X2, Wait_Y2 );
  gotoxy( Wait_X1+3, Wait_Y1+1 );
  _setcursortype(_NOCURSOR);
  gotoxy( Wait_X1+3, Wait_Y1+1 );
  cputs( "Remove the disk from the drive and use RIA to copy
    the' );

  gotoxy( Wait_X1+3, Wait_Y1+2 );
  cputs( "data onto the Zenith for processing." );
  gotoxy( Wait_X1+3, Wait_Y1+4 );
  cputs( "Press F10 when ready to start again." );
  while ( !Done() ) {
  }
  puttext( Wait_X1, Wait_Y1, Wait_X2, Wait_Y2, box_save );
  _setcursortype(_NORMALCURSOR);
  lowvideo();
}

// *-----*
// | Procedure: Done |
// |-----|
// | Checks to see if a key was pressed, and returns 0 if no key |
// | pressed. If a key was pressed, and it wasn't F10, it beeps and |
// | returns 0 as well. If F10 was pressed, returns -1. |
// |-----|
// | Parameters: (none) |
// | Returned: 0 if F10 not pressed |
// |           -1 if F10 pressed. |
// | Variables: (none) |
// *-----*
int Done() {
  # define DoneX1 24
  # define DoneX2 56
  # define DoneY1 11
  # define DoneY2 13
  int keypressed, Bad_Key=1;
  char save_buf[(DoneX2-DoneX1+1) * (DoneY2-DoneY1+1)
  * 2];
  if ( kbhit() ) {
    if ( (keypressed=getch()) != 0 )
      {
        /* Non-extended key pressed, so make */
        if (keypressed == 27 )
          {
            gettext( DoneX1, DoneY1, DoneX2,
              DoneY2, save_buf );
            highvideo();
            draw_box( DoneX1, DoneY1, DoneX2,
              DoneY2 );
            gotoxy( 26, 12 );
            cputs( "Do you really want to abort?" );
            while ( Bad_Key ) {
              keypressed = getch();
              tolower(keypressed);
              switch ( keypressed ) {
                case 'n':
                  Bad_Key = 0;
                  break;
                case 'y':
                  Disable_INT_Handler();
                  exit(0);
                  break;
                default:
                  /* a noise, then return 0.
                  sound(110);
                  delay(200);
                  nosound();
                  break;
            }
            puttext( DoneX1, DoneY1, DoneX2,
              DoneY2, save_buf );
            lowvideo();
          }
        else

```

```

// a noise, then return 0.
sound(110);
delay(200);
nosound();
return ( 0 );
}
else
{
    if ( getch() != 68 )
    {
        // An extended key was pressed, but it
        // wasn't F10, so make a rude noise and
        // return 0.
        sound(110);
        delay(200);
        nosound();
        return ( 0 );
    }
    else
    {
        if ( COM_Data_Pending ||
Output_Data_Waiting )
        {
            // F10 was pressed, but we but there
            // is still data to process. Make a
            // rude noise and return 0.
            sound(110);
            delay(200);
            nosound();
            return ( 0 );
        }
        else
            // F10 key pressed, so signal done.
            return (-1);
    }
}
return(0);          /* If no key pressed, return 0 */
}

/* ----- */
/* | PROCEDURE: Get_Tokens |
/* | ----- |
/* | PURPOSE: Get the desired number of tokens from a string. |
/* | ON CALL: String - String to extract tokens from |
/* |           Num_Tokens - Number of tokens to extract |
/* |           Token - Array to store tokens in. |
/* | RETURNS: -1 if everything OK or |
/* |           zero if not enough tokens found. |
/* | ----- */
int
Get_Tokens( char *String, int Num_Tokens, char *Token[] ) {
int i;
if ( (Token[0] = strtok( String, ';' )) == NULL )
    return (0);
for ( i=1; i<Num_Tokens; i++ ) {
    if ( (Token[i] = strtok( NULL, ';' )) == NULL )
        return (0);
}
return (-1);
}

/* ----- */
/* | PROCEDURE: Error_Exit |
/* | ----- |
/* | PURPOSE: Prints an error message on the screen and exits. |
/* | ON CALL: Format - Formatting string, or Formatted error |

```

```

/* | ----- |
/* | message. |
/* | Name - Single parameter to use with format. |
/* | RETURNS: Does not return. |
/* | ----- */
void
Error_Exit( char *Format, char *Name ) {
window( 1, 1, 80, 25 );
gotoxy( 1, 25 );
if ( Name == NULL )
    printf( Format );
else
    printf( Format, Name );
Disable_INT_Handler();
exit( 1 );
}

/* ----- */
/* | PROCEDURE: Open_File |
/* | ----- |
/* | PURPOSE: Open recovery, unknown, or date file. |
/* | ON CALL: Code - file type to open. |
/* |           Offset - Current offset in output buffer. |
/* | RETURNS: Nothing |
/* | ----- */
void
Open_File( int Code, unsigned int Offset ) {
int Day_Code, Month_Code, i;
char *Data, U_Number[3] = "";
char *Wd, *Da, *Mo, *Yr, *Pointer;
char *Abriev_Month[] = { 'JAN',
                        'FEB',
                        'MAR',
                        'APR',
                        'MAY',
                        'JUN',
                        'JUL',
                        'AUG',
                        'SEP',
                        'OCT',
                        'NOV',
                        'DEC' };
char *Month[] = { 'January',
                  'February',
                  'March',
                  'April',
                  'May',
                  'June',
                  'July',
                  'August',
                  'September',
                  'October',
                  'November',
                  'December' };
char *Abriev_Weekday[] = { 'MON',
                            'TUE',
                            'WED',
                            'THU',
                            'FRI',
                            'SAT',
                            'SUN' };
char *Weekday[] = { 'Monday',
                    'Tuesday',
                    'Wednesday',
                    'Thursday',
                    'Friday',
                    'Saturday',
                    'Sunday' };
}

```

```

if( Code == 1 ){
    /*-----*/
    /*| This section opens a new recovery sub file. |*/
    /*-----*/
    /*| If this is not the recovery User Number (6) |
    /*| then something has gone really wrong. |
    /*-----*/
    if( User_Number != 6 )
        Error_Exit( "Bad call to subroutine
        Open_File()\n", NULL );
    if( REC_Records != 0 ){
        /*-----*/
        /*| Wrap the minor recovery number |
        /*| if needed. |
        /*-----*/
        if( REC_Sub_Code == 99 )
            REC_Sub_Code = 0;
        /*-----*/
        /*| Create the recovery file name. |*/
        /*-----*/
        fclose( Output_File );
        sprintf( Output_File_Name, "%sREC%02d-
        %02d.REC", Output_Path,
        ++REC_Sub_Code, REC_Code );
        Bytes_Free = Cluster_Size;
        Display_Filename( Output_File_Name );
        Records = 0;
        /*-----*/
        /*| Open the recovery file for output. |*/
        /*-----*/
        if( ( Output_File = fopen( Output_File_Name,
        "w" ) ) == NULL )
            Error_Exit( "I can not open the file *%s*
            recovery file (%s)
            !!!\n", Output_File_Name );
        REC_Records = 0;
    }
    return;
}
else
{
    Data = Output_Buffer + Offset;
    if( strlen( Data ) == 6 ){
        /*-----*/
        /*| Oops! We seem to have slipped on a |
        /*| bug. Try to get data from next line |
        /*-----*/
        /* Point to next line
        Data = Data + 7;
        /* Search for first comma in line.
        Pointer = strchr( Data, ',' );
        if( Pointer - Data > 2 || Pointer - Data < 1 ){
            /*-----*/
            /*| Death by Bunga!!!! |
            /*-----*/
            strcpy( U_Number, "13" );
        }
        else
            strcpy( U_Number, Data + Pointer - Data );
    }
    else
        strcpy( U_Number, Data + 2 );
    User_Number = atoi( U_Number );
    if( User_Number < 1 || User_Number > 10 )
        User_Number = 13;
    fclose( Output_File );
}

```

```

if( User_Number == 6 ) {
    if( REC_Code == 99 )
        REC_Code = 0;
    sprintf( Output_File_Name, "%sREC%02d-
    %02d.REC", Output_Path, 1,
    ++REC_Code );
    REC_Sub_Code = 1;
}
else
{
    if( Use_Code[User_Number] == 99 )
        Use_Code[User_Number] = 0;
    sprintf( Output_File_Name, "%sUNK%1d-
    %02d.DTE", Output_Path,
    User_Number, ++Use_Code[
    User_Number] );
}
Records = 0;
if( User_Number != 6 ){
    Bytes_Free = Cluster_Size;
    Display_Filename( Output_File_Name );
    if( ( Output_File = fopen( Output_File_Name,
    "w" ) ) == NULL )
        Error_Exit( "I can not open the file *%s*
        date file (%s) !!!\n",
        Output_File_Name );
    /* Strip off user number
    strtok( Data, " " );
    /* Strip off User number description
    Wd = strtok( NULL, " " );
    strcpy( Assay_Name, Wd );
    if( Wd == NULL )
        strcpy( Assay_Name, "ERROR" );
    for( i = 0; i < strlen( Assay_Name ); i++ ){
        if( Assay_Name[i] == ' ' )
            Assay_Name[i] = 0;
            i = 100;
    }
    strtok( NULL, " " ); // Strip off counting time
    /* Get the day of week abbreviation.
    Wd = strtok( NULL, " " );
    /* Get day of month
    Da = strtok( NULL, " " );
    if( Da == NULL )
        Da = "1";
    /* Get month abbreviation.
    Mo = strtok( NULL, " " );
    Yr = strtok( NULL, " " ); // Get the year
    if( Yr == NULL )
        Yr = "1980";
    for( i = 0; i < 7; i++ ){
        Day_Code = 0;
        if( !strcmp( Wd, Abriev_Weekday[i] ) ){
            Day_Code = i;
            i = 10;
        }
    }
    for( i = 0; i < 12; i++ ){
        Month_Code = 0;
        if( !strcmp( Mo, Abriev_Month[i] ) ){
            Month_Code = i;
            i = 15;
        }
    }
}
if( ( fprintf( Output_File, "%s\n%s\n%s\n%s\n",
    Weekday[Day_Code],
    Month[Month_Code], Da, Yr ) ) == EOF )

```

```

        Error_Exit("Error during creation of datefile
        (%s)\n", Output_File_Name );
        fclose( Output_File );
    }
    if ( User_Number == 6 )
    {
        REC_Records = 0;
    }
    else
        strcpy( Output_File_Name + strlen(
        Output_File_Name ) - 3, 'UNK' );
    Records = 0;
    Bytes_Free = Cluster_Size;
    Display_Filename( Output_File_Name );
    if ( (Output_File = fopen( Output_File_Name, 'w' ))
    == NULL )
        Error_Exit("I can not open the file! *%s output
        file (%s) !!!\n", Output_File_Name);
}

// *-----*
// | PROCEDURE: Write_Data |
// |-----|
// | PURPOSE: Write data to the disk |
// | ON CALL: Nothing |
// | RETURNS: Nothing |
// *-----*
void Write_Data() {
    unsigned int Next, Current=0, Data_Offset;
    char *Dose, *CPM, Scratch[20];

    Dose = NULL;
    /* if there really is no data, then return */
    if ( Output_Tail == 0 )
        return;
    if ( strlen(Output_File_Name) != 0 )
        if ( ( Output_File = fopen( Output_File_Name, 'a' ) ) ==
        NULL )
            Error_Exit("I can not open the file! *%s output file
            (%s) !!!", Output_File_Name);
    while ( TRUE ) {
        /* Scan for end of string */
        if ( (Next = Scan_for_NULL( Current )) == 0 ) {
            /*-----*/
            /* No terminating NULL found */
            /*-----*/
            No_NULL_Found( Current );
            return;
        }
        if ( strlen( Output_Buffer + Current ) == 6 ) {
            switch ( Get_Code( Current ) ) {
                case 200: /* Beginning of User Number
                */
                    Data_Offset = Next;
                    if
                    ((Next=Scan_for_NULL(Data_Offset))!= 0 ) {
                        /* No NULL so not complete line */
                        No_NULL_Found( Current );
                        return;
                    }
                    Open_File( 0, Data_Offset );
                    Display_Status( 200 );
                    break;
                case 999: /* End of User Number */
                    fclose( Output_File );
                    Output_File_Name[0] = 0;
                    Records = 0;
                    Display_Status( 999 );
                    break;
                case 52: /* Replicate Average UNK */
                    Data_Offset = Next;
                    if ( (Next = Scan_for_NULL(
                    Data_Offset )) == 0 ) {
                        /* No NULL so not complete line */
                        No_NULL_Found( Current );
                        return;
                    }
                    strtok( Output_Buffer + Data_Offset, '' );
                    /* Strip off CV */
                    strtok( NULL, '' ); /* Strip off % Bound */
                    if (Dose == NULL )
                        printf("Data_offset=%s",
                        Output_Buffer + Data_Offset);
                    Dose = strtok( NULL, '' );
                    sprintf( Output_File, "%s\n", Dose );
                    Bytes_Free = ( strlen( Dose ) + 2 );
                    Display_Num_Records( ++Records );
                    Display_Status( 52 );
                    break;
                case 100: /* Recovery Count */
                    Data_Offset = Next;
                    if ( (Next=Scan_for_NULL(Data_Offset))
                    == 0 ) {
                        /* No NULL so not complete line */
                        No_NULL_Found( Current );
                        return;
                    }
                    /* Strip out Sample Number */
                    strtok( Output_Buffer + Data_Offset, '' );
                    /* Strip off Position */
                    strtok( NULL, '' );
                    /* Strip off First selected channel # */
                    strtok( NULL, '' );
                    /* Get counts per minute. */
                    CPM = strtok( NULL, '' );
                    if ( atof( CPM ) > 1200.0 ) {
                        Display_Status( 200 );
                        Open_File( 1, 0 );
                    }
                    printf(Output_File, "%2f\n", atof(CPM) );
                    sprintf( Scratch, "%2f\n", atof( CPM ) );
                    Bytes_Free = strlen( Scratch );
                    Display_Num_Records( ++Records );
                    ++REC_Records;
                    Display_Status( 100 );
                    break;
                default:
                    Display_Status( 69 );
                    break;
            }
        }
        Current = Next;
    } /* End while loop. */
}

void
No_NULL_Found ( unsigned int Current ) {
    if ( Current != 0 )
        Move_Data_Up( Current );
    Output_Data_Waiting = 0;
    if ( Output_File != NULL ) {
        fclose ( Output_File );
    }
}

```

```

int Get_Code( unsigned int Code_Offset ){
    int Block_Code, Check_Sum, Calc_Sum=0, i;

    sscanf( Output_Buffer+Code_Offset, "%d,%X", &Block_Code,
            &Check_Sum );
    for ( i=0; i<4; i++ )
        Calc_Sum += Output_Buffer[Code_Offset+i];
    if ( Calc_Sum != Check_Sum )
        Check_Sum_Error++;
    return ( Block_Code );
}

void
Move_Data_Up ( unsigned int Start ) {
    int i;
    int j = 0;

    /* ----- */
    /* | Move the incomplete data to beginning of the buffer | */
    /* ----- */
    for ( i=Start; i < Output_Tail; i ++ )
        Output_Buffer[j++] = Output_Buffer[i];
    /* ----- */
    /* | Reset the Output Buffer tail pointer. | */
    /* ----- */
    Output_Tail = j;
}

/* ----- */
/* | Procedure: Reset_Timer | */
/* ----- */
/* | Resets the Last_Time variable | */
/* ----- */
/* | Parameters: (none) | */
/* | Returned: (none) | */
/* | Variables: time_t Last_Time Global variable | */
/* | containing | */
/* | | last time routine called. | */
/* ----- */
/*
void
Reset_Timer() {
    Last_Time = 0;
}

/* ----- */
/* | Procedure: Elapsed_Time | */
/* | ----- | */
/* | Determines how many seconds have elapsed since last call. | */
/* | ----- | */
/* | Parameters: (none) | */
/* | Returned: Elapsed time in seconds since last call. | */
/* | Variables: time_t Current_Time Current time in seconds. | */
/* | | time_t E_Time Elapsed time. | */
/* | | time_t Last_Time Global variable containing | */
/* | | last time routine called. | */
/* | ----- */
time_t
Elapsed_Time() {
    time_t Current_Time, E_Time;

    Current_Time = time( NULL );
    if ( Last_Time == 0 )
        Last_Time = Current_Time;
}

return (0);
}
else
{
    E_Time = Current_Time - Last_Time;
    return ( E_Time );
}
}

void
Initialize_Video( void ) {
    draw_box(1, 1, 80, 25);
    gotoxy( 33, 1 );
    cputs("LSCdata (1.2)");
    draw_box( 3, 3, 23, 23 );
    gotoxy( 5, 3 );
    cputs("Filename");
    gotoxy( 16, 3 );
    cputs("Records");
    gotoxy( 53, 2 );
    cprintf("Bytes Received:%10u.0 ");
    draw_box( 58, 19, 78, 24 );
    gotoxy( 60, 20 );
    cputs("Approximate Bytes");
    gotoxy( 62, 21 );
    cputs("Free on Disk");
    draw_box(32, 15, 78, 17);
    gotoxy( 50, 15 );
    cputs("Status");
    Display_Status( 0 );
    gotoxy(6,25);
    cputs("Press the F10 key when ready to remove the disk");
}

int
Control_Break() {
    /* a noise, then return 0. */
    sound(110);
    delay(200);
    nosound();
    return ( TRUE );
}

void
Capture_Interrupts() {
    _harderr( Hardware_Error );
    ctrlbrk( Control_Break );
    Enable_INT_Handler();
}

/* ----- */
/* | Procedure: Allocate_Buffers | */
/* | ----- | */
/* | Allocates serial input and output buffers | */
/* | ----- | */
/* | Parameters: (none) | */
/* | Returned: (void) | */
/* | Variables: char *COM_buffer Global pointer to the | */
/* | | allocated input buffer. | */
/* | | char *Output_Buffer Global pointer to the | */
/* | | allocated output buffer. | */
/* | ----- */
void
Allocate_Buffers() {
}

```

```

if ((COM_Buffer = (char *)malloc(BUFFER_SIZE)) ==
    NULL) {
    fprintf(stderr, Fatal error: Unable to allocate serial
    communications buffer.\n");
    exit (100);
}
if ((Output_Buffer = (char *)malloc(BUFFER_SIZE)) ==
    NULL) {
    fprintf(stderr, Fatal error: Unable to allocate output
    buffer.\n");
    exit (100);
}

void promptForNewDisk() {
    struct fblk File_Block;
    FILE *dummy;
    char box_save[( BOX_LR_X-BOX_UL_X + 2) * (BOX_LR_Y
    - BOX_UL_Y + 2) * 2 ];
    char Search_String[20];

    Bytes_Received=0;
    Window_Line=1;
    Initialize_Video();
    // *-----*
    // | Set global variable to tell error handling routine |
    // | that we will want to prompt for a disk if none |
    // | is in the drive. |
    // *-----*
    #pragma warn -aus // Stop annoying warning
    Prompt_Disk = 1;
    #pragma warn +aus
    // *-----*
    // | Check for disk in drive, and if not there. |
    // | force an error so we can get the user to |
    // | Put it in. |
    // *-----*
    strcpy(Search_String, Disk_Drive);
    strcat(Search_String, "lscdisk.txt");
    while ((dummy=fopen ( Search_String, "w" )) == NULL)
        ; /*Loop until we found disk */
    fclose (dummy);
    unlink ( Search_String );
    strcpy(Search_String, Disk_Drive);
    strcat ( Search_String, "lscdata.exe");
    while ( !findfirst( Search_String , &File_Block, 0 )) {
        gettext( BOX_UL_X, BOX_UL_Y, BOX_LR_X,
        BOX_LR_Y+1, box_save );
        highvideo();
        draw_box( BOX_UL_X, BOX_UL_Y, BOX_LR_X,
        BOX_LR_Y+1 );
        gotoxy( BOX_UL_X+3, BOX_UL_Y+1 );
        _setcursortype(_NOCURSOR);
        cputs( ' This is the LSC data acquisition program
        disk.' );
        gotoxy( BOX_UL_X+3, BOX_UL_Y + 2 );
        cputs( 'Please insert a blank formatted disk, then
        push the ENTER key.' );
        while ( getch() != 13 ) {
            sound(110);
            delay(200);
            nosound();
        }
        puttext( BOX_UL_X, BOX_UL_Y, BOX_LR_X,
        BOX_LR_Y+1, box_save );
        _setcursortype(_NORMALCURSOR);
        lowvideo();
        biosdisk( RESET_DRIVE, 0, 0, 0, 0, 0, NULL);
        flushall();
        _dos_getdiskfree( Drive, &Disk_Data);
        Display_Free();
    }
    _dos_getdiskfree( Drive, &Disk_Data );
    Bytes_Free = ( unsigned long int )Disk_Data.avail_clusters
    * ( unsigned long int )Disk_Data.bytes_per_sector *
    ( unsigned long int )Disk_Data.sectors_per_cluster;
    Cluster_Size = Disk_Data.sectors_per_cluster *
    Disk_Data.bytes_per_sector;
    #pragma warn -aus // Stop annoying warning
    Prompt_Disk = 0;
    #pragma warn +aus
}

```

3. Serial Port Interrupt Handler for LSCDATA.EXE

```

*-----*
| SOURCE FILE: COMHNDLRASM |
|-----*
| Serial port data collection routine for use by |
| LSCdata.exe. |
*-----*

```

Title COMHNDLRASM Serial port interrupt handler routines.
Page 55,132

```

; Assembler routines for use with C program.
;
.MODEL small
Comm_Port EQU 0 ; Set = 0 for COM1, 1 for COM2
BUFFER_SIZE EQU 10240
; *
;| Masks for COM Port initialization. |
; *
P_NONE EQU 0
P_ODD EQU 00001000B
P_EVEN EQU 00011000B
STOP1 EQU 00000000B
STOP2 EQU 00000100B
WORD7 EQU 00000010B
WORD8 EQU 00000011B
BAUD0110 EQU 0
BAUD0150 EQU 00100000B
BAUD0300 EQU 01000000B
BAUD0600 EQU 01100000B
BAUD1200 EQU 10000000B
BAUD2400 EQU 10100000B
BAUD4800 EQU 11000000B
BAUD9600 EQU 11100000B

Pic_Mask EQU 21H ; Port address of 8259 mask register.
Pic_Eoi EQU 20H ; Port address of 8259 EOI instr.
LCR_DLAB EQU 10000000B

If Comm_Port ; Define physical port assignments.
Comm_Data EQU 02F8h ; for COM2.
Comm_Ier EQU 02F9h ;
Comm_Iir EQU 02FAH ; Interrupt Identification Register.
Comm_Lcr EQU 02FBH ;
Comm_Mcr EQU 02Fch ;
Comm_Stat EQU 02Fdh ;
Com_Int EQU 08h ;
Int_Mask EQU 08H ; Mask for 8259, COM2 is IRQ3

Else ; Define physical port assignments
Comm_Data EQU 03F8h ; for COM1.
Comm_Ier EQU 03F9h ;
Comm_IIR EQU 03FAH ; Interrupt Identification Register.
Comm_LCR EQU 03FBH ;
Comm_Mcr EQU 03Fch ;
Comm_Stat EQU 03Fdh ;
Com_Int EQU 0Ch ;
Int_Mask EQU 10H ; Mask for 8259, COM1 is IRQ4.
Endif

.DATA
EXTRN_COM_Tail:WORD
EXTRN_COM_Head:WORD
EXTRN_COM_Buffer:WORD
EXTRN_COM_Errors:WORD
EXTRN_Prompt_Disk:BYTE
EXTRN_COM_Data_Pending:BYTE

Intc_Seg DW 0
Intc_Offs DW 0
Init_Msg DB 'Please place a blank formatted DS/DD disk into the drive.'
Len_Init_Msg EQU $ - Init_Msg
Init_Msg_2 DB 'Press any key to continue.'
Len_Init_Msg_2 EQU $ - Init_Msg_2
Abort_Msg DB 'Correct error then press a key: A)abort, R)etry, I)gnore, or F)ail.'
Len_Abort_Msg EQU $ - Abort_Msg
Video_Segment DW 0
Vid_Offset DW 0
Cursor_Type DW 0
Cursor_Position DW 0

```



```

                MOV     AL,Com_Int      ; Load INT number for COM port.
                INT     21H             ; Reset INT handler.
Disable_INT_Handler_1:
                POP     DS
                POP     DX
                POP     AX
                RET

_Disable_INT_Handler ENDP

Get_Video_Mode EQU 0FH
Get_Cursor     EQU 03H
IGNORE        EQU 0
RETRY         EQU 1
ABORT         EQU 2
FAIL          EQU 3

;-----*
;| Procedure : COM_INT_Handler
;|
;| Asynchronous Communications Interrupt routine.
;| (@!)
;-----*
COM_INT_Handler PROC FAR
                STI                     ; Enable interrupts.
                PUSH    AX              ; Save effected registers.
                PUSH    BX
                PUSH    CX
                PUSH    DX
                PUSH    DS
                PUSH    ES
                PUSH    DI

                MOV     AX,@data        ; Set the Data segment to the data segment.
                MOV     DS,AX
                MOV     ES,AX

;-----*
;| Interrupt Bus 2.1
;| 11 RX Error condition (Highest Priority)
;| 10 RX Character available.
;| 01 TXD Register empty.
;| 01 Modem Interrupt (Lowest Priority.)
;-----*
                MOV     DX,Comm_IIR     ; Load the Interrupt Identification Register.
                IN      AL,DX           ; Get the interrupt identification form the UART.
                AND     AL,0000110B     ; Was the interrupt caused by an error?
                CMP     AL,00000110B
                JNE     COM_INT_Handler_1 ; If no, then continue.
                XOR     AH,AH           ; Clear high order byte.
                MOV     _COM_Errors,AX ; Store COM error.
                JMP     SHORT COM_INT_Handler_3 ; End Interrupt.
COM_INT_Handler_1:
                MOV     DX,Comm_Data    ; Load COM port into DX
                MOV     DI,_COM_Tail    ; Load the Data Buffer Offset into DI
                ADD     DI,_COM_Buffer
                CLC                     ; Clear the Carry Flag.
                CLI                     ; Clear Interrupts.
                IN      AL,DX           ; Get a character from the COM port.
                MOV     [DI],AL         ; Store it in the data buffer.
                CMP     DI,BUFFER_SIZE - 1 ; Was this the last character in th buffer?
                JNE     COM_INT_Handler_2 ; If no, then continue.
                ;
                ; We are at the end of the COMM Buffer, so we need to
                ; wrap back to the beginning.
                ;
                MOV     _COM_Tail,0     ; Wrap the COMM buffer pointer.
                JMP     SHORT COM_INT_Handler_2a ; Jump over other tail calcs.
COM_INT_Handler_2:

```

```

        INC     _COM_Tail           ; Increment our data pointer to the next location.
COM_INT_Handler_2a:
        OR     _COM_Data_Pending,1 ; Signal that there is data waiting.
COM_INT_Handler_3:
        STI                     ; Enable interrupts.
        MOV     AL,20H           ; Signal end of interrupt to controller.
        OUT     Pic_Eoi,AL

        POP     DI
        POP     ES
        POP     DS               ; Restore registers.
        POP     DX
        POP     CX
        POP     BX
        POP     AX
        IRET

```

```
COM_INT_Handler ENDP
```

```

;-----*
;| PROCEDURE : Enable_INT_Handler |
;| |                               |
;| Enables the Asynchronous Communications Routine. |
;| |                               |
;|-----*

```

```

        PUBLIC _Enable_INT_Handler
_Enable_INT_Handler PROC NEAR

```

```

        PUSH   AX
        PUSH   BX
        PUSH   DX
        PUSH   DS
        PUSH   ES

        MOV    AH,35H           ; Load DOS function 'Get Vector'.
        MOV    AL,Com_Int       ; Load interrupt number for Com port.
        INT    21H             ; Get the vector.
        MOV    Intc_Seg,ES      ; Save the Vector segment.
        MOV    Intc_Offs,BX     ; Save the Vector offset
        PUSH   CS               ; Make DS point to our code segment.
        POP    DS
        MOV    DX,OFFSET COM_INT_Handler ; Load Offset of COM_INT_Handler.
        MOV    AH,25H           ; Load DOS function 'Set Vector'.
        MOV    AL,Com_Int       ; Load interrupt number for COM port.
        INT    21H             ; Take over the COM interrupt.
;
;   Set baud rate
;
        MOV    AH,00H
        MOV    AL,BAUD0300 OR STOP1 OR P_EVEN OR WORD8
        MOV    DX,Comm_Port
        INT    14H

        MOV    DX,Comm_Mcr      ; Modem controller DTR and OUT2;
        MOV    AL,0Bh           ;
        OUT    DX,AL           ;

        MOV    DX,Comm_Ier      ; Interrupt enable register
        MOV    AL,1             ; on asynch controller.
        OUT    DX,AL

        IN     AL,Pic_Mask      ; Read current 8259 int. mask.
        AND    AL,NOT Int_Mask ; Reset mask for this COM port.
        OUT    Pic_Mask,AL     ; Write back 8259 int. mask.

        POP    ES
        POP    DS
        POP    DX

```

```

        POP     BX
        POP     AX

        RET

.Enable_INT_Handler ENDP

END

```

; Done here.

G. RIA data file copying Module

1. Header File for RIACOPY.C

```

#ifndef RIACOPY_H_
#define RIACOPY_H_

/*
 * SOURCE FILE: RIACOPY.H
 * Header file for RIACOPY.C - Copy RIA files.
 * RIA program version 1.1
 */

#define RIGHT      77
#define LEFT       75
#define DELETE     83
#define HOME       71
#define END        79
#define CR         13
#define LF         10

struct Window_Style Main_Window = {RED, CYAN, RED, 1,
    1, 80, 25, BLACK, CYAN, 'RIAcopy (1.0)- Copies RIA files
    from floppy' };
struct Window_Style File_Window = {WHITE, BLUE, WHITE,
    2, 2, 17, 24, WHITE, LIGHTGRAY, 'Copied Files' };
struct Window_Style Records_Window = {WHITE, BLUE,
    WHITE, 18, 2, 28, 24, WHITE, LIGHTGRAY, 'Records' };
struct Window_Style Desc_Window = {WHITE, BLUE,
    WHITE, 29, 2, 70, 24, WHITE, LIGHTGRAY,
    'Description' };
struct Window_Style Data_Window = {YELLOW, LIGHTGRAY,
    YELLOW, 67, 2, 79, 13, WHITE, RED, 'Data' };
struct Window_Style Name_Window = {WHITE, CYAN,
    YELLOW, 16, 10, 62, 12, WHITE, CYAN, 'Enter Your Last
    Name [ Max 12 character ]' };
struct Window_Style Get_Descript = {WHITE, CYAN,
    YELLOW, 18, 20, 63, 22, WHITE, CYAN, * };

void Delete_File( FILE *, char * );
void Describe( char *, int );
void Print_Stat( int, char * );

/*
 * Global Constants
 */
#define TRUE      -1
#define FALSE     0
#define INPUT_UL_X 4
#define INPUT_UL_Y 4
#define INPUT_LR_X 22
#define INPUT_LR_Y 22
#define IGNORE    0
#define RETRY     1
#define ABORT     2

/*
 * Data from Prompt for Disk
 */
#define RESET_DRIVE 0

```

```

#define VERIFY_SECTORS 4
#define HEAD           0
#define DISK           0
#define TRACK          0
#define SECTOR         1
#define NUMBER_SECTORS 1
#define BOX_UL_X       6
#define BOX_UL_Y       11
#define BOX_LR_X       73
#define BOX_LR_Y       13
#endif

```

2. Source for RIA file copying program

```

/*
 * SOURCE FILE: RIACOPY.C
 * Copies files from the RIA data floppy disks
 */
#include <stdio.h>
#include <stdlib.h>
#include <conio.h>
#include <string.h>
#include <dos.h>
#include <bios.h>
#include <ctype.h>
#include <dir.h>
#include <process.h>
#include <alloc.h>

#include 'rialib.h'
#include 'riacopy.h'

/*
 * Program - RIACopy.Exe ( Version 1.1 )
 * Copies raw data files from the LSC RIA data disks onto the
 * hard disk and then prompts the user to create +DOS
 * compatible descriptions for the files.
 * Version 1.1 - Delete zero length files from RIA data diskettes.
 */

char datapath[120]="C:\\LOTUS\\LSC\\";
char *Time_Stamp="C:\\LOTUS\\RIADATA\\timestamp.p";
char User_Name[13]="";
FILE *Stats_File;
struct fblk fblk;
int start = 1,
    Copied_Files=0;
char Error_Msg[80];
char Prompt_Disk=0;
char Show_Data[10][12];
char *file_desc[150];
char *new_desc[150];
int fcount=1;
FILE *Desc_File;

```

```

struct Window_Style *ScreenWindows[] = { &Main_Window,
&File_Window, &Desc_Window, &Records_Window,
NULL };

#ifdef DEBUG
char *Drive="A:";
int _drive=0;
#else
char *Drive="B:";
int _drive=1;
#endif

int
main (int argc /*, char *argv*/) {
FILE *current_file;
char *tmp_desc;
char Filename[80];
int Records;

if ((tmp_desc = getenv("RIADRIVE")) != NULL ) {
Drive[0] = tmp_desc[0];
Drive[1] = ':';
Drive[2] = 0;
_drive = toupper( tmp_desc[0] ) - 'A';
}
if ((tmp_desc = getenv("RIAPATH")) != NULL )
strcpy( datapath, tmp_desc );
if (argc > 1) {
printf("RiaCopy.Exe - Moves RIA files from a floppy
disk to the\n");
printf("LSC directory on the hard
disk.\n\n");
printf("It will copy from the B: drive by default, but
this can be \n");
printf("overridden by setting the environment
variable RIADRIVE to a \n");
printf("drive. It will store the files on the default
drive, in the \n");
printf("directory \\LOTUS\\LSC, unless the
environment variable RIAPATH\n");
printf("is set.\n");
exit(0);
}
if (( Stats_File = fopen( 'C:\\System\\RiaStats.Dat','w' ) ==
NULL ) {
fprintf( stderr, 'Can't open
C:\\System\\RiaStats.Dat\n');
exit(1);
}
setcbrik( 1 );
Read_Descriptions( datapath, &fcount, file_desc );
Screen_Setup( ScreenWindows );
_harderr( Hardware_Error );
Prompt_New_Disk( &Prompt_Disk, Drive );
Get_User_Name( &Name_Window, User_Name,
Time_Stamp );
while ( ( current_file = Get_File( Filename, Drive, &start ) )
!= NULL ) {
if ( (Records = Copy_File( current_file, Filename,
datapath, &Copied_Files,
&File_Window, &Records_Window,
Show_Data ) ) != 0 )
{
Delete_File( current_file, Filename );
if ( Records > 0 ) {
Describe( Filename, Records );
Print_Stats( Records, Filename );
}
}
}
fclose(current_file);
}
Save_Desc( datapath, file_desc, new_desc, Copied_Files,
fcount );
// Save_Desc();
fclose( Stats_File );
window(1,1,80,25);
_wscroll=1;
clrscr();
return(0);
}

void
Delete_File( FILE *File, char *Filename ) {
char *Name;
Name = allocate( 120 );
fclose( File );
strcpy( Name, Drive );
strcat( Name, Filename );
unlink( Name );
free( Name );
}

#pragma warn -par
char *
Copy_Desc( char *Desc ) {
char *tmp;
tmp = strdup( Desc );
if ( tmp == NULL )
die( 'Can't allocate string space for description\n' );
return (tmp);
}

int
Date_File( char * filename ) {
char * pointer;

pointer = strchr( filename, '.' );
if (pointer == NULL) return (0);
++pointer;
if ( strcmp( pointer, 'dte', 3 ) )
return(0);
else
return(-1);
}

void
Describe( char *Filename, int Records ) {
int i, length, Line;
struct text_info ti;
char msg[80];
char Description[41]="";
char *savedesc, *savedata;
/* char delete[3]="(32,8,0);
char backspace[5]="(8,32,8,0);
*/
gettextinfo( &ti );
savedata = Window_Save(&Data_Window);
savedesc = Window_Save( &Get_Descript );
if ( Date_File( Filename ) )
strcpy( Description, 'Date file.' );
else

```

```

Window_Box(&Data_Window);
window(Data_Window.x1+1, Data_Window.y1+1,
Data_Window.x2-1, Data_Window.y2-1);
_wscroll=0;
textcolor( Data_Window.textcolor);
textbackground( Data_Window.backcolor);

length = ( Records < 10 ) ? Records : 10 ;
for ( i=0; i<length; i++){
    gotoxy(1,i);
    cprintf("%10s",&Show_Data[i-1][0]);
}
_wscroll=1;
window(1,1,80,25);
textcolor( Get_Descript.textcolor);
textbackground( Get_Descript.backcolor);
sprintf( msg, 'Describe %s [40 characters max.],
Filename );
strcpy( Get_Descript.title,msg);
Window_Box( &Get_Descript );
strcpy(Description, User_Name );
strcat(Description, ': ');
gotoxy(Get_Descript.x1+1, Get_Descript.y1+1);
cputs(Description);
length = 40 - strlen( Description );
while ( strlen( Description ) == 40 - length ) {
    Input_String( Description + strlen( Description
), length, 0, 0 );
    if ( strlen( Description ) == 40 - length ) {
        Beep();
    }
}
Window_Restore( &Get_Descript, savedesc );
Window_Restore( &Data_Window, savedata );
}

window( Desc_Window.x1+1, Desc_Window.y1+1,
Desc_Window.x2-1, Desc_Window.y2-1);
textcolor( Desc_Window.textcolor);
textbackground( Desc_Window.backcolor);
Line = Copied_Files;
if (Copied_Files > (Desc_Window.y2 - Desc_Window.y1-1) ) {
    Line = Desc_Window.y2 - Desc_Window.y1 - 1;
    gotoxy( 1,Line );
    cputs("\r\n");
}
gotoxy( 1, Line );
cprintf( "%s",Description );
window( 1,1,80,24);
textattr( ti.attribute );
strcpy( msg, Filename );
strcat( msg, '' );
strcat( msg, Description );
new_desc[Copied_Files-1] = Copy_Desc( msg );
}

void
Print_Stat( int Records, char *Filename ) {
    char *tmp;
    char msg[50];
    if ((tmp = strchr( new_desc[Copied_Files-1], ' '))--NULL ) {
        sprintf( msg, 'Bad description for %s.\n',Filename );
        die( msg );
    }
    ++tmp;
    fprintf( stdprn, "%-13s %-40s \n Contains %d records\n",
    Filename, tmp, Records );
}

```

H. RIA File description Module - RIADDESC.EXE

1. Header File For RIADDESC.C

```

#ifndef RIADDESC_H
#define RIADDESC_H
/*
// | SOURCE FILE: RIADDESC.H
// |-----|
// | Header File for RIADDESC.C - Describe RIA files.
// |-----|
// | RIA program version 1.1
// |-----|
*/
struct Window_Style Main_Window = (RED, CYAN, RED,1,
1, 80, 25, BLACK, CYAN, 'RIAdesc (1.01) - Describe RIA
worksheets' );
struct Window_Style File_Window = (WHITE, BLUE, WHITE,
10, 5, 70, 20, WHITE, LIGHTGRAY, 'File Actions' );
struct Window_Style Get_Descript = (WHITE, BROWN,
YELLOW, 2, 20, 78, 23, WHITE, BROWN, ' ');
struct Window_Style Name_Window = (WHITE, CYAN,
YELLOW, 16, 10, 62, 12, WHITE, CYAN, 'Enter Your Last
Name [ Max 12 character ] ');

int Get_Time_Stamp( void );
time_t relative_time( struct fblk );

GetFileToDescribe( struct fblk *fileBlock );
char * get_123_title( char * );
void Delete_File( FILE *, char * );
void Describe( void );
void Print_Stat( int , char * );
/*-----*/
/* | Global Constants | */
/*-----*/
#define TRUE -1
#define FALSE 0
#define INPUT_UL_X 4
#define INPUT_UL_Y 4
#define INPUT_LR_X 22
#define INPUT_LR_Y 22
#define IGNORE 0
#define RETRY 1
#define ABORT 2
/*-----*/
/* | Data from Prompt for Disk | */
/*-----*/
#define RESET_DRIVE 0
#define VERIFY_SECTORS 4
#define HEAD 0
#define DISK 0
#define TRACK 0
#define SECTOR 1
#define NUMBER_SECTORS 1
#define BOX_UL_X 6
#define BOX_UL_Y 11
#define BOX_LR_X 73
#define BOX_LR_Y 13
#endif

```

2. Source for RIADDESC.C

```

/*-----*/
// | SOURCE FILE: RIADDESC.C
// |-----|

```

```

// |-----|
// | Assigns descriptions to RIA files. |
// |-----|
/* #define TESTING */
#include <stdio.h>
#include <stdlib.h>
#include <conio.h>
#include <string.h>
#include <dos.h>
#include <bios.h>
#include <ctype.h>
#include <dir.h>
#include <process.h>
#include <alloc.h>
#include <time.h>

#include 'rialib.h'
#include 'riadesc.h'
// |-----|
// | Program - RIAdesc.Exe (Version 1.1) |
// |-----|
// | Has the user create 4DOS compatible descriptions for |
// | newly created RIA 1-2-3 worksheet files. |
// |-----|
// | Version 1.1 - Make sure we don't try to describe |
// | worksheets that are already described. |
// |-----|

struct Window_Style *screenWindows[]={ &Main_Window,
&File_Window, NULL };
char datapath[120]~"C:\\\\LOTUS\\\\RIADATA\\\\";
char User_Name[13]~";
FILE *Stats_File;
struct fblk fblk;
char *searchname;
int start = 1,
F_Line = 1,
Copied_Files=0;
char Error_Msg[80];
char Prompt_Disk=0;
char Show_Data[10][12];
char *file_desc[500];
char *new_desc[100];
time_t Time_Stamp;
int fcount=1;
FILE *Desc_File;
int _Describe=0;
int _First=1;

#ifdef TESTING
#define DEBUGWAIT(A) puts(A); getch()
#else
#define DEBUGWAIT(A)
#endif

#ifdef DEBUG
char *Drive="A:";
int _drive=0;
#else
char *Drive="B:";
int _drive=1;
#endif

int
main(int argc /*, char *argv[] */){
char *tmp_desc;

if((tmp_desc = getenv("RIADRIVE")) != NULL){

```

```

Drive[0] = tmp_desc[0];
Drive[1] = ':';
Drive[2] = 0;
_drive = toupper( tmp_desc[0] ) - 'A';
}
if((tmp_desc = getenv("RIAPATH")) != NULL )
strcpy( datapath, tmp_desc );
if (argc > 1 ){
printf("RiaDesc.Exe - Moves RIA files from a floppy
disk to the\n");
printf(" LSC directory on the hard
disk.\n\n");
printf(" It will copy from the B: drive by default, but
this can be \n");
printf(" overridden by setting the environment
variable RIADRIVE to a \n");
printf(" drive. It will store the files on the default
drive, in the \n");
printf(" directory \\\\LOTUS\\\\LSC, unless the
environment variable RIAPATH\n");
printf(" is set.\n");
exit(0);
}
setcbk( 1 );
Read_Descriptions( datapath, &fcount, file_desc );
Screen_Setup( screenWindows );
if (!Get_Time_Stamp())
exit(1);
searchname = allocate( 150 );
strcpy( searchname, datapath );
strcat( searchname, ".wk1" );
while ( GetFileToDescribe( &fblk ) ){
Describe( );
}
Save_Desc( datapath, file_desc, new_desc, Copied_Files,
fcount );
fclose( Stats_File );
window(1,1,80,25);
_wscroll=1;
clrscr();
return(0);
}

int
GetFileToDescribe( struct fblk *fileBlock ){
time_t file_time;
int done=0;

while ( !done ){
if ( start ){
done = findfirst( searchname, fileBlock, 0 );
start = 0;
}
else
done = findnext( fileBlock );
if ( done )
return( NULL );
file_time = relative_time( *fileBlock );
if ( file_time > Time_Stamp ){
return( 1 );
}
}
/* Should never get here */
return( 0 );
}
int

```

```

Get_Time_Stamp() {
    char *Time_Stamp_Name;

    Time_Stamp_Name = allocate( 140 );
    strcpy( Time_Stamp_Name, datapath );
    strcat( Time_Stamp_Name, 'timestam.p' );
    if ( findfirst( Time_Stamp_Name, &ffblk, 0 ) != 0 ) {
        free( Time_Stamp_Name );
        return ( 0 );
    }
    Time_Stamp = relative_time( ffbk );
    free( Time_Stamp_Name );
    return ( 1 );
}

time_t
relative_time( struct ffbk file ) {
    struct tm Time;

    Time.tm_sec = ( file.ff_ftime & 0x1F ) * 2;
    Time.tm_min = ( file.ff_ftime & 0x7e0 ) >> 5;
    Time.tm_hour = ( file.ff_ftime & 0xF800 ) >> 11;
    Time.tm_mday = ( file.ff_fdate & 0x1F );
    Time.tm_mon = ( file.ff_fdate & 0x1e0 ) >> 5;
    Time.tm_year = (( file.ff_fdate & 0xfe00 ) >> 9 ) + 80;
    return( mktime(&Time) );
}

char *
Copy_Desc( char *Desc ) {
    char *tmp;
    tmp = strdup( Desc );
    if ( tmp == NULL )
        die( "Can't allocate string space for description\n" );
    return ( tmp );
}

void
Describe() {
    int length, Line, part_len, len;
    struct text_info ti;
    char msg[80];
    char Description[41] = "";
    char *savedesc, *killfile;
    char *wrksh_t_title;
    char *wrksh_t_file;
    int _described_ = FALSE;

    if ( !_First ) {
        Get_User_Name( &Name_Window, User_Name,
            'C:\\LOTUS\\RIADATA\\timestam.p' );
        _First++;
    }
    _Describe=1;
    gettextinfo( &ti );
    /*-----**/
    /*| See if already described |*/
    /*-----**/
    len = strlen( ffbk.ff_name );
    for ( j=0; j<fcount; j++ ) {
        if ( strncmp( ffbk.ff_name, file_desc[j], len ) == 0 ) {
            _described_ = TRUE;
            break;
        }
    }
}

}
if ( !_described_ ) {
    savedesc = Window_Save( &Get_Descript );
    _wscroll=1;
    window( 1, 1, 80, 25 );
    textcolor( Get_Descript.textcolor );
    textbackground( Get_Descript.backcolor );
    sprintf( msg, "Describe %s (40 characters max.) Press
        F2 to delete file.", ffbk.ff_name );
    strcpy( Get_Descript.title, msg );
    Window_Box( &Get_Descript );
    if ( (wrksh_t_file = (char *)malloc( 128 )) == NULL )
        {
            printf( "Error allocating string space in
                procedure DESCRIBE of RIAdesc.c
                module.\n" );
            printf( "Press any key to continue:" );
            getch();
            wrksh_t_title = NULL;
        }
    else
        {
            strcpy( wrksh_t_file, datapath );
            strcat( wrksh_t_file, ffbk.ff_name );
            wrksh_t_title = get_123_title( wrksh_t_file );

            gotoxy( Get_Descript.x1+1, Get_Descript.y1+1 );
            cprintf( "Worksheet Title: %40.40s", wrksh_t_title ==
                NULL ? "Not titled." : wrksh_t_title );
            strcpy( Description, User_Name );
            strcat( Description, " " );
            part_len = strlen( Description );
            length = 40 - part_len;
            if ( wrksh_t_title != NULL )
                strcat( Description, wrksh_t_title, 40-part_len );
            gotoxy( Get_Descript.x1+1, Get_Descript.y1+2 );
            textcolor( Get_Descript.forecolor );
            cputs( Description );
            while ( part_len == 40 - length ) {
                if ( (Input_String( Description + 40 - length,
                    length, strlen( Description ) - part_len,
                    _Describe )) == INPUT_BAD ) {
                    _Describe=0;
                    Window_Restore( &Get_Descript,
                        savedesc );
                    window( File_Window.x1+1,
                        File_Window.y1+1,
                        File_Window.x2-1,
                        File_Window.y2-1 );
                    textcolor( File_Window.textcolor );
                    textbackground(
                        File_Window.backcolor );
                    killfile = allocate ( 130 );
                    strcpy( killfile, datapath );
                    strcat( killfile, ffbk.ff_name );
                    unlink( killfile );
                    Line = F_Line;
                    if ( Copied_Files > (File_Window.y2
                        - File_Window.y1 - 1) ) {
                        Line = File_Window.y2 -
                            File_Window.y1 - 1;
                    }
                    gotoxy( 1, Line );
                    cputs( "\r\n" );
                }
            }
            gotoxy( 1, Line );
            cprintf( "%s deleted", killfile );
            ++F_Line;
            window( 1, 1, 80, 24 );
        }
}

```

```

        free( killfile );
        textattr( ti.attribute );
        return;
    }
    else
        part_len = strlen( Description );
    if ( part_len == 40 - length ){
        Beep();
    }
} // End While
_Describe=0;
Window_Restore( &Get_Descript, savedesc );
window( File_Window.x1+1, File_Window.y1+1,
        File_Window.x2-1, File_Window.y2-1);
textcolor( File_Window.textcolor);
textbackground( File_Window.backcolor);
Line = F_Line;
if (Copied_Files > (File_Window.y2 - File_Window.y1
- 1)) {
    Line = File_Window.y2 - File_Window.y1 - 1;
    gotoxy( 1,Line );
    cputs("\r\n");
}
gotoxy( 1, Line );
cprintf( "%s described.", fblk.ff_name );
++F_Line;
window( 1,1,80,24);
textattr( ti.attribute );
strcpy( msg, fblk.ff_name );
strcat( msg, "" );
strcat( msg, Description );
new_desc(Copied_Files++) = Copy_Desc( msg );
} // End if not described.
} // End Procedure DescribeDescribe
#pragma warn +par

```

I. RIADIR.EXE - Generates Directory Listings for raw RIA data files.

1. Header File for RIADIR.C

```

#ifndef RIADIR_H_
#define RIADIR_H_
// *-----*
// | SOURCE FILE: RIADIR.H |
// |-----|
// | Header file for RIADIR.C - Get RIA Directory |
// |-----|
// | RIA program version 1.1 |
// *-----*
#define RIGHT      77
#define LEFT       75
#define DELETE     83
#define HOME       71
#define END        79
#define CR         13
#define LF         10
#define SPACE     32
#define F10        68
#define UP         72
#define DOWN       80

#define LINE       0
#define ALL        1
#define NONE       2

```

```

struct File_Data {
    char *name[150];
    char *desc[150];
    char tag[150];
};

typedef struct File_Stats {
    char *name;
    char *desc;
    char tag;
} File_Stats;

struct Window_Style Main_Window = {RED, CYAN, RED, 1,
    1, 80, 25, BLACK, CYAN, 'RIADir (1.0) - Sorts RIA data
    files for LOTUS.'};
struct Window_Style Info_Window = {WHITE, BLUE, WHITE,
    30, 10, 50, 12, WHITE, RED, "."};

void Highlight( struct Window_Style );
void Normal( struct Window_Style );
void Print( File_Stats * );
void activate_window( struct Window_Style Window );
void Tag( void );
int get_key( void );
int Scroll_Up( void );
int Scroll_Down( void );
void Update_Window( int );
File_Stats * structalloc( void );
unsigned long Get_Sort_Code( char * );
int sort_function( const void *, const void * );
char * Find_Desc( char * );
void Delete_File( FILE *, char * );
void Describe( char *, int );
void Print_Stats( int, char * );
char *getFileName( char *Extension );
#endif

```

2. Source for RIADIR module

```

// *-----*
// | SOURCE FILE: RIADIR.C |
// |-----|
// | Makes sorted listings of various raw RIA data |
// | files for use by other modules. |
// *-----*
#include <stdio.h>
#include <stdlib.h>
#include <conio.h>
#include <string.h>
#include <dos.h>
#include <ctype.h>
#include <dir.h>
#include <process.h>
#include <alloc.h>

#include 'rialib.h'
#include 'riadir.h'
// *-----*
// | Program - RIADir.Exe (Version 1.0) |
// |-----|
// | Sorts the directory of the RIA files and creates the files |
// | used by LOTUS to allow the user to select RIA files. |
// |-----|
// | Compiled using Borland C/C++ 3.1. |
// *-----*
char datapath[120]="C:\\\\LOTUS\\\\LSC\\";

```

```

struct fblk fblk;
File_Stats *File_Info[150];
char *extensions[] = { 'unk', 'rec', 'dte' };
int file_count=0;
int start = 1;
char Error_Msg[128];
char *file_desc[150];
int fcount=1;
int Window_Start,
    win_line,
    win_length,
    file_line;
extern struct text_info TEXT;
char Prompt_Disk=0;

int
main (int argc /*, char *argv[] */) {
    char *tmp_desc, outfile[128];
    char *screen;
    int i, j;
    FILE *output;

    gettextinfo( &TEXT );
    if ((tmp_desc = getenv("RIAPATH")) != NULL)
        strcpy( datapath, tmp_desc );
    if (argc > 1) {
        printf("Riadir.Exe - Sorts RIA data files for use with
            the LOTUS RIA worksheet.\n\n");
        exit(0);
    }
    setcbkr( 1 );
    Read_Descriptions( datapath, &fcount, file_desc );
    screen = Window_Save( &Main_Window );
    Window_Box( &Main_Window );
    Window_Box( &Info_Window );
    activate_window( Info_Window );
    gotoxy( 4,1);
    cpus("Sorting files");
    File_Info[file_count] = structalloc();
    for (i = 0; i < 3; i++) {
        start = 1;
        while ( ( File_Info[file_count]->name = getFileName(
            extensions[i] ) ) != NULL ) {
            File_Info[file_count]->desc = Find_Desc(
                File_Info[file_count]->name );
            File_Info[file_count]->tag = 0;
            ++file_count;
            File_Info[file_count] = structalloc();
        }
        strcpy( outfile, datapath );
        strcat( outfile, extensions[i],1);
        strcat(outfile, "files.dat");
        if ((output = fopen( outfile, "w" )) == NULL) {
            sprintf( Error_Msg, "Error opening %s", outfile );
            die(Error_Msg);
        }
        if (file_count > 0) {
            qsort( (void *) File_Info, file_count, sizeof(
                File_Info[0] ), sort_function );
            for ( j=0; j<file_count; j++) {
                sprintf( output,
                    "\x22%\x22,\x22%\x22\n",
                    File_Info[j]->name, File_Info[j]-
                    >desc );
            }
        }
        fclose( output );
        file_count = 0;
    }

    Window_Restore( &Main_Window, screen );
    window(1,1,80,25);
    gotoxy( TEXT.cursx, TEXT.cury );
    return(0);
}

File_Stats *
structalloc() {
    File_Stats *temp;

    if (( temp = (File_Stats *) malloc( sizeof(File_Stats) )) ==
        NULL )
        die("Ran out of memory during 'malloc' call.\n");
    return ( temp );
}

#pragma warn -aus
int sort_function( const void *a, const void *b ) {
    unsigned long code_1=0, code_2=0;
    File_Stats **case1;
    File_Stats **case2;

    case1 = (File_Stats*)a;
    case2 = (File_Stats*)b;
    code_1 = Get_Sort_Code( (*case1)->name );
    code_2 = Get_Sort_Code( (*case2)->name );
    if (code_1 < code_2) return(-1);
    if (code_1 == code_2) return( 0 );
    return( 1 );
}

#pragma warn +aus

unsigned long
Get_Sort_Code( char *filename ) {
    unsigned long code;
    if (filename[0] == 'U' || filename[0] == 'u') {
        code = 200000L;
        code += 1000L * (unsigned long) (
            filename[3] - '0' );
        code += 10L * (unsigned long) (filename[5] -
            '0') * 10 + (filename[6] - '0' );
        if (toupper(filename[8]) == 'D')
            ++code;
    }
    else /* Recovery file name */
        code = 100000L;
        code += 10L * (unsigned long) ( (filename[3] -
            '0') * 10 + (filename[4] - '0' ) );
        code += 1000L * (unsigned long) (filename[6] -
            '0') * 10 + (filename[7] - '0' );
        if (toupper(filename[8]) == 'D')
            ++code;
    }
    return ( code );
}

char *
Find_Desc( char *filename ) {
    int j, len, i=0;
    char *pointer;
}

```

```

static char *empty="";

len = strlen( filename );
for ( j=0; j <= fcount; j++){
    if (strncmpi(filename, file_desc[j], len) == 0){
        pointer = file_desc[j] + len;
        while (pointer[i] == ' ')
            i++;
        return ( pointer + i );
    }
}
return ( empty );
}

void
Highlight( struct Window_Style Window ){
    textcolor( Window.backcolor );
    textbackground( Window.textcolor );
}

void
Normal( struct Window_Style Window ){
    textcolor( Window.textcolor );
    textbackground( Window.backcolor );
}

void
activate_window( struct Window_Style Window ){
    window( 1,1,80,25);
    window( Window.x1+1, Window.y1+1, Window.x2-1,
            Window.y2-1 );
    textcolor( Window.textcolor );
    textbackground( Window.backcolor );
    return;
}

char *
getFileName( char *Extension ){
    char *Search;
    int done;

    Search = allocate( 129 );
    strcpy( Search, datapath );
    strcat( Search, "*" );
    strcat( Search, Extension );
    if ( start ){
        done = findfirst( Search, &fblk, 0 );
        start = 0;
    }
    else
        done = findnext( &fblk );
    if ( done )
        return( NULL );
    free( Search );
    return (strstore( fblk.ff_name ));
}

```

J. RIAKILL.EXE - Deletes raw RIA data files that are no longer needed.

1. Header File for RIAKILL.C

```
#ifndef _RIAKILL_H_
```

```

#define _RIAKILL_H_
// *-----*
// | SOURCE FILE: RIAKILL.H |
// |-----|
// | Header file for RIAKILL.C - File deletion |
// |
// | RIA Program - Version 1.1 |
// *-----*

#define RIGHT 77
#define LEFT 75
#define DELETE 83
#define HOME 71
#define END 79
#define CR 13
#define LF 10
#define SPACE 32
#define F10 68
#define UP 72
#define DOWN 80

#define LINE 0
#define ALL 1
#define NONE 2

struct File_Data {
    char *name[150];
    char *desc[150];
    char tag[150];
};

typedef struct File_Stats {
    char *name;
    char *desc;
    char tag;
} File_Stats;

struct Window_Style Main_Window = (RED, CYAN, RED, 1,
    1, 80, 25, BLACK, CYAN, 'RIAKILL (1.0) - Deletes temp LSC
    files for next assay. ');
struct Window_Style File_Window = (WHITE, BLUE, WHITE,
    5, 2, 73, 24, WHITE, RED, 'Use Arrow Keys andspace
    bar to select files. (* selects all) ');

void activate_window( struct Window_Style Window );
void Delete_File( FILE *, char * );
void Describe( char *, int );
char * Find_Desc( char * );
int get_key( void );
char * getNextFile( char * );
unsigned long Get_Sort_Code( char * );
void Highlight( struct Window_Style );
void Normal( struct Window_Style );
void Print( File_Stats * );
void Print_Stats( int, char * );
void Tag( void );
int Scroll_Up( void );
int Scroll_Down( void );
int sort_function( const void *, const void * );
File_Stats * strcalloc( void );
void Update_Window( int );
#endif

```

2. Source for RIAKILL Module

```

// *-----*
// | SOURCE FILE: RIAKILL.C |
// |-----|

```

```

// | Presents the raw data RIA files to the user |
// | and allows them to delete those that have been |
// | used and are no longer needed for calculations. |
// *-----*
#include <stdio.h>
#include <stdlib.h>
#include <conio.h>
#include <string.h>
#include <dos.h>
#include <ctype.h>
#include <dir.h>
#include <process.h>
#include <alloc.h>

#include 'rialib.h'
#include 'riakill.h'

char datapath[120]="C:\\LOTUS\\LSC\\";
struct fblk fblk;
File_Stats *File_Info[150];
char *extensions[] = { 'unk', 'rec', 'dte' };
int file_count=0;
int start = 1;
char Error_Msg[80];
char *file_desc[150];
int fcount=-1;
int Window_Start,
    win_line,
    win_length,
    file_line;
extern struct text_info TEXT;
struct Window_Style *screenWindows[]={ &Main_Window,
    &File_Window, NULL };
char Prompt_Disk=0;

int
main (int argc /*, char *argv[] */) {
    char *tmp_desc, ch;
    char Filename[80];
    int i, Update;

    gettextinfo( &TEXT );
    if ((tmp_desc = getenv("RIAPATH")) != NULL )
        strcpy( datapath, tmp_desc );
    if (argc > 1) {
        printf("RiaKill.Exe - Presents the RIA data files to the
            user and displays\n");
        printf("    the descriptions, and allows the
            user to select the.\n\n");
        printf("    files which need to be deleted.
            \n\n");
        printf("    It will search for files on the default drive,
            in the directory\n");
        printf("    \\LOTUS\\LSC, unless the environment
            variable RIAPATH is set.\n");
        exit(0);
    }
    setcbkr( 1 );
    Read_Descriptions( datapath, &fcount, file_desc );
    File_Info[file_count] = strualloc();
    for (i = 0; i < 3; i++) {
        start = 1;
        while ( ( File_Info[file_count]->name = getNextFile(
            extensions[i] ) ) != NULL ) {
            File_Info[file_count]->desc = Find_Desc(
                File_Info[file_count]->name );
            File_Info[file_count]->tag = 0;
            ++file_count;
            File_Info[file_count] = strualloc();
        }
    }
    if ( file_count == 0 ) {
        textattr( TEXT.attribute );
        clrscr();
        printf( "No temporary LSC data files found.\n");
        exit(0);
    }
    qsort( (void *) File_Info, file_count, sizeof( File_Info[0] ),
        sort_function );
    if (file_count >= 21)
    {
        File_Window.y1 = 2;
        File_Window.y2 = 24;
    }
    else
    {
        File_Window.y1 = ( 21 - file_count ) / 2 + 2;
        File_Window.y2 =
            File_Window.y1 + file_count + 1;
    }
    Screen_Setup( screenWindows );
    window(1,1,80,25);
    textcolor( Main_Window.titlefore );
    textbackground( Main_Window.titleback );
    gotoxy( 1, 25);
    Center_Text( "Push the F10 key to begin deleting selected
        files", Main_Window );
    activate_window( File_Window );
    _wscroll = 0;
    win_length = File_Window.y2 - File_Window.y1 - 1;
    for (i = 0; i < win_length; i++) {
        gotoxy( 1, i+1 );
        Print( File_Info[i] );
    }
    _wscroll = 1;
    gotoxy( 1, 1);
    Highlight( File_Window );
    Print( File_Info[0] );
    win_line = 1;
    file_line = 0;
    Window_Start = 0;
    gotoxy( 1, 1);
    Update_Window( ALL );
    while ( ( ch = get_key() ) != F10 ) {
        switch ( ch ) {
            case UP:
                Update = Scroll_Up();
                break;
            case SPACE:
                Tag();
                break;
            case DOWN:
                Update = Scroll_Down();
                break;
            case '*':
                for (i = 0; i < file_count; i++) {
                    File_Info[i]->tag = 1;
                }
                Update = ALL;
                break;
            default:
                Beep();
                Update = NONE;
                break;
        }
    }
}

```

```

    }
    Update_Window( Update );
}
window(1,1,80,25);
_wscroll=1;
for(i = 0; i < file_count; i++){
    if( File_Info[i]>tag ){
        strcpy(Filename, datapath);
        strcat(Filename, File_Info[i]>name);
        unlink( Filename );
    }
}
clrscr();
return(0);
} // END MAIN

int
get_key( void ){
    int ch;
    if ((ch = getch()) == 0)
    {
        ch = getch();
        switch( ch ){
            case F10:
            case UP:
            case DOWN:
                return( ch );
            default:
                return( 0 );
        }
    }
    else
    {
        switch(ch){
            case SPACE:
            case '*':
                return( ch );
            default:
                return(0);
        }
    }
} // End get_key

int
Scroll_Up( void ){
    if( win_line == 1 )
        if( file_line == 0 )
        {
            file_line = file_count - 1;
            win_line = win_length;
            Window_Start = file_count > 21 ?
                file_count - 21 : 0;
            return( ALL );
        }
    else
    {
        --file_line;
        --Window_Start;
        return( ALL );
    }
}
else
{
    Normal( File_Window );
    Update_Window( LINE );
    --win_line;
    --file_line;
    Highlight( File_Window );
}

```

```

        Update_Window( LINE );
        return ( NONE );
    }
} // End Scroll_Up

int
Scroll_Down( void ){
    if( win_line == win_length )
        if( file_line == file_count - 1 )
        {
            file_line = 0;
            win_line = 1;
            Window_Start = 0;
            return( ALL );
        }
    else
    {
        ++file_line;
        ++Window_Start;
        return( ALL );
    }
}
else
{
    Normal( File_Window );
    Update_Window( LINE );
    ++win_line;
    ++file_line;
    Highlight( File_Window );
    Update_Window( LINE );
    return ( NONE );
}
} // End Scroll_Down

void
Update_Window( int code ){
    int i;

    char morech[2];
    if( code == NONE ){
        gotoxy(1, win_line );
        return;
    }
    if( code == LINE ){
        gotoxy( 1, win_line );
        _wscroll = 0;
        Print( File_Info[file_line] );
        _wscroll = 1;
    }
    if( code == ALL ){
        if( Window_Start != 0 )
        {
            textattr( RED*16+LIGHTGRAY);
            strcpy(morech, '*');
        }
        else
        {
            textcolor( File_Window.forecolor );
            textbackground( File_Window.backcolor );
            strcpy(morech, '*');
        }
    }
}
window(1,1,80,25);
gotoxy( File_Window.x1+1, File_Window.y1 );
cputa( morech );
activate_window( File_Window );
if( Window_Start+win_length < file_count )
{

```

```

        textattr( RED*16+LIGHTGRAY );
        strcpy(morech,"");
    }
    else
    {
        textcolor( File_Window.forecolor );
        textbackground(File_Window.backcolor);
        strcpy(morech,"");
    }
    window (1,1,80,25);
    gotoxy( File_Window.x1+1, File_Window.y2 );
    cputs( morech );
    activate_window( File_Window );
    Normal( File_Window );
    _wscroll=0;
    for ( i = 0; i<win_length; i++){
        gotoxy(1, i+1);
        Printf File_Info{Window_Start + i};
    }
    Highlight( File_Window );
    gotoxy( 1, win_line );
    Printf File_Info{file_line};
    _wacroll=1;
}
// End Update_Window

void
Tag( void ){
    File_Info{file_line}>tag ^= 1;
}

void
Print( File_Status *File ){
    printf( " %c %12s %c%-40s      'File->tag==0?':",
        File->name, "", File->desc==NULL? " 'File->desc";
}

File_Status *
strucalloc() {
    File_Status *temp;

    if (( temp = (File_Status *) malloc( sizeof(File_Status) )) ==
        NULL )
        die("Ran out of memory during 'malloc' call.\n");
    return ( temp );
}

#pragma warn -sus
int sort_function( const void *a, const void *b ){

    unsigned long code_1=0, code_2=0;
    File_Status **case1;
    File_Status **case2;

    case1 = (File_Status*)a;
    case2 = (File_Status*)b;
    code_1 = Get_Sort_Code( (*case1)->name );
    code_2 = Get_Sort_Code( (*case2)->name );
    if (code_1 < code_2 ) return( -1 );
    if (code_1 == code_2 ) return( 0 );
    return( 1 );
}

#pragma warn +sus

unsigned long
Get_Sort_Code( char *filename ) {
    unsigned long code;
    if (filename[0] == 'U' || filename[0] == 'u' ){
        code = 200000L;
        code += 1000L * (unsigned long) ( filename[3] - '0' );
        code += 10L * (unsigned long) ( filename[5] -
            '0')*10+(filename[6]-'0');
        if (toupper(filename[8]) == 'D' )
            ++code;
    }
    else /* Recovery file name */
        code = 100000L;
        code += 10L * (unsigned long) ( (filename[3] - '0')*10 +
            (filename[4] - '0') );
        code += 1000L * (unsigned long) ( filename[6] -
            '0')*10+(filename[7]-'0');
        if (toupper(filename[8]) == 'D' )
            ++code;
    }
    return ( code );
}

char *
Find_Desc( char *filename ){
    int j, len,i=0;
    char *pointer;

    len = strlen( filename );
    for ( j=0; j <= fcount; j++){
        if (strcmpi(filename, file_desc[j], len) == 0 ){
            pointer = file_desc[j] + len;
            while (pointer[i] == ' ') i++;
            return ( pointer + i );
        }
    }
    return ( NULL );
}

char *
getNextFile( char *Extension ){
    char *Search;
    int done;

    Search = allocate( 129 );
    strcpy( Search, datapath );
    strcat( Search, "." );
    strcat( Search, Extension );
    if ( start ){
        done = findfirst( Search, &ffblk, 0 );
        start = 0;
    }
    else
        done = findnext( &ffblk );
    if ( done )
        return( NULL );
    free( Search );
    return (strstore( ffblok.ff_name ));
}

void
Highlight( struct Window_Style Window ){
    textcolor( Window.backcolor );
    textbackground( Window.textcolor );
}

```

```

void
Normal( struct Window_Style Window ) {
    textcolor( Window.textcolor );
    textbackground( Window.backcolor );
}

void
activate_window( struct Window_Style Window ) {
    window( 1,1,80,25);
    window( Window.x1+1, Window.y1+1, Window.x2-1,
            Window.y2-1 );
    textcolor( Window.textcolor );
    textbackground( Window.backcolor );
    return;
}

```

K. Raw Data Editor - FIXIT.EXE

1. Main body of FIXIT.BAS

```

*-----*
| SOURCE FILE: FIXIT.BAS
|-----|
| Program for editing Raw data files from the
| LSC. Compiles using TurboBasic 1.0 and
| PowerBasic 2.0-3.1
|-----*

DEFINT A-Z
DIM ColorPalette(10,2)
DIM FileData(3000), FileName$(40), FileBreak%(20)
$INCLUDE 'SCREEN.INC'

*-----*
| PROCEDURE: Main
|-----|
| PURPOSE: Loop and process files until done.
|-----*

Finished%=0
CALL DefineColors
CALL DefineCurrentPalette
WHILE NOT FINISHED
    CALL ChooseFileType(Extension$)
    IF Extension$='REC' THEN
        Head$='Edit Recovery Files'
    ELSE
        Head$='Edit Unknown Files'
    END IF
    CALL ScreenSetUp(Head$,7,3,2,32)
    CALL WaitDraw(10,30,10,10)
    A$ = 'C:\LOTUS\LSC\PROGRAMS\RIADIR.EXE'
    SHELL A$
    CALL GetFileNames(Extension$)
    CALL ChooseAFile
    CALL GetData(Extension$)
    CALL EditFile
    CALL StoreTheData
    CALL ScreenSetUp('LSC File Editor',7,3,2,32)
    LineNumber% = 0 : ColumnNumber%=0
    CALL DrawWindow(3,43,4)
    CALL CenterText(12,'Do you want to edit another file?'
    (Y/N),2)
    CALL CheckKey(PushedKey$)
    IF PushedKey$<>'Y' AND PushedKey$<>'N' THEN
        Finished%=-1
    END IF
WEND
CLS

```

END

```

SUB DefineCurrentPalette
    SHARED Black, Blue, Green, Cyan, Red, Magenta, Brown,
        White, Gray
    SHARED LightBlue, LightGreen, LightCyan, LightRed,
        LightMagenta
    SHARED Yellow, HighIntensityWhite, ColorPalette%()
    CALL DefinePalette(1,HighIntensityWhite,Cyan)
    CALL DefinePalette(2,White,Blue)
    CALL DefinePalette(3,Red,Cyan)
    CALL DefinePalette(4,Yellow,Blue)
    CALL DefinePalette(5,Brown,Cyan)
    CALL DefinePalette(6,Magenta,Cyan)
    CALL DefinePalette(7,LightRed,Cyan)
    CALL DefinePalette(8,Blue,Yellow)
    CALL DefinePalette(9,Yellow,Cyan)
    CALL DefinePalette(10,Red+16,White)
END SUB

```

```

*-----*
| Subroutine - ChooseFileType
|-----|
| This subroutine allows the user to choose whether a
| Recovery or an Unknown file will be edited.
| Extension$ is set to REC if the file is a recovery file
| and if UNK the file is an unknown file.
|-----*

```

```

SUB ChooseFileType(Extension$)
    SHARED LineNumber%, ColumnNumber%
    CALL ScreenSetUp('LSC File Editor',7,3,2,32)
    LineNumber%-8 : ColumnNumber%=13
    CALL DrawWindow(5,40,4)
    LOCATE 9,15
    PRINT 'Use the Left and Right Arrow Keys to';
    LOCATE 10,15 : PRINT 'Highlight the type of file to edit.';
    LOCATE 11,15 : PRINT 'Then push RETURN.'
    LineNumber%-15 : ColumnNumber%-13
    CALL DrawWindow(3,40,4)
    Code%=1
    CALL ExtensionDisplay(Code%)
    ChooseExtension:
    CALL CheckKey(KeyPushed$)
    IF LEN(KeyPushed$)>1 THEN GOTO TestExtendedASCII
    IF KeyPushed$=CHR$(13) THEN GOTO ExtensionChosen
    NotValidKey:
    BEEP : GOTO ChooseExtension
    TestExtendedASCII:
    ExtendCode%=ASC(MID$(KeyPushed$,2,1))
    IF ExtendCode%<75 AND ExtendCode%<77 THEN
        GOTO NotValidKey
    IF Code%=2 THEN Code%=1 ELSE Code%=2
    CALL ExtensionDisplay(Code%)
    GOTO ChooseExtension
    ExtensionChosen:
    IF Code%=1 THEN Extension$='REC' ELSE
        Extension$='UNK'
END SUB

```

```

*-----*
| Subroutine - ExtensionDisplay
|-----|
| This subroutine displays the words Recovery and
| Unknown. If Code%=1 then Recovery is in reverse
| video and if Code%=2 then unknown is in reverse
| video.
|-----*

```



```

*-----*
1      Subroutine - AddDataToFile      |
1      This Subroutine adds one or two records to the data |
1      matrix.                        |
*-----*
SUB AddDataToFile(NumberToAdd%, RecordNumber%,A,B)
  SHARED FileData(), NumberOfRecords%
  FOR Record% = NumberOfRecords% TO RecordNumber%
    STEP -1
    FileData(Record%+NumberToAdd%) = FileData(Record%)
  NEXT Record%
  NumberOfRecords% = NumberOfRecords% +
    NumberToAdd%
  FileData(RecordNumber%) = A
  IF NumberToAdd% > 2 THEN GOTO EndAddDataToFile
  FileData(RecordNumber%+1) = B
  EndAddDataToFile:
END SUB

SUB AddFileBreak(BreakInFile%,Increment%)
  SHARED FileBreak(), NumberFileBreaks%
  NumberFileBreaks% = NumberFileBreaks% + 1
  FileBreak%(NumberFileBreaks%) = BreakInFile%
  CALL SortFileBreaks
  CALL FindBreak(BreakInFile%,BreakNumber%)
  CALL MoveFileBreaks(BreakNumber%+1,Increment%)
END SUB

SUB MoveFileBreaks(Begin%,Increment%)
  SHARED NumberFileBreaks%,FileBreak%()
  IF Begin% > NumberFileBreaks% THEN GOTO
    EndMoveFileBreaks
  FOR Break% = Begin% TO NumberFileBreaks%
    FileBreak%(Break%) = FileBreak%(Break%) + Increment%
  NEXT Break%
  EndMoveFileBreaks:
END SUB

SUB DeleteFileBreak(BreakInFile%,Increment%)
  SHARED NumberFileBreaks%, FileBreak%()
  CALL FindBreak(BreakInFile%,BreakNumber%)
  FOR Break% = BreakNumber% TO NumberFileBreaks%
    FileBreak%(Break%) = FileBreak%(Break%+1)
  NEXT Break%
  CALL MoveFileBreaks(BreakNumber%,Increment%)
  NumberFileBreaks% = NumberFileBreaks% - 1
END SUB

SUB FindBreak(BreakInFile%,BreakNumber%)
  SHARED NumberFileBreaks%, FileBreak%()
  FOR Break% = 1 TO NumberFileBreaks%
    IF FileBreak%(Break%) = BreakInFile% THEN GOTO
      EndFindBreak
  NEXT Break%
  PRINT "Error - Invalid Break Line"
  STOP
  EndFindBreak:
  BreakNumber% = Break%
END SUB

SUB ChooseAFile
  SHARED FileCount%,
    FileName$( ),LineNumber%,ColumnNumber%
  SHARED FileNumber%, FileToOpen$, FileOpen$
  LineNumber% = 8 : ColumnNumber% = 32
  CALL ScreenSetUp(Head$,8,3,2,32)
  CALL DrawWindow(FileCount%+2,16,4)
  LineNumber%=4 : ColumnNumber%=0
  CALL DrawWindow(3,50,4)
  CALL CenterText(5,Highlight the desired file, then push
    RETURN,2)
  FOR FileNumber% = 1 TO FileCount%
    LOCATE FileNumber%+8,34 : PRINT
      FileName$(FileNumber%)
  NEXT FileNumber%
  FileNumber%=1
  CALL HighlightFile(1)
  CheckForKey:
  CALL CheckKey(KeyPushed$)
  IF LEN(KeyPushed$)=2 THEN GOTO ExtendedASCII
  IF KeyPushed$=CHR$(13) THEN GOTO
    ReturnKeyPushed
  KeyError:
  BEEP : GOTO CheckForKey
  ExtendedASCII:
  Code%=ASC(RIGHT$(KeyPushed$,1))
  IF Code% < 72 AND Code% < 80 THEN GOTO
    KeyError
  OldFileNumber%=FileNumber%
  IF Code%=72 THEN FileNumber%=FileNumber%-1
  IF Code%=80 THEN FileNumber%=FileNumber%+1
  IF FileNumber% > FileCount% THEN
    FileNumber%=1
  IF FileNumber% < 1 THEN
    FileNumber%=FileCount%
  CALL HighlightFile(OldFileNumber%)
  GOTO CheckForKey
  ReturnKeyPushed:
  FileOpen$ = FileName$(FileNumber%)
  FileToOpen$="C:\LOTUS\LSC\'+FileName$(FileNumber%
  )
  CLOSE
  OPEN FileToOpen$ FOR INPUT AS #1
END SUB

SUB HighlightFile(OldFileNumber%)
  SHARED FileName$( ),FileNumber%
  LOCATE OldFileNumber%+8,34,0 :
  CALL SetColor (2) : PRINT FileName$(OldFileNumber%);
  LOCATE FileNumber%+8,34,0
  CALL SetColor (8)
  PRINT FileName$(FileNumber%);
  CALL SetColor (2)
END SUB

SUB GetData(Extension$)
  SHARED FileData(),NumberOfRecords%, FileBreak%(),
    NumberFileBreaks%
  NumberOfRecords%=0 : NumberFileBreaks% = 0
  WHILE NOT EOF(1)
    NumberOfRecords% = NumberOfRecords% + 1
    INPUT #1, FileData(NumberOfRecords%)
    IF FileData(NumberOfRecords%) >= 0 AND
      Extension$="UNK" OR NumberFileBreaks%=1
      THEN GOTO EndWhileLoop
    IF FileData(NumberOfRecords%) < 1000 AND
      Extension$="REC" THEN GOTO
      EndWhileLoop
    NumberFileBreaks% = NumberFileBreaks% + 1
    FileData(NumberOfRecords%+1) =
      FileData(NumberOfRecords%)
    FileData(NumberOfRecords%) = - 1
  END SUB

```

```

        NumberOfRecords% = NumberOfRecords% + 1
        FileBreak%(NumberFileBreaks%) =
            NumberOfRecords% - 1
    EndWhileLoop:
WEND
END SUB

SUB DisplayData
    SHARED FileData(), LineNumber%, ColumnNumber%,
        NumberOfRecords%
    SHARED Start%, NumberFileBreaks%,
        FileBreak%().Extension$
    LineNumber% = 4 : ColumnNumber% = 4 : Increment% = 14
    IF Start% + Increment% > NumberOfRecords% THEN
        Increment% = NumberOfRecords% - Start%
    CALL DrawWindow(17,29,4)
    FileRecord% = 0
    FOR Record% = Start% TO Start% + Increment%
        CALL DisplayARecord(Record%,1)
    NEXT Record%
END SUB

SUB HighlightData
    SHARED FileData(), DataRecord%, Start%,
        OldRecordNumber%
    Flag% = 1
    Beginning:
    IF Flag% = 1 THEN Record% = OldRecordNumber%
    IF Flag% > 1 THEN Record% = DataRecord%
    CALL DisplayARecord(Record%,Flag%)
    IF Flag% = 1 THEN Flag% = 2 : GOTO Beginning
END SUB

SUB DisplayARecord(Record%,Flag%)
    SHARED FileData(), NumberFileBreaks%, Start%,
        FileBreak%(), Extension$
    IF Flag% = 1 THEN CALL SetColor(2) ELSE Call
        SetColor(8)
    LOCATE Record% - Start% + 5,9,0
    IF FileData(Record%) >= 0 THEN GOTO NotAFileBreak
    PRINT 'Begin New File'
    GOTO EndDisplayARecord
    NotAFileBreak:
    CALL DetermineRecordNumber(Record%.FileRecord%)
    PRINT USING '###:FileRecord%';
    PRINT ' ';
    PRINT USING '####,#####:FileData(Record%)';
    EndDisplayARecord:
    CALL SetColor(2)
END SUB

SUB DetermineRecordNumber(Record%.FileRecord%)
    SHARED NumberFileBreaks%,FileBreak%().Extension$
    IF NumberFileBreaks% = 0 THEN StartRec% = 0 : GOTO
        SetFileRecord
    IF Record% = 1 AND Extension$ = "REC" THEN StartRec% = 0
        : GOTO SetFileRecord
    FOR Break% = 1 TO NumberFileBreaks%
        IF Record% < FileBreak%(Break%) AND Break% > 1
            THEN StartRec% = FileBreak%(Break% - 1) :
                GOTO SetFileRecord
        IF Record% < FileBreak%(Break%) AND Break% = 1
            THEN StartRec% = 0 : GOTO SetFileRecord
    NEXT Break%
    StartRec% = FileBreak%(NumberFileBreaks%)
    SetFileRecord:
        FileRecord% = Record% - StartRec%
    END SUB

SUB SortFileBreaks
    SHARED NumberFileBreaks%,FileBreak%()
    FOR StartPoint% = 1 TO NumberFileBreaks% - 1
        FOR Break% = StartPoint% TO NumberFileBreaks% - 1
            IF FileBreak%(Break% + 1) > FileBreak%(Break%)
                THEN GOTO EndBreakSortingLoop
            SWAP FileBreak%(Break% + 1),
                FileBreak%(Break%)
        EndBreakSortingLoop:
    NEXT Break%
    NEXT StartPoint%
END SUB

SUB FindFileBreaks
    SHARED NumberFileBreaks%, FileBreak%(),
        NumberOfRecords%, FileData()
    NumberFileBreaks% = 0
    FOR Record% = 1 TO NumberOfRecords%
        IF FileData(Record%) >= 0 THEN GOTO
            ContinueLoop
        NumberFileBreaks% = NumberFileBreaks% + 1
        FileBreak%(NumberFileBreaks%) = Record%
    ContinueLoop:
    NEXT Record%
END SUB

SUB EditFile
    DIM KeyCode%(10)
    DATA 71,72,73,79,80,81,82,83
    SHARED FileData(), NumberOfRecords%, LineNumber%,
        ColumnNumber%
    SHARED Start%, DataRecord%, OldRecordNumber%,
        FileOpen$,Extension$
    SHARED NumberFileBreaks%, FileBreak%()
    IF Extension$ = "REC" THEN ExtCode% = 2 ELSE ExtCode% = 1
    RESTORE
    FOR Code% = 1 TO 8
        READ KeyCode%(Code%)
    NEXT Code%
    Start% = 1 : DataRecord% = 1 : OldRecordNumber% = 1
    CALL ScreenSetUp("Editing "+FileOpen$,8,3,2,32)
    LineNumber% = 4 : ColumnNumber% = 35
    CALL DrawWindow(13,43,4)
    LOCATE 5,37 : PRINT "Ins  Insert before the current line.;"
    LOCATE 6,37 : PRINT "Del  Delete the current line.;"
    LOCATE 7,37 : PRINT "PgUp  Back data up one screen full.;"
    LOCATE 8,37 : PRINT "PgDn  Advance data one screen full.;"
    LOCATE 9,37 : PRINT "Home  Goto beginning of data.;"
    LOCATE 10,37 : PRINT "End    Goto end of data.;"
    LOCATE 11,37 : PRINT "Return Add data to end of data.;"
    LOCATE 12,37 : PRINT CHR$(24); "    Goto the previous line.;"
    LOCATE 13,37 : PRINT CHR$(25); "    Goto the next line.;"
    LOCATE 14,37 : PRINT "Esc  Write data to Hard Disk.;"
    LOCATE 15,37 : PRINT "Insert a -1 for a file break (UNK only).;"
    CALL DisplayData
    CALL HighlightData
    TestKey:
    CALL CheckKey(KeyPushed$)
    IF LEN(KeyPushed$) = 2 THEN GOTO ExtendedCodes
    Code% = ASC(KeyPushed$)
    IF Code% = 27 THEN GOTO StoreData
    IF Code% = 13 AND DataRecord% = NumberOfRecords% THEN
        GOTO AddToEnd
    IF Code% > 47 AND Code% < 58 OR Code% = 45 THEN GOTO
        ChangeData

```

```

BadKey:
  BEEP : GOTO TestKey
ExtendedCodes:
  Code%=ASC(RIGHTS(KeyPushed$,1))
  FOR Test% = 1 TO 8
    IF KeyCode%(Test%) = Code% THEN GOTO
GoodKey
  NEXT Test%
  GOTO BadKey
GoodKey:
  SELECT CASE Test%
CASE - 1 Home Key
  Start%=1 : DataRecord%=1 : OldRecordNumber%=1
  CALL DisplayData
  CALL HighlightData
CASE - 2 Up Arrow Key
  OldRecordNumber%=DataRecord%
  DataRecord% = DataRecord% - 1
  IF DataRecord%<1 THEN BEEP : DataRecord%=1
  IF DataRecord%>=Start% THEN GOTO
  StillOnScreen
  Start%=DataRecord%
  CALL DisplayData
  StillOnScreen:
  CALL HighlightData
CASE - 3 Page Up Key
  LineNumber%=DataRecord%-Start%+1
  Start%=Start%-15
  IF Start%>=1 THEN GOTO NotPastBeginningOfFile
  Start%=1 : DataRecord%=LineNumber% :
  OldRecordNumber%=DataRecord%
  GOTO DisplayTheData
NotPastBeginningOfFile:
  DataRecord%=DataRecord%-15
  OldRecordNumber%=DataRecord%
  DisplayTheData:
  Call DisplayData
  CALL HighlightData
CASE - 4 End Key
  IF NumberOfRecords%<15 THEN Start%=1 ELSE
  Start%=NumberOfRecords%-14
  DataRecord%=NumberOfRecords%
  OldRecordNumber% = DataRecord%
  CALL DisplayData
  CALL HighlightData
CASE - 5 Down Arrow Key
  OldRecordNumber% = DataRecord%
  DataRecord% = DataRecord% + 1
  IF DataRecord%>NumberOfRecords% THEN BEEP :
  DataRecord%=OldRecordNumber% : GOTO
  TestKey
  IF DataRecord%<=Start%+14 THEN GOTO
  StillOnScreen2
  Start%=Start%+1
  CALL DisplayData
  StillOnScreen2:
  CALL HighlightData
CASE - 6 Page Down Key
  LineNumber%=DataRecord%-Start%+1
  Start% = Start% + 15
  IF Start%+14<=NumberOfRecords% THEN GOTO
  NotPastEndOfFile
  Start% = NumberOfRecords% - 14
  IF Start%<1 THEN Start%=1
  DataRecord%=LineNumber%-Start%-1 :
  OldRecordNumber%=DataRecord%
  GOTO DisplayTheData
  NotPastEndOfFile:
  DataRecord% = DataRecord% + 15
  OldRecordNumber% = DataRecord%
  GOTO DisplayTheData
CASE - 7 Insert Key
  CALL AddDataToFile(1,DataRecord%,0,0)
  InsertMode:
  CALL DisplayData
  LOCATE DataRecord%-Start%+5,9,0
  CALL DetermineRecordNumber(
  DataRecord%,FileRecord%)
  CALL SetColor(8)
  PRINT USING '###:FileRecord%';
  PRINT STRING$(16,32);
  CALL SetColor(2)
  LineNumber%=17 : ColumnNumber%=42
  CALL DrawWindow(5,19,4)
  LOCATE 18,43 : PRINT 'Enter the value';
  LOCATE 19,43 : PRINT 'to insert at ':FileRecord%;
  LOCATE 20,43 : INPUT ";NewValue
  Increment%=2
  GotNewValue:
  FileData(DataRecord%) = NewValue
  IF NewValue<1000 OR Extension$<<'REC' THEN
  GOTO HighlightTheData
  IF OldValue>1000 AND Extension$='REC' THEN
  GOTO HighlightTheData
  CALL AddDataToFile(1,DataRecord%,-1,0)
  HighlightTheData:
  CALL FindFileBreaks
  CALL DisplayData
  CALL HighlightData
  LineNumber%=17 : ColumnNumber%=42
  CALL EraseWindow(5,19,2,32)
CASE - 8 Delete Key
  TypeCode%=1
  IF DataRecord%<NumberOfRecords% THEN GOTO
  NotLastRecord
  NumberOfRecords% = NumberOfRecords%-1
  DataRecord%=DataRecord% - 1 :
  OldRecordNumber% = DataRecord%
  GOTO CheckUp:
NotLastRecord:
  BreakCode% = 1
  IF FileData(DataRecord%)>1000 AND
  Extension$='REC' THEN GOTO DeleteBreak
  IF FileData(DataRecord%)>=0 THEN
  NotDeleteFileBreak
  IF Extension$='REC' THEN GOTO BadKey
  DeleteBreak:
  IF ExtCode% = 2 THEN BreakCode% = 2
  NotDeleteFileBreak:
  CALL DeleteDataFromFile( BreakCode%,
  DataRecord%-BreakCode%+1)
  CheckUp:
  IF NumberOfRecords%=0 THEN GOTO StoreData
  CALL FindFileBreaks
  GOTO DisplayTheData
END SELECT
GOTO TestKey
AddToEnd:
  Escape%=1
  DataRecord% = DataRecord% + 1
  IF DataRecord%-Start%>14 THEN Start%=DataRecord%-
  14
  NumberOfRecords% = NumberOfRecords% + 1
  GOTO InsertMode:
ChangeData:

```



```

CALL SetColor (BorderColor%)
PRINT CHR$(219);
NEXT X%
LOCATE 25,2: COLOR White : PRINT CHR$(32);
CALL SetColor (BorderColor%)
PRINT CHR$(219)+STRING$(76,220)+CHR$(219);
END SUB

```

```

-----*
| Subroutine - WaitDraw |
|-----|
| This subroutine draws the figure : *-----* |
| | Please Wait. | |
| *-----* |
| on the screen at the location X%,Y%, where X%,Y% is the |
| upper left corner of the box. The words 'Please Wait.' |
| blink. |
|-----*

```

```

SUB WaitDraw(X%,Y%,TextColor,BorderColor)
  SHARED Black, Blue, Green, Cyan, Red, Magenta, Brown,
  White, Gray
  SHARED LightBlue, LightGreen, LightCyan, LightRed,
  LightMagenta
  SHARED Yellow, HighIntensityWhite
  CALL SetColor (BorderColor)
  IF X%=0 THEN X%=11
  IF Y%=0 THEN Y%=32
  LOCATE X%, Y%
  PRINT CHR$(218)+STRING$(15,196)+CHR$(191)
  LOCATE X%+1,Y%
  PRINT CHR$(179)+STRING$(15,32)+ CHR$(179)
  LOCATE X%+2,Y%
  PRINT CHR$(192)+STRING$(15,196)+CHR$(217)
  LOCATE X%+1,Y%+2 :CALL SetColor (TextColor)
  PRINT 'Please Wait.';
END SUB

```

```

-----*
| Subroutine - WaitErase |
|-----|
| This subroutine erases the figure draw by the WaitDraw |
| subroutine, covering it over with the character(ASCII 176). |
|-----*

```

```

SUB WaitErase(X%, Y%, BackgroundColor,
  BackgroundCharCode%)
  SHARED Black, Blue, Green, Cyan, Red, Magenta, Brown,
  White, Gray
  SHARED LightBlue, LightGreen, LightCyan, LightRed,
  LightMagenta
  SHARED Yellow, HighIntensityWhite
  CALL SetColor (BackgroundColor)
  LOCATE X%,Y%
  PRINT STRING$(20,BackgroundColor%);
  LOCATE X%+1,Y%
  PRINT STRING$(20,BackgroundColor%);
  LOCATE X%+2,Y%
  PRINT STRING$(20,BackgroundColor%);
END SUB

```

```

-----*
| Subroutines : Hi |
| Low |
| Reverse |
| ReverseLow |
| ReverseBlink |
|-----*

```

```

| Blink |
|-----|
| These short subroutines are for use on monochrome screen |
| computers, but can be used with computers equipped with |
| color monitors. They control the way the characters are |
| printed on the screen. |
|-----|

```

```

SUB Hi
  COLOR 14,1
END SUB

SUB Low
  COLOR 15,1
END SUB

SUB Reverse
  COLOR 1,7
END SUB

SUB ReverseLow
  COLOR 1,8
END SUB

SUB ReverseBlink
  COLOR 17,7
END SUB

SUB Blink
  COLOR 31,1
END SUB

```

APPENDIX C. - DIVERSE: A Utility for calculating Finite State Diversities

1. General Description:

The DIVERSE utility is a Microsoft EXCEL worksheet which uses macros to calculate the diversity indices of Shannon (Shannon and Weaver, 1949) and Brillouin (Brillouin, 1962) for data sets. For a detailed discussion of the indices, see Pielou, 1969.

2. Macro Listing (DIVERSE.XLM)

| | |
|---------------|---|
| DiversityCalc | -RESULT(1) |
| | -ARGUMENT('dataField',8) |
| | -VOLATILE(TRUE) |
| SummedArea | -SUM(dataField) |
| | -IF(NOT(SummedArea)) |
| | - RETURN(0) |
| | -END.IF() |
| | -SET.VALUE(SummedDiversity,0) |
| | -FOR.CELL('CurrentCell',dataField,TRUE) |
| | -IF(NOT(ISTEXT(CurrentCell))) |
| Proportion | - CurrentCell/SummedArea |
| | - IF(Proportion<=0) |
| | - SET.VALUE(SummedDiversity, SummedDiversity- |
| | (Proportion)*LN(Proportion)) |
| | - END.IF() |
| | -END.IF() |
| | -NEXT() |
| | -RETURN(DIVERSE.XLM!SummedDiversity) |

| | |
|------------------------|---|
| | |
| RecalculateTable | -CALCULATE.NOW() |
| | -CALCULATE.NOW() |
| | -RETURN() |
| | |
| EmptyTable | -SELECT(!\$B\$5:\$M\$16) |
| | -CLEAR(3) |
| | -SELECT(!\$B\$5) |
| | -RETURN() |
| | |
| | |
| SummedDiversity | |
| SummedCount | |
| ProductedDiversity | |
| H | |
| | |
| | |
| BrillouinDiversityCalc | -RESULTY(1) |
| | -ARGUMENT('dataField',8) |
| | -VOLATILE(TRUE) |
| SummedBArea | -SUM(dataField) |
| Factorial | -FACT(SummedBArea) |
| | -SET.VALUE(ProductedDiversity,1) |
| | -FOR.CELL('CurrentCell',dataField,TRUE) |
| | - SET.VALUE(ProductedDiversity, ProductedDiversity*FACT(CurrentCell)) |
| | -NEXT() |
| | -SET.VALUE(H,1/SummedBArea*LN(Factorial/ProductedDiversity)) |
| | -RETURN(DIVERSE.XLMIH) |
| | |
| | |
| | |

| | |
|-----------|---------------------------------|
| | -FACT(3) |
| | |
| Auto_Open | -VOLATILE(TRUE) |
| Path | -GET.DOCUMENT(2) |
| | -HIDE() |
| | -OPEN(Path&'\DIVERSE.XLS') |
| | -WINDOW.MAXIMIZE('DIVERSE.XLS') |
| | =SELECT(!\$B5) |
| | =RETURN() |

3. Worksheet Design (DIVERSE.XLS)

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S |
|----|---|-----------------|----------|---|---|---|---|---|---|---|---|----|----|----|---|---|-------------------------------|------------------------|-----------|
| 1 | | | | | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | | | | | | |
| 4 | | | Interval | | | | | | | | | | | | | | | | |
| 5 | | State | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Weighted Diversity of Intervals (Shannon) | Weighted Diversity of Intervals (Brillouin) | | | |
| 6 | | 1 | | | | | | | | | | | | | | | Total | 0 | |
| 7 | | 2 | | | | | | | | | | | | | | | Diversity Index | 1.000 | |
| 8 | | 3 | | | | | | | | | | | | | | | Hmax | | |
| 9 | | 4 | | | | | | | | | | | | | | | H | | |
| 10 | | 5 | | | | | | | | | | | | | | | X | 0 | |
| 11 | | 6 | | | | | | | | | | | | | | | Y | 1 | |
| 12 | | 7 | | | | | | | | | | | | | | | z | 12 | |
| 13 | | 8 | | | | | | | | | | | | | | | r | 0 | |
| 14 | | 9 | | | | | | | | | | | | | | | H | | |
| 15 | | 10 | | | | | | | | | | | | | | | | | |
| 16 | | 11 | | | | | | | | | | | | | | | | | |
| 17 | | 12 | | | | | | | | | | | | | | | | Shannon | Brillouin |
| 18 | | Clear Table | | | | | | | | | | | | | | | Mean State Interval Diversity | 0.000 | 0.000 |
| 19 | | Proportion | | | | | | | | | | | | | | | Calculate Table | Equally Good Intervals | |
| 20 | | Interval Totals | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | Temporal State Diversity | | |
| 21 | | | | | | | | | | | | | | | | | Temporal Evenness | 0.000 | |

The "Clear Table" button is linked to the EmptyTable Function, and the Calculate Table" button is linked to the RecalculateTable Function.

4. Macro Sheet Named Cells (DIVERSE.XLM)

| Name | Reference | Type |
|------------------------|------------------------------|-----------|
| Auto_Open | =\$B\$54 | Function |
| BrillouinDiversityCalc | =\$B\$38 | Function |
| CurrentCell | =DIVERSE.XLS!\$O\$19 | Reference |
| dataField | =DIVERSE.XLS!\$B\$18:\$M\$18 | Argument |
| Diversity | =\$B\$10 | Variable |
| DiversityCalc | =\$B\$2 | Function |
| EmptyTable | =\$B\$25 | Function |
| Factorial | =\$B\$42 | Variable |
| H | =\$B\$35 | Variable |
| Path | =\$B\$55 | Variable |
| ProductedDiversity | =\$B\$34 | Variable |
| Proportion | =\$B\$12 | Variable |
| RecalculateTable | =\$B\$21 | Function |
| SummedArea | =\$B\$5 | Variable |
| SummedBArea | =\$B\$41 | Variable |
| SummedCount | =\$B\$33 | Variable |
| SummedDiversity | =\$B\$32 | Variable |

4. Worksheet Named Cells (DIVERSE.XLS)

| Name | Reference | Type |
|-----------|-----------------|-----------|
| dataArea | =\$B\$5:\$M\$16 | Reference |
| Diversity | =\$Q\$6 | Variable |
| EGI | =\$Q\$18 | Variable |
| EGI_B | =\$R\$18 | Variable |
| H | =\$Q\$14 | Variable |
| Hmax | =\$Q\$8 | Variable |
| Hprime | =\$Q\$9 | Variable |
| Intervals | =\$B\$4:\$M\$4 | Variable |
| MSTD | =\$Q\$17 | Variable |
| MSTD_B | =\$R\$17 | Variable |
| r_ | =\$Q\$13 | Variable |
| s | =\$Q\$12 | Variable |
| Total | =\$Q\$5 | Variable |
| X | =\$Q\$10 | Variable |
| Y | =\$Q\$11 | Variable |

5. Worksheet Cell Functions

| Weighted Diversity of Intervals (Shannon) | Weighted Diversity of Intervals (Brillouin) |
|--|--|
| =IF(Total,SUM(B5:M5)/Total*EXP("DIVERSE.XLM!DiversityCalc(B5:M5)),") | =IF(SUM(B5:M5),SUM(B5:M5)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B5:M5)),") |
| =IF(Total,SUM(B6:M6)/Total*EXP("DIVERSE.XLM!DiversityCalc(B6:M6)),") | =IF(SUM(B6:M6),SUM(B6:M6)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B6:M6)),") |
| =IF(Total,SUM(B7:M7)/Total*EXP("DIVERSE.XLM!DiversityCalc(B7:M7)),") | =IF(SUM(B7:M7),SUM(B7:M7)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B7:M7)),") |
| =IF(Total,SUM(B8:M8)/Total*EXP("DIVERSE.XLM!DiversityCalc(B8:M8)),") | =IF(SUM(B8:M8),SUM(B8:M8)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B8:M8)),") |
| =IF(Total,SUM(B9:M9)/Total*EXP("DIVERSE.XLM!DiversityCalc(B9:M9)),") | =IF(SUM(B9:M9),SUM(B9:M9)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B9:M9)),") |
| =IF(Total,SUM(B10:M10)/Total*EXP("DIVERSE.XLM!DiversityCalc(B10:M10)),") | =IF(SUM(B10:M10),SUM(B10:M10)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B10:M10)),") |
| =IF(Total,SUM(B11:M11)/Total*EXP("DIVERSE.XLM!DiversityCalc(B11:M11)),") | =IF(SUM(B11:M11),SUM(B11:M11)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B11:M11)),") |
| =IF(Total,SUM(B12:M12)/Total*EXP("DIVERSE.XLM!DiversityCalc(B12:M12)),") | =IF(SUM(B12:M12),SUM(B12:M12)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B12:M12)),") |
| =IF(Total,SUM(B13:M13)/Total*EXP("DIVERSE.XLM!DiversityCalc(B13:M13)),") | =IF(SUM(B13:M13),SUM(B13:M13)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B13:M13)),") |
| =IF(Total,SUM(B14:M14)/Total*EXP("DIVERSE.XLM!DiversityCalc(B14:M14)),") | =IF(SUM(B14:M14),SUM(B14:M14)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B14:M14)),") |
| =IF(Total,SUM(B15:M15)/Total*EXP("DIVERSE.XLM!DiversityCalc(B15:M15)),") | =IF(SUM(B15:M15),SUM(B15:M15)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B15:M15)),") |
| =IF(Total,SUM(B16:M16)/Total*EXP("DIVERSE.XLM!DiversityCalc(B16:M16)),") | =IF(SUM(B16:M16),SUM(B16:M16)/Total*EXP("DIVERSE.XLM!BrillouinDiversityCalc(B16:M16)),") |

| | A | B | C | D | E | F | G |
|-----------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|---|
| Proportion | =IF(Total,SUM(B5:B16)/Total,") | =IF(Total,SUM(C5:C16)/Total,") | =IF(Total,SUM(D5:D16)/Total,") | =IF(Total,SUM(E5:E16)/Total,") | =IF(Total,SUM(F5:F16)/Total,") | =IF(Total,SUM(G5:G16)/Total,") | |
| Interval Totals | =SUM(B5:B16) | =SUM(C5:C16) | =SUM(D5:D16) | =SUM(E5:E16) | =SUM(F5:F16) | =SUM(G5:G16) | |

| | H | I | J | K | L | M |
|--|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | =IF(Total,SUM(H5:H16)/Total,") | =IF(Total,SUM(I5:I16)/Total,") | =IF(Total,SUM(J5:J16)/Total,") | =IF(Total,SUM(K5:K16)/Total,") | =IF(Total,SUM(L5:L16)/Total,") | =IF(Total,SUM(M5:M16)/Total,") |
| | =SUM(H5:H16) | =SUM(I5:I16) | =SUM(J5:J16) | =SUM(K5:K16) | =SUM(L5:L16) | =SUM(M5:M16) |

| | | | | | |
|--|--|--|--|--|--|
| | | | | | |
|--|--|--|--|--|--|

| | Shannon | Brillouin |
|-------------------------------|---|---|
| Mean State Interval Diversity | =SUM(N5:N16) | =SUM(O5:O16) |
| Equally Good Intervals | =IF(Total,EXP("D:\THESIS\APPENDIX\ DIVERSE.XLM!IDiversityCalc(B18:M18)"), "") | =IF(Total,EXP("D:\THESIS\APPENDIX\ DIVERSE.XLM!BrillouinDiversityCalc(B19:M19)"), "") |
| Temporal State Diversity | =IF(Total,EGI/MSTD,"") | =IF(Total,EGI_B/MSTD_B,"") |
| Temporal Evenness | =Hprime/LN(COUNT(Intervals)) | =IF(Total<COUNT(Intervals),"",H/Hmax) |

| | |
|-----------|--|
| Total | =SUM(B5:M16) |
| Diversity | =DIVERSE.XLM!IDiversityCalc(dataArea) |
| Index | =EXP(Q6) |
| Hmax | =IF(Total<COUNT(Intervals),"",1/Total*LN(FACT(Total)/(FACT(X)^(s-r)*FACT(Y)^r))) |
| H' | =DIVERSE.XLM!IDiversityCalc(B18:M18) |
| X | =INT(Total/s) |
| Y | =INT(Total/s)+1 |
| s | =COUNT(Intervals) |
| r | =MOD(Total,COUNT(Intervals)) |
| H | =IF(Total,'DIVERSE.XLM!BrillouinDiversityCalc(B19:M19),"") |

Vita

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