

GROWTH AND AGE STUDY OF PACIFIC HAGFISH  
(Eptatretus stoutii) OFF THE CENTRAL CALIFORNIA COAST

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## I. Executive Summary

The recent development of a short term, but very intense fishery for Pacific hagfish (Eptatretus stoutii) on the Pacific Coast of North America prompted concern over the absence of management and conservation guidelines for this species. However, very little is known of the biology of hagfish, particularly with regard to information applicable to fishery resource management. Growth and population age-size structure data are among critical categories of information that are non-existent to date.

This project obtained primary growth information from field and laboratory studies as well as comprehensive population size composition data. Field growth information consisted of data from mark and recapture studies and the collection of a large volume of size class information encompassing all seasons. The latter provided a detailed, composite illustration of the size class structure of the hagfish population throughout the year from which inferences regarding stock recruitment and size class distributions were made. Field growth data were augmented by growth and behavioral observations on small groups of fish maintained under controlled conditions in the laboratory. A biochemical assay for so-called Fluorescent Age Pigments (FAPs) was conducted in conjunction with the primary growth studies as a possible means of confirming, and perhaps predicting size-at-age of Pacific hagfish.

Growth studies were based on direct measurements of absolute size changes of marked fish released in the field. These were then used to determine annual and instantaneous growth rates for an array of individual hagfish sizes and the generation of overall predictive annual and instantaneous growth rate curves. These were ultimately used to extrapolate and predict "size-at-age" over wide range of lengths and construct a hypothetical absolute growth curve for the species encompassing a size range of 50 to 450-500+ mm total length. Several growth pattern scenarios and extrapolated growth curves for these jawless fish were developed to accommodate a possible unique growth characteristic of hagfish where negative growth occurs on a consistent basis. Aside from this deviation from the norm, hagfish appear to show similar trends to bony and cartilaginous fishes in that growth appears to be most rapid in smaller and younger individuals and that growth rates are affected by temperature as well as other factors associated with confinement in an artificial environment.

Several growth curves and subsequent size-at-age predictions were developed. These varied depending on the use of annual or instantaneous growth rate projections and the inclusion or exclusion of atypical (negative and zero) growth data. Given that annual rates have no statistical additive properties, extrapolations and predictions based on annual rates were used only as a comparison with instantaneous rates and a comparison between growth curves using two different hatch sizes. Greater reliance was placed on instantaneous rate based extrapolations and predictions. The most comprehensive of these were those utilizing all available and appropriate data (positive, negative, zero growth). This model predicted older ages per size category while the statistically more conservative one predicted younger ages per size class.

Analysis of length-frequency distribution patterns based on thousands of fish collected in twelve fishing trips throughout the year indicated that recruitment to the stock occurred at a size of about 180-200 mm total length and revealed the presence of distinct size class groups. In most instances size cohorts were difficult to separate by visual inspection, particularly among the mid-sized lengths. In some cases, however, good separation of modal size groups could be seen. In the best of these, the modal size classes were related to the size-at-age predictions based on the more reliable instantaneous growth rate data and tentative hypothetical age assignments were made, albeit with appropriate reservations. The statistical separation of mixed distributions from the larger overall length-frequency distributions of hagfish is presently being pursued as a separate project using other approaches including so-called Cassie Curves (Cassie, 1954; Summerfield and Olds, 1987) and maximum likelihood probability analyses (Agha and Ibrahim, 1984).

On the bases of the instantaneous growth rate projections it is tentatively estimated that the ages of the lower and upper size ranges (180-450+ mm Total Length) of hagfish captured in this project ranged in age from 4-8 years and 15-25 years respectively depending on the inclusion or exclusion of zero or negative growth data. Estimates using only positive growth data yield younger ages per size class while inclusion of all data produces older ages per size group. Female hagfish attain sexual maturity at about 325 mm TL and are 7-12 years old. The original targeted minimum size by the commercial fishery in California of 354+ mm TL represent fish between 8-12 years of age.

FAP analysis from three types of tissues (brain, muscle, heart) displayed wide variations despite efforts to standardize protocols according to the most rigorous review of the method to date (Hill and Wormsley,1991;Hill,1992). However, separation of FAP levels according to sexes indicated only limited potential for application with brain and heart tissues while measures of FAP titers for muscle varied too greatly to show a useful trend for possible confirmation of size-at-age. Statistically valid trends where FAP levels were directly proportional to size were shown for brain tissue FAPs for males but not for females. Conversely, a definitive trend was shown for heart tissue from females but not for males. Thus, while definable trends were found in this study, the wide overall variation in the data between and within tissue types and apparent differences between sexes limits FAP analysis as a simple, reliable tool for size-at-age confirmation in field populations. The problems noted are similar to those found in other previous work with FAPs on a variety of animals (Crossland, et al.,1989; Sheehy,1989; Vernet, Hunter and Vetter,1988). These limit the potential application of FAP analysis on hagfish. Unless variation from field samples can be reduced, FAP analysis may not be a fruitful avenue to pursue on a whole population level.

In general, this project provides a composite picture of hagfish growth patterns and makes tentative predictions of size-at-age within a Central California population of Pacific Hagfish. These were made using a number of different scenarios based on differing biological assumptions and serve as a useful starting point from which other working hypotheses can be generated. This is particularly important in view of the fact that there are no other information or data of this type available on Pacific Hagfish to date. It should be noted that efforts to improve upon the information gathered through this project will be continued through other smaller projects of narrower focus (e.g. Maximum Likelihood Analyses of size class distributions; statolith analysis). It is noteworthy that the interest in commercial hagfish fishing for the "eelskin" industry has recently shifted to the Northeastern United States and that hagfish research in the context of fisheries ecology is generating considerable attention in that region. Hopefully, the information contained here will be of use and also provide insights with regard to similar species being considered for exploitation in the Northwest Atlantic Ocean.

## II. INTRODUCTION

The Pacific hagfish (Eptatretus stoutii) is one of several species of primitive jawless fishes which have historically been ignored by fisheries biologists and ichthyologists for a variety of reasons. Thus it is not surprising that there are wide informational gaps on the biology of this species. In North America, most ichthyologists and fisheries scientists have historically considered hagfishes as mere biological oddities while the fishing industry looked upon these fish as pests or "trash-fish" of no economic value. However, while this perception prevailed for decades in North America, hagfish became the basis of the lucrative Asian "eel-skin wallet" industry (Kato, 1989; Gorbman, et al, 1990) which produces western fashion accessories and had quietly grown to a multimillion dollar industry abroad. During the late 1980's, foreign buyers canvassed Pacific coast of North America in an effort to establish new sources of hagfish as it appeared that the Asian fisheries for hagfish based on Eptatretus burgeri and E. atami was in decline while the demand for hagfish skins continued to grow. By 1988, a new fishery for Pacific hagfish (E. stoutii) was established and appeared to be growing explosively as many fishers were drawn to a lucrative market for a seemingly abundant product which required little investment and risk. Analysis of California catch data at the peak of the fishery in 1990 (Figure 1,2) revealed that millions of fish were being harvested. These did not account for high levels of waste of sub-optimal sized hagfish which were apparently discarded on a routine basis (Novak, 1990) The explosive growth and intensity of the California hagfish fishery was of great concern to fisheries biologists and resource policy makers as it was immediately apparent that virtually no information on the biology and ecology of this fish existed. This was particularly true of information directly applicable to the development of resource management and conservation guidelines. These factors coupled with the documented low fecundity (Gorbman, 1990; Nakamura, 1991; Johnson, 1992) and sudden economic value of the species indicated a potential for another "Boom and Bust" fishery on the Pacific coast.

Figure 1

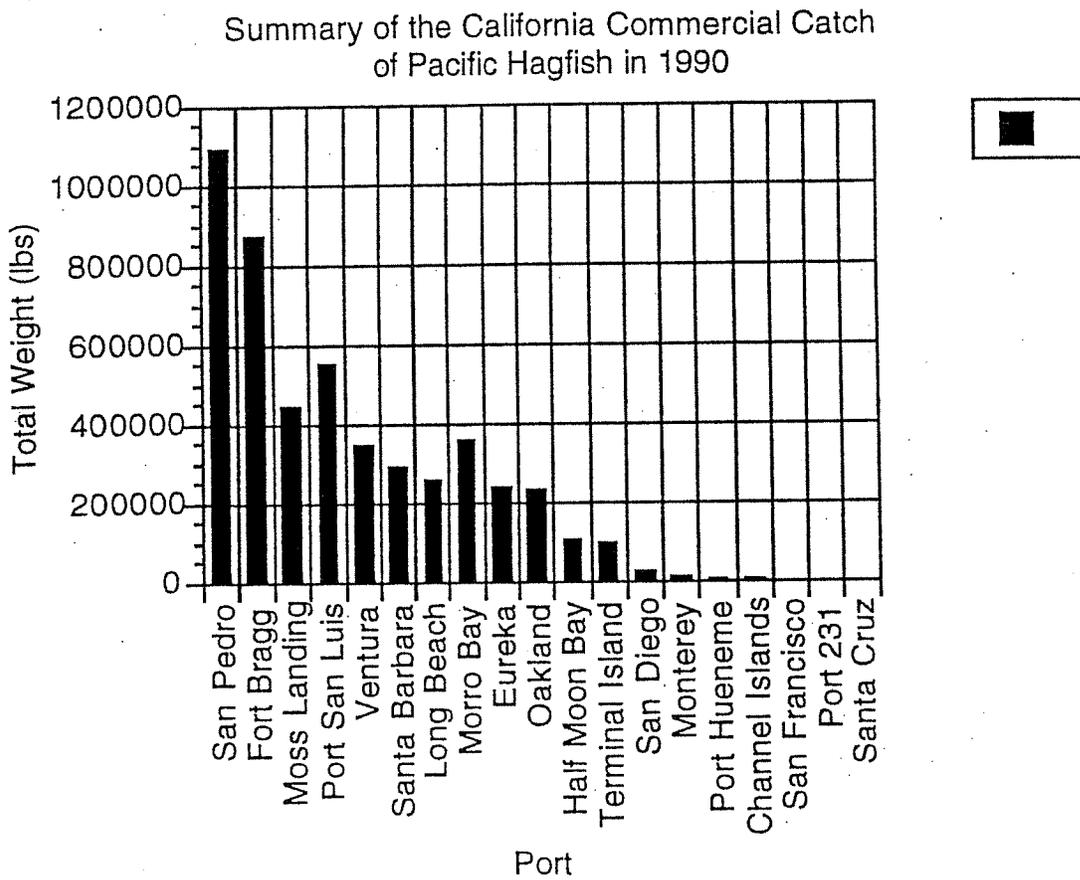
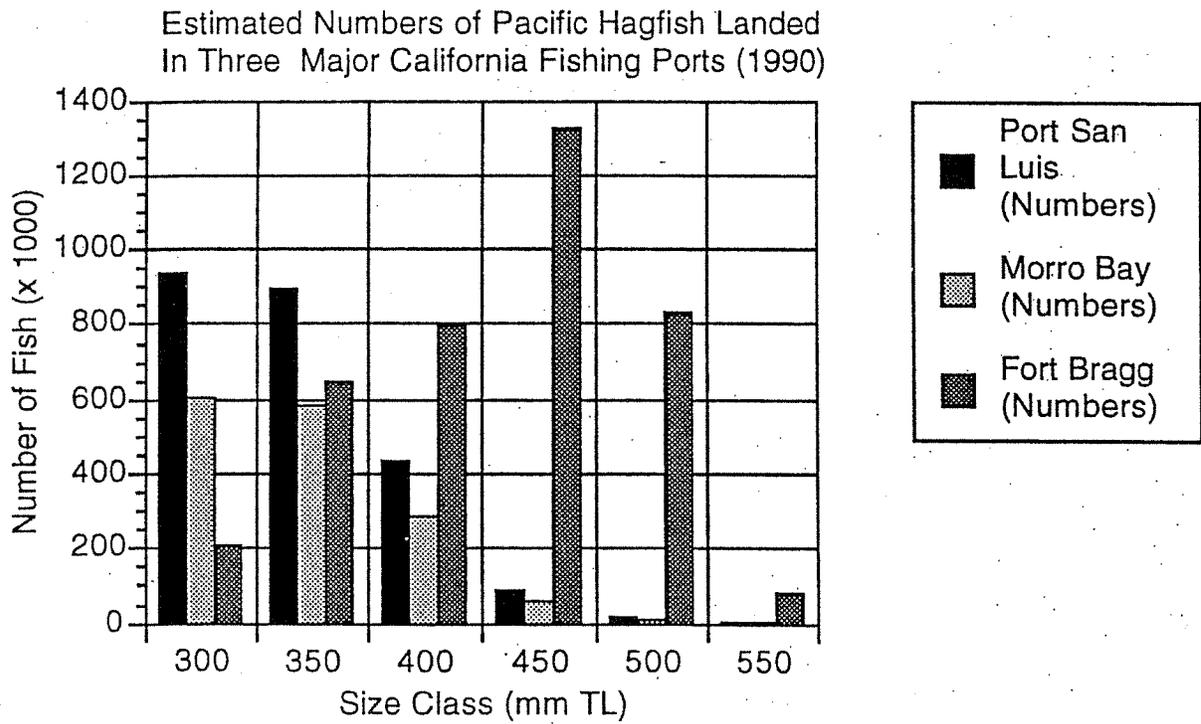


Figure 2



A number of research projects were quickly funded by state and federal agencies to conduct research in hagfish biology (Brooks and Love,1990;Calliet,1991) and fisheries technology (Melvin,1992) beginning around 1989-1990. This particular project is an offshoot of one of the larger of the initial hagfish research projects (Nakamura, 1991) funded in 1989-1990. Our effort which was funded by the former Office of Environmental Affairs for the state of California, was a broad-based study on an array of aspects of reproductive biology and ecology including seasonal population size-depth distributions. Importantly, the initial hagfish project served to define some critical informational areas for future investigation. One of these involved the acquisition of growth-age data and related population size(age) composition information which was non-existent at the time. The project described in this report was an effort to obtain this information.

### III. PURPOSE

#### A. Basic Problem or Impediment Addressed.

The purpose of this project was to obtain growth data on hagfishes for extrapolation and prediction of hypothetical size and age relationships. Conventional wisdom among fisheries scientists dictates that ideally at least three independent means of confirming size-at-age relationships are necessary for unequivocal substantiation. It should be noted, however, that in practice satisfying all these requirements is more the exception than the rule in the real world of applied fisheries science. Specifically with regard to hagfishes, there are constraints in applying some of the more common fisheries research methodologies for age/growth studies due to the unique biology of the species.

Confirmation of size at age relationships include a variety of approaches and methods (Weatherly,1972;Summerfelt and Hall,1987). These include use of natural or induced growth rings in hard tissues, use of growth data from mark and recapture studies in the field, analysis of population size-frequency distributions for discreet modal size(age) groups and the monitoring of growth of laboratory held populations, preferably of known age. Hagfish are evolutionarily, structurally and physiologically atypical compared to most other groups such as the bony fishes(Osteichthyes) and cartilaginous fishes(Chondrichthyes). In these groups, determination

of age and growth relationships relies primarily on traditional methods based on analyses of hard tissues for annual growth rings. Primitive jawless fishes (Agnatha) such as hagfishes lack hard endoskeletal tissues or superficial epidermal bony structures which could deposit seasonal growth rings (annuli). While non-skeletal tissues in the form of statoliths have been anecdotally mentioned in the literature (Hardisty and Potter, 1982; Bond, 1982) these have never been established as a possible indicator or growth rings for hagfishes.

With regard to the widely practiced method of applying external tags to facilitate growth monitoring in individually identifiable fish, the lack of a substantive hard endoskeletal structures as anchoring points for tags in hagfishes precludes use of these devices. This, in addition to the well known extreme body contortions and knotting behavior of hagfishes (Hardisty, 1979) were shown to cause major tag loss in our initial work.

The absence of historic commercial catch data for Pacific hagfish appropriately sampled for length-frequency analyses precluded examination of the population for presence of discreet modal size (age) groups for clues or a basis for inferences on population size class structure and growth (Petersen Method). Previous work by other researchers using captive hagfish in the laboratory have never focused specifically on growth. These factors and the consideration of the fact that the deep benthic environment inhabited by hagfish is relatively inaccessible and a difficult place to obtain the desired data necessitated the use of some unconventional and indirect means of achieving the goals of this project.

## B. Objectives

The objectives of the study were to obtain data on absolute growth rates from live hagfish under different conditions and to use these data for extrapolation and prediction of hypothetical size-at-age assignments based on annual and instantaneous rate conversions. Absolute growth curves were to be constructed based on these conversions. "Walford Plots" were also to be constructed as a gross check for consistency of growth rate estimates and predicted age relative to observations of large hagfish and hypothetical maximum size for the species. The application of a biochemical assay for Fluorescent Age Pigments (FAPs) as a possible means of confirming size and age predictions was to be used in conjunction with the

primary growth information. Examination of length frequency distributions for the presence of distinct modal size(age) class groups was to be conducted with subsequent tracking of these for clues to size/age relationships. Length-frequency distributions were to be compared and related to growth rate derived size-at-age predictions

#### IV. APPROACH

A. The basic approach involved the use of field and laboratory studies incorporating application of a specially developed internal tagging system for hagfish and some non-traditional or infrequently used approaches for extrapolating growth curves, predicting size-at-age exclusively on the basis of growth curves and confirming age-size estimates through an infrequently used biochemical assay.

##### 1. Field Studies

Field studies revolved around 12 one-day fishing trips on a chartered commercial fishing vessel to a selected study site off Port San Luis (San Luis Obispo County, CA). Sampling was uniformly carried out by setting 48 standard Korean hagfish traps baited with fish carcasses. Once all traps were set, gear was allowed to fish for two hours before set line retrieval was begun. Fish were removed from traps and placed in a holding tank which functioned as a live well. Individual fish were anesthetized (MS-222) and measured for length on deck and tagged with minute stainless steel, binary coded, wire tags (Northwest Technologies, Inc., Olympia, WA) inserted into the caudal musculature with a modified hypodermic syringe. Binary coding of tags permitted the identification of individual fish released in the field. In all instances fish were checked for presence of previous tags with a metal tag detector (Northwest Technologies Inc., Olympia, WA) customized for work on wet ship deck conditions. Any previously tagged fish were measured and the tag removed for subsequent identification. All newly tagged fish on board the fishing vessel were allowed to recover in another holding tank and subsequently returned to their natural habitat. Such fish were returned to the ocean bottom via a specially constructed canister which opened on contact with the substrate, thereby releasing the fish on the ocean bottom. This was to minimize likely predation loss had they been forced to make their way down through the water column in excess of 180 meters. All measurements of field tagged fish were used for length-

frequency analysis in which distributions were sorted and graphically analyzed by individual sampling dates throughout the year.

Absolute growth rates (mm/da) derived from size changes between time of tagging and time of recapture were then adjusted for actual post-tagging time (days) at large. These data were traditional fisheries procedures (Ricker,1958; Krebs,1978). Conversions from absolute annual growth increments were made using the following relationships:

$$L_r - L_t = INC = [\text{Absolute Increment (mm)}] \quad (1)$$

Where:  $L_t = \text{Length at Tagging}$   
 $L_r = \text{Length at Recapture}$

$$INC/da @ \text{ large} = Inc = [\text{Absolute Growth Rate(mm/day)}] \quad (2)$$

$$IncX365 = I = [\text{Absolute Annual Growth Increment (mm)}] \quad (3)$$

$$\text{Finite Rate} = L_{t+1}/L_t \quad (4)$$

Where:  $L_{t+1} = (L_t) + (I)$

$$\text{Annual Rate} = I/L_t = (L_{t+1}-L_t)/L_t \quad (5)$$

$$\text{Instantaneous Rate} = \text{Log } e(L_{t+1}/L_t) \quad (6)$$

Relationships between growth rates and sizes were initially obtained by using Linear Regression and Correlation statistics with analysis of residuals (Ryan, Joiner and Ryan,1976; Doudy and Wearden,1991) and/or data transformations as appropriate. In all instances where annual and instantaneous rates were used, wide variations in data resulted in modest to small r-square values common in this type of field data as opposed to that from rigidly controlled experiments. With one exception, beta coefficient analysis based on t-tests indicated significant slope values ( $P=0.05$ ) and that observed growth trends were real.

Predicted growth rates were obtained from the derived curves at one-year intervals beginning with a starting fish size of 50 mm total length (TL). The latter was established as a size at hatching or length at time t ( $L_t$ ) for Pacific hagfish based on early embryological work of Dean (1899) which represents the

only known specimens of prehatch hagfish, and Stockard (1906) who re-examined Dean's histological specimens and data. In these very old studies, it was found that hagfish embryos completely encircle the large (25mm+) oblong mature egg along its longitudinal axis, thus providing estimates of hatchling hagfish between 43 and 58mm total length. More recent research on the evolutionary significance of the deciduous hagfish teeth (conodonts) have resulted in some ancillary tooth/fish length relationship information suggesting a 50mm hatch size for Pacific hagfish (Kresja, et al,1990,1989;Kresja,1993 pers. comm.) Thus with a Lt of 50mm total length as a reference and starting point, extrapolations of size at successive yearly intervals were made based on annual and instantaneous growth rate curves previously generated from field data.

All captured fish were measured to provide a basis for population length-frequency analysis and inspection for definitive modal size class groupings. These were examined for possible information on size class growth rates and related to predicted size-at-age. These data also provided stock recruitment information.

## 2. Laboratory Studies

Laboratory studies involved the maintenance of live hagfish in marine laboratory outfitted with a running sea water system. All fish used in these studies were caught in the same manner as described for field tagged animals and were held in a live tank with circulating sea water pending return to port. Upon arrival in the laboratory the fish were placed in one of two large tanks (8'x4'x2') with a constant flow through of water at approximately 5 gpm. After acclimation to the tanks, fish were anesthetized, measured and tagged. Two small groups were maintained under different conditions of light and temperature (ambient sea temperature, natural photoperiod;14 degrees C., light excluded) in an effort to have conditions more similar to the natural environment. Fish were fed carcasses of fresh frozen rock fish (Sebastes spp.) at three to four day intervals rather than on a daily basis as previous studies (Hardisty,1979;Nakamura,1991) had shown hagfish do not eat every day and the daily placement of food ad libitum resulted in uneaten food and potentially unhealthy conditions. The fish were maintained and fed over several months (depending on group) and were ultimately

sacrificed and measured for growth changes. Growth data obtained from laboratory maintained fish were treated in the same manner as described for field caught tagged fish and similar size at age projections were made.

Additional laboratory studies involved the dissection of brain, muscle and heart tissues from fish sacrificed in the field and laboratory for FAP analysis. Size measurements of sacrificed fish were made and tissue samples were immediately frozen with liquid nitrogen for assay or stored in an ultra low temperature freezer (-80 Degrees C. for later assay. The assay protocol followed the comprehensive work of Hill (1991) who refined the procedures working with controlled populations of laboratory reared fish. The protocols adapted to this study are described in appendix 1. FAP levels are quantified on the basis of measurable fluorescence at specific Fluorescence Index (RFI) which also takes into account tissue sample size. These are then correlated to size on the assumption that the metabolically inert FAPs accumulate with age (size) as has been shown for other invertebrate organisms (Crossland, et al.,1989;Ettershank,1983;Hirsche and Anger,1987;Sheehy,1989; et al) and a few fish species (Oguri,1986;Hill.1991;Vernet, Hunter and Vetter,1988; Mullin, 1988).

## B. Project Management

- a. The F/V Gus-D captained by Mr. Frank Donahue (c/o 200 Lamp Lighter Lane, Arroyo Grande, CA 93420) provided a fully equiped fishing vessel for all capture, marking and release experiments in the field.
- b. Two graduate student research assistants (Mr. Benjamin Stephens, Ms Myra Artana (Biological Sciences Department Graduate Program, Cal Poly University), were instrumental in the collection of all specimens and data from the field. The laboratory assignments were partitioned with Mr.Stephens in charge of maintaining the PG&E marine laboratory hagfish populations and basic logistics of field surveys. Ms. Artana was in charge of biochemical analyses on the Cal Poly University campus.

- c. The principal investigator for the project was Royden Nakamura (Biological Sciences Department, Cal Poly University, San Luis Obispo, CA). Primary responsibility involved overall project direction and coordination, supervision of research assistants and research priorities, budget and manpower administration, data reduction and analyses, progress and final data analysis and report composition.
- d. Histological preparations on hagfish epidermal tissues were prepared by San Luis Histoprocessing Company (3388 Broad Street, San Luis Obispo, CA, 93401).
- e. Specialized tagging equipment requirements were met by Northwest Technologies Inc (Shaw Island and Olympia, WA) on an as-needed basis
- g. Overall budgetary administration and oversight requirements were met by the California Polytechnic State University Research Foundation Accounting Office (Cal Poly University, San Luis Obispo, CA 93407) through Ms. Sue Crouch

## V. FINDINGS

### A. Actual Accomplishments and Findings

#### 1. Field Population Tag Returns, Population Estimates and Growth Data

A total of 4275 fish were caught, marked and released during the course of this project. Forty-three tagged fish were recaptured which provided usable growth information. A few tag returns came from fish which had been at large in the same sampling area for long periods of time. This suggested that the population, which consists of a benthic, burrowing form, is less mobile than other schooling or active demersal species and may be restricted to a definitive location. Previous studies by Love, et. al.(1991) on stock identification of the Santa Barbara population using mitochondrial DNA analysis suggests hagfish populations off California exist as discreet sub-populations. Aside from natural mortalities and recruitment, it was assumed that the population was relatively static and might be regarded

a "closed" one in the context of immigration and emigration. The Schnabel mark and recapture procedure (Krebs,1989) was used and resulted in an estimate of 411,177 hagfish in the immediate area of the study with a 95% Confidence Interval of 317,445 to 571,259.

## 2. Growth Data From Field Population

### a. Absolute growth

Out of a total of 43 recaptured fish, 32 tags with usable information were obtained. In addition, data from 7 tagged fish recovered from our earlier project were included, giving a total of 50 recaptured fish for growth analysis. While most size changes were positive, negative (5%) and zero (5%) growth was consistently observed throughout the project. Initial response to these observations was to address possible causes or explanations for these unusual and unforeseen results. These included: (1) errors in decoding of tags (2) inconsistency in measurement protocols (3) stress related effects on tagged hagfish (4) possibility that negative growth was a real phenomenon in hagfish.

Reexamination of decoded tag data revealed that tags were properly identified. Review of measurement methods in the laboratory did not suggest changes in protocols, nonetheless, an alternative measure referred to as "Duct Length" was established as a routine back-up measurement and a means of confirming the occurrence of negative growth. This alternative measurement is the distance from the tip of snout extending to the posterior-most gill area opening on the left side and has been used in other studies on jawless fish such as lampreys (Hardisty,1979). Since this portion of the hagfish anatomy has more semi-rigid endoskeletal components, it is assumed to be less apt to show variations in measurements as the section immediately posterior to gill slits which have no cartilaginous endoskeletal elements save the notochord (chorda).

Serious consideration of the possibility that negative growth was a real phenomenon was based on several rationales: (1) The consistency and isometry of Duct Length to the basic Total Length measurement was statistically tested

(figure 3,4) and shown to be an acceptable measure since it is isometric with total length. Importantly duct length measures also showed consistent negative growth values (2) Negative growth values occurred in both field and laboratory studies (3) Project tasks were allotted so all size measurements were made by the same individual (4) All tags were re-read and checked for decoding errors (5) Changes in growth rates normally occur among fishes and include the common seasonal growth associated with temperature changes or with physiological factors (e.g. ovarian maturation,nutrition,stress) as well as ecologically influenced biphasic growth among certain elasmobranchs and teleost fishes (6) Ontogenetic,ecological and behaviorally associated negative growth has been described (Hardisty,1978;Larsen,1962;Larsen and Dufors,1993) for other jawless fishes evolutionarily and structurally similar to Hagfishes [e.g. European River Lamprey(Lampetra fluviatilis)]. Importantly,such negative growth has been attributable to actual reduction of the notochord (chorda) in addition to the expected muscle tissue resorbtion (Larsen and Dufors,1993; Larsen,1962) (7) Recent reexamination of methodologies of age and growth studies for fish has led to the recognition that fish may undergo long periods of zero and even negative growth which has led to underestimates of age among certain groups of commercially important fishes (McFarlane and Beamish,1987). Indeed, negative growth has been shown to occur in Sablefish (Anoplopoma fimbria), a teleost fish.

Because of the atypical nature of primitive jawless fishes such as hagfish and the fact that negative growth is not the norm among the more typical bony fishes (Osteichthyes) or cartilaginous fishes (Chondrichthyes) separate data conversions and extrapolations were made where one growth series was based only on positive field growth data and a second series was based on all available information (ie.positive, negative and zero growth data). The former accommodates the view that perhaps negative growth are not truly reflective of growth in the strict sense and that negative growth is an artifact of tagging and handling stress. The other model accepts the view that hagfish, like certain other primitive jawless fishes do indeed normally display negative growth manifested in actual constriction of the notochord (chorda) and absorption of caudal

Figure 3

Relationship of Duct Length (Gill-Head Length)  
to Total Length (cm)

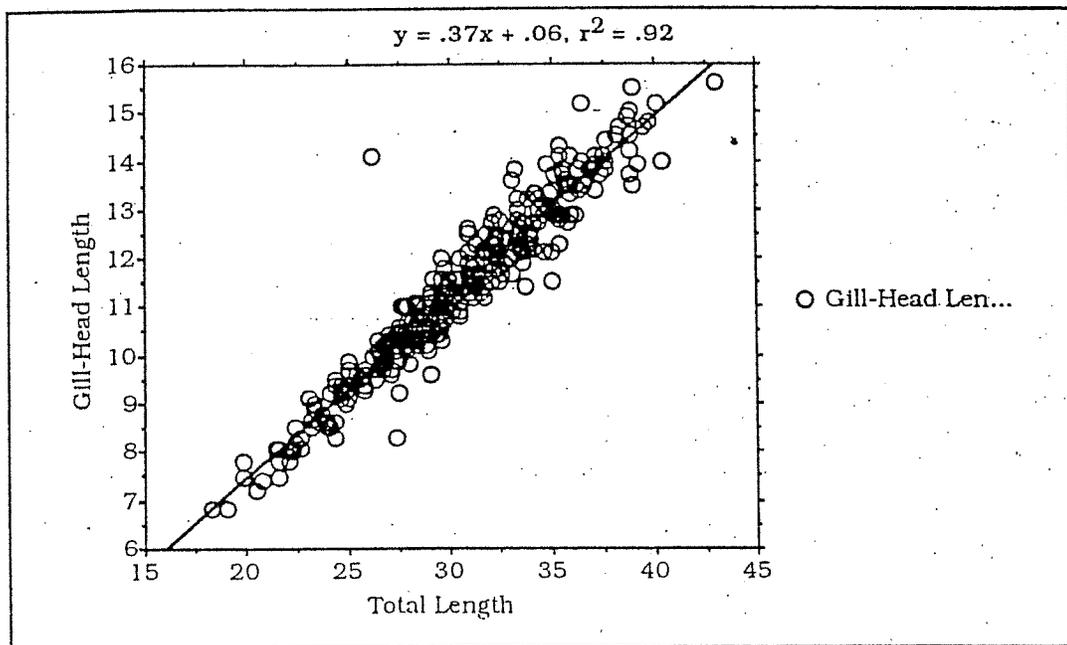
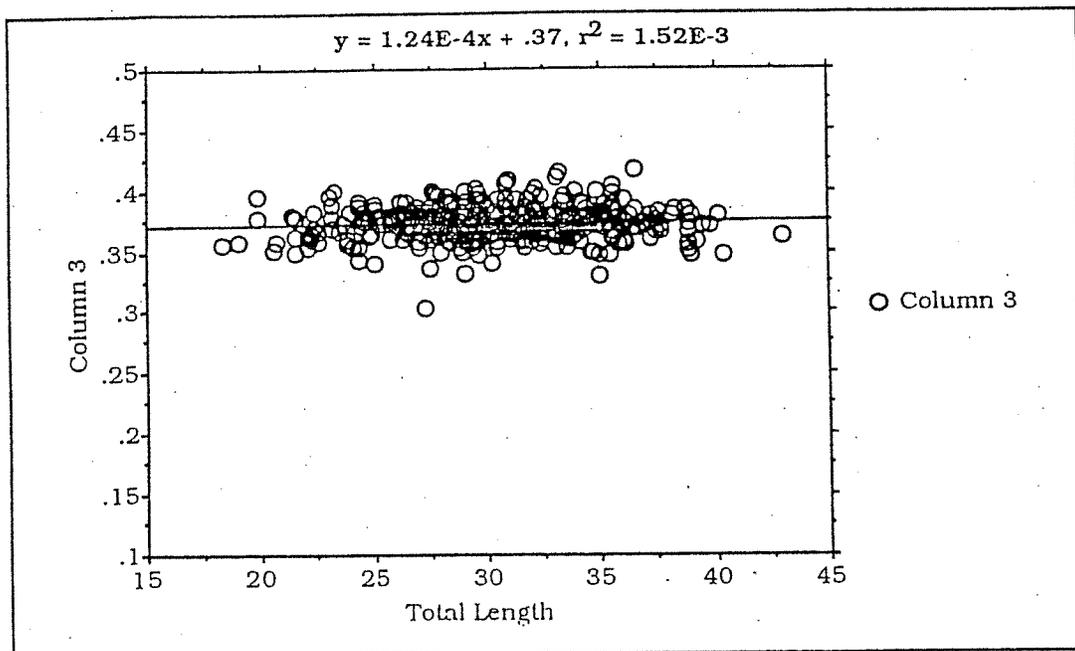


Figure 4

Relationship of Duct Length-Total Length Ratio  
to Total Length (cm)



muscle tissues (Larsen and Dufors,1993;Larsen,1962). A third series of conversions and extrapolations was made from data gathered from the small groups of hagfish in the laboratory.

The absolute growth increments in field data series were adjusted to the period of time the fish remained at large between initial tagging and recapture to provide absolute growth rates in mm/da. A diagram of absolute growth rates [mm/da = Inc of formula (2)] versus total length for positive growth values from the field illustrates the expected trend of high rates for smaller fish (Figure 5). This trend was also obvious in other plots using duct length instead of total length. However, since duct length measurements were used mainly as an alternative measurement and to detect negative growth, total length was the primary basis for all subsequent growth data conversions used to describe hagfish growth patterns. This decision was also made because the duct length measurement was routinely established after the project was well underway and because the greatest volume of information is in the form of total length.

On the average, these directly observed absolute growth rates for E. stoutii (0.33-0.01mm/da) are higher but comparable to other estimates (0.11-0.14,0.94mm/da) for a similar northwest Pacific species (E. Burgeri) which were indirectly obtained and anecdotally described in studies on size class specific reproductive cycles(Tsuneki, O uji and Saito,1983; Patzner,1978). Hagfish maintained under laboratory conditions consistently displayed lower growth rates than tagged fish in the field with values consistently <0.01mm per day (Figure 6). Laboratory fish held at presumably more natural deep water conditions of lower temperature(14 Degrees C.) and greatly reduced light displayed lower growth rates (0.0022-0.0111 mm/da) than fish held at ambient surface seawater temperature (0.0042-0.0622 mm/da). The average absolute growth rates for hagfish in the field were 0.0110 mm/day (positive growth data only) and 0.0650 mm/day (positive, negative and zero growth data) while laboratory averages were 0.0263 and 0.0067mm/day for ambient and low temperature groups respectively.

Figure 5

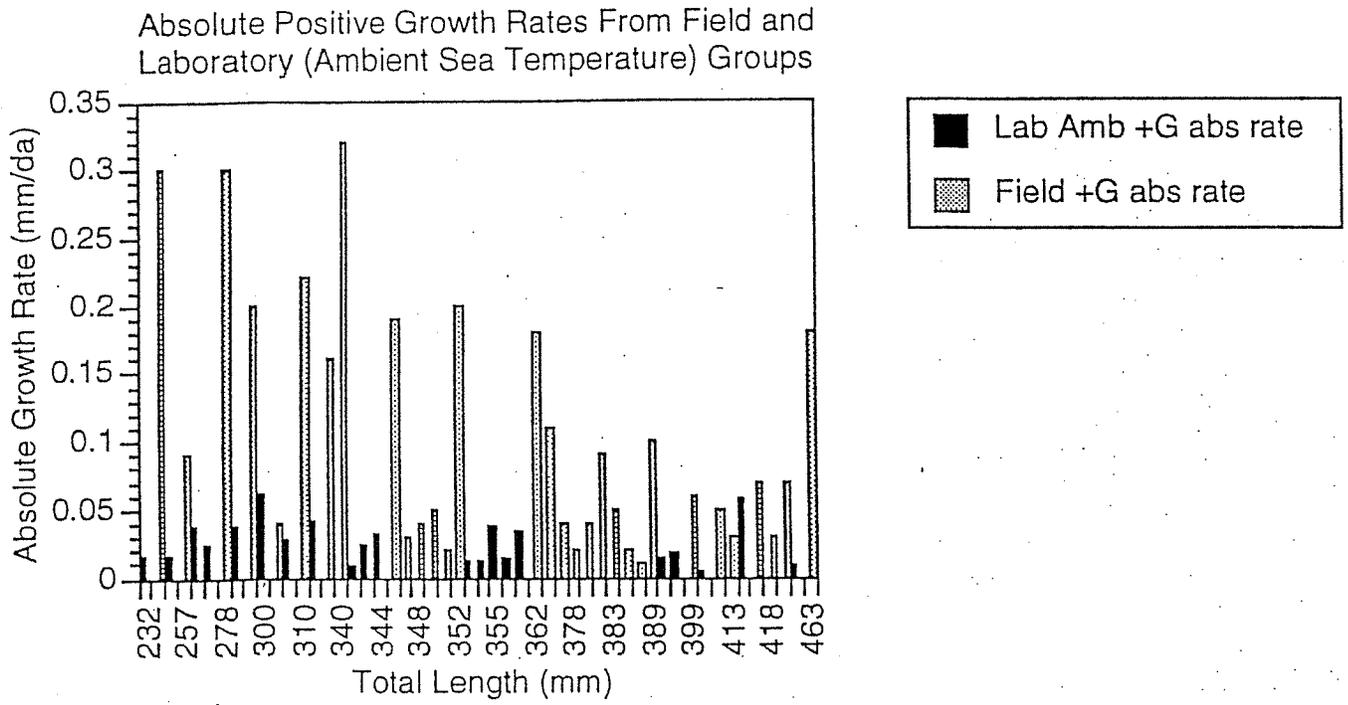
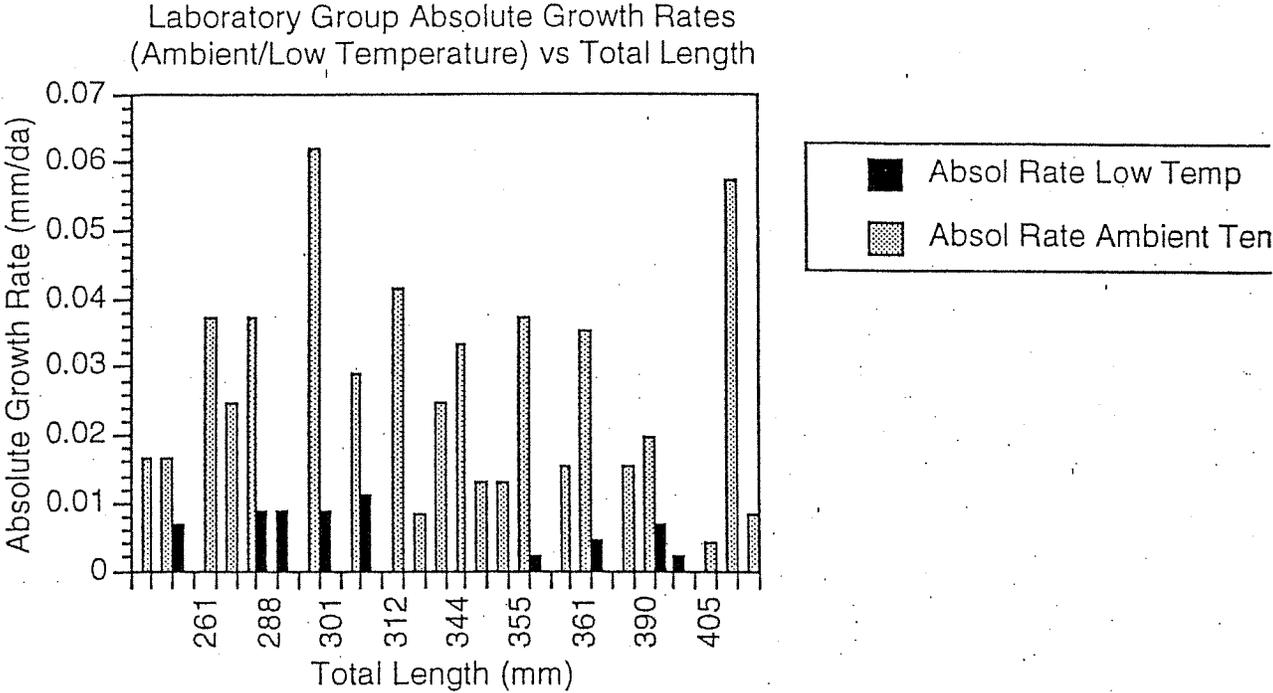


Figure 6



#### b. Annual growth rates

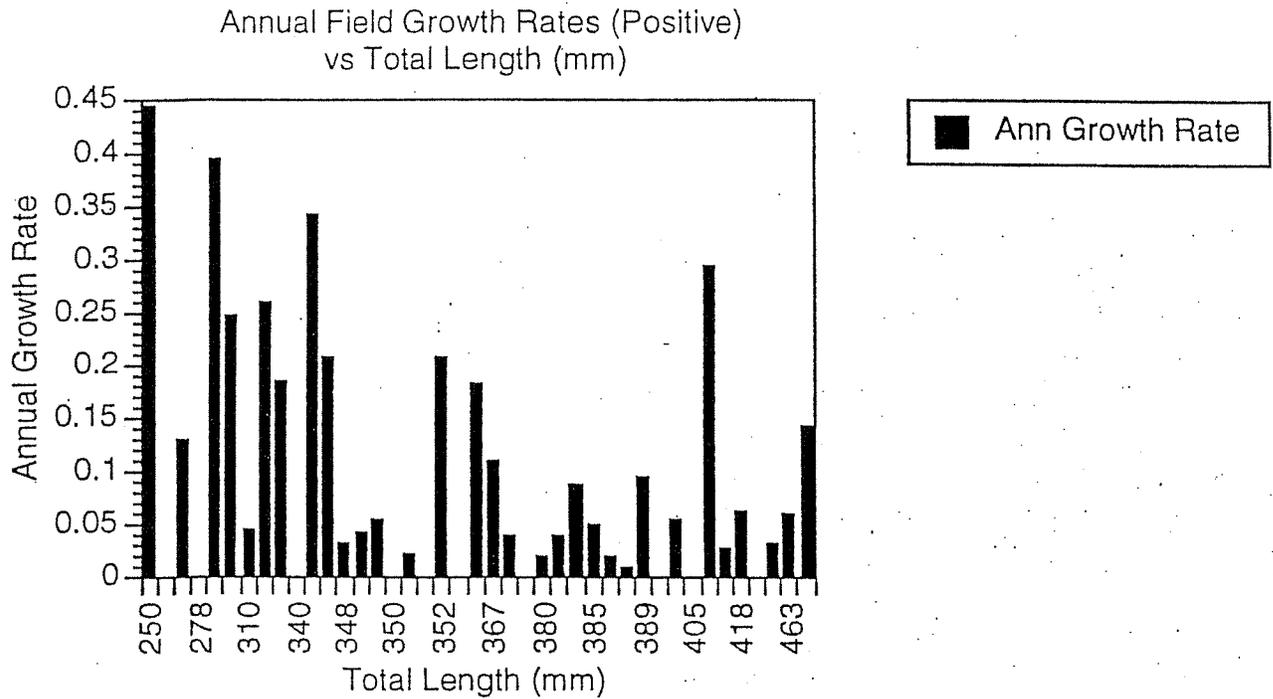
Absolute growth measurements were converted to annual growth rates as indicated in formula (5), nomenclaturally modified from Ricker(1958). Annual positive growth rates in the field ranged from 0.0096 to 0.445 with an average of 0.091 (9.1%). This range is extended to a low of -0.2363 with the inclusion of zero and negative growth data which results in an average annual rate of 0.039 (3.9%). Field data plots of only positive growth rates against total and duct length measurements indicate a marked trend of higher rates for smaller(younger) fish similar to the absolute growth data previously described in Figure 7. Laboratory group annual rates (positive growth only) are lower than those obtained from field data with values of 0.033 (3.3%) at ambient sea surface temperatures and 0.007 (0.7%) at 14 degrees C.

The positive growth observations are consistent with well documented growth patterns of most bony and cartilaginous fish and the conventional wisdom that smaller, younger fish generally grow faster, an important underlying assumption of most fish growth models. In this study the annual rate information was also used to compare differences between curves based on annual versus instantaneous rates and those using two hatching sizes (Figure 11).

#### c. Instantaneous growth

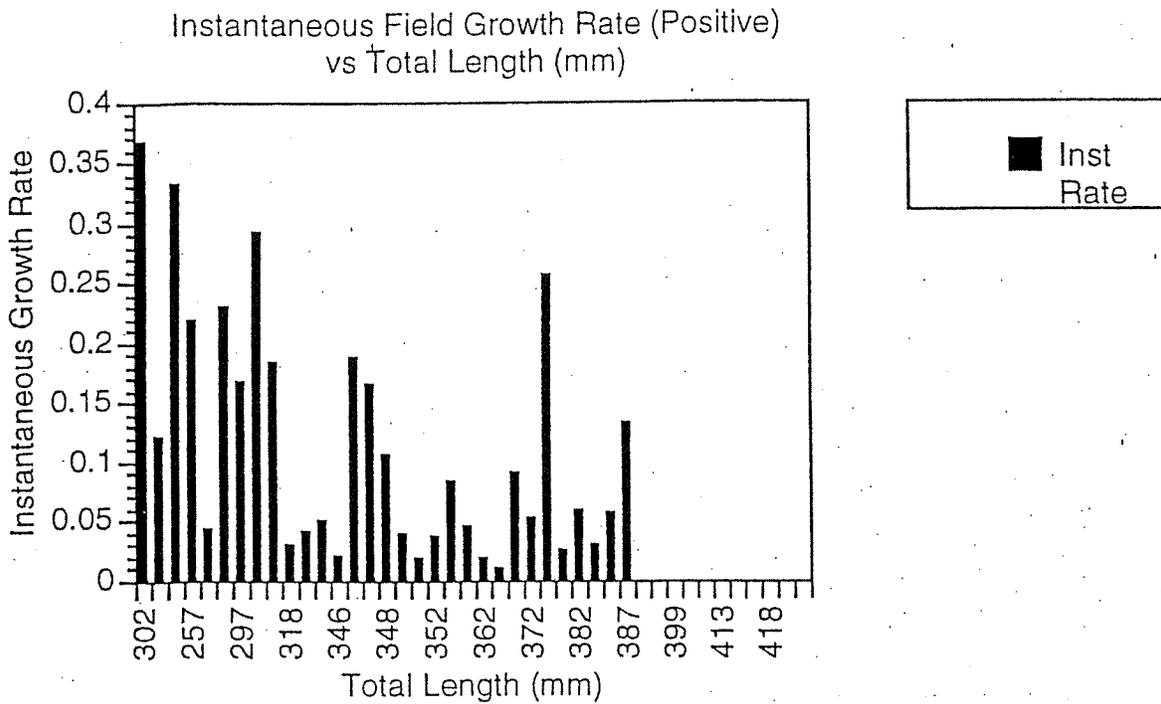
While size-at-age predictions were made using only positive annual growth rates, more diverse and reliable estimates were made using instantaneous rate data given the additive properties of instantaneous rates and capacity to generate appropriate linear relationships. The data clearly show higher rates for smaller individuals in most instances (Figures 8,9,10). Positive instantaneous rates from field data ranged from 0.010 to 0.368 while inclusion of zero and negative growth data extended the low value to -0.2696. Plots of these field data against size resulted in curves with slope values of -0.0010 and -0.0008 respectively. In the case of the positive-rates-only data, Beta coefficient analyses indicate rejection ( $P < 0.05$ ) of the null hypothesis for zero slope which would have indicated no relationship between growth rate and

Figure 7



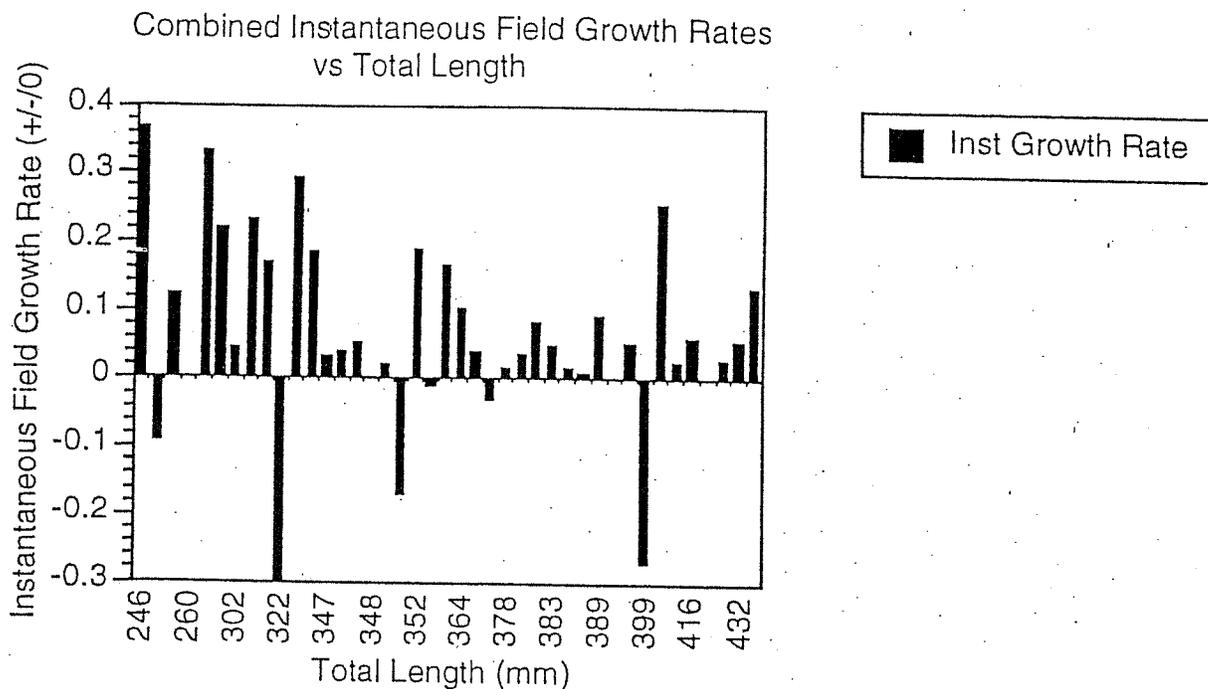
Regression analyses of these data indicated wide variations and relatively low r-square values (0.16+) while tests for a definitive trend (slope) resulted in rejection of the Null Hypothesis where Beta = 0 and acceptance of an Alternative Hypothesis for a slope of -0.0008 (p=0.05)

Figure 8



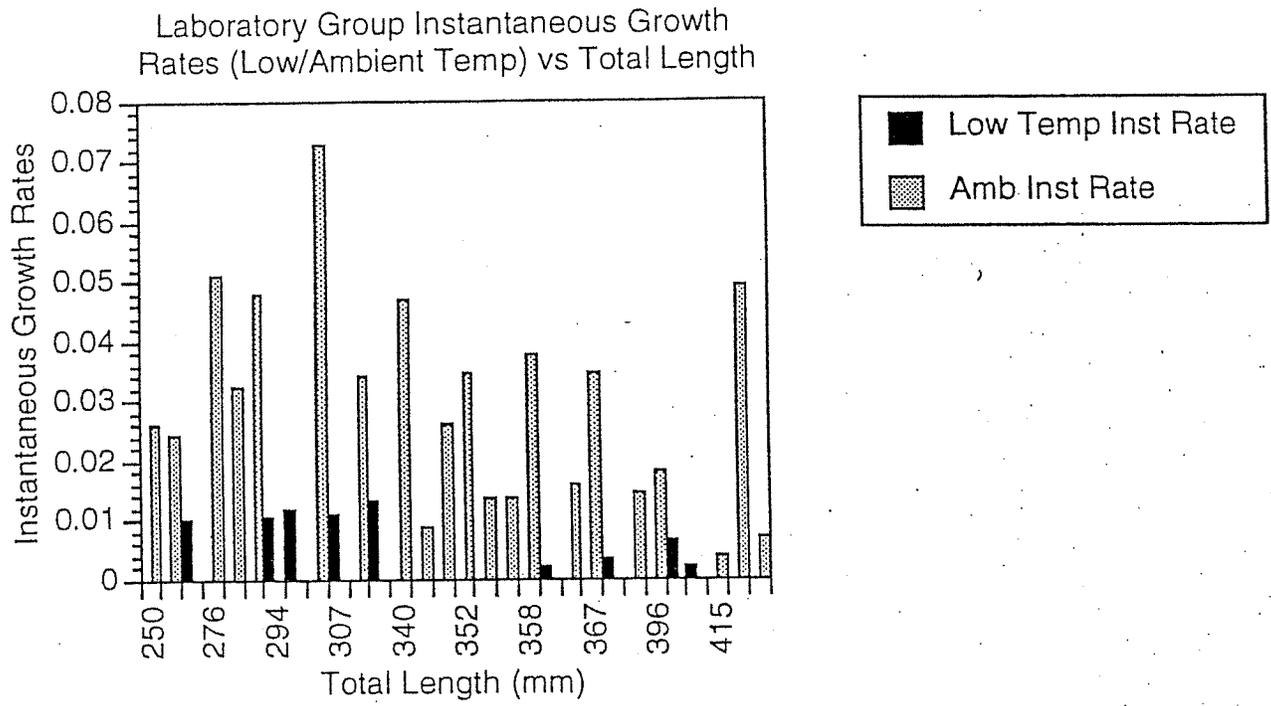
Regression analyses of these data indicate less variation and increased r-square values (0.31+) compared to annual rate data (0.16+). Residuals analysis did not warrant further data transformation while beta coefficient analysis for a definitive trend (slope) resulted in rejection of the Null Hypothesis where Beta = 0 and the acceptance of an Alternative Hypothesis for a slope of -0.001.

Figure 9



Regression analyses of these pooled data show wider variations and relatively low r-square values (0.05+). However, residuals analysis did not indicate additional data transformations while beta coefficient analysis for a definitive trend (slope) resulted in a marginal value for the Null Hypothesis where  $\text{Beta} = 0$  ( $p=0.10$ ) for slope value of -0.0006.

Figure 10



size. The data including zero and negative growth result also showed a definitive slope (-0.0008), however the increased variance resulted in marginal probability value of 0.10 which precludes outright rejection of the null hypothesis for a zero slope. However the relationship was utilized in subsequent extrapolations for comparison and to accommodate the possibility that the zero and negative growth observations are valid. In both instances modest to low r-square values (0.31-0.05) are reflective of wider variations that generally appear in field data as opposed to that obtained in rigidly controlled situations. As expected curves based on only positive rates resulted in steeper growth curves and larger size-at-age predictions than those using positive, negative and zero growth data.

Examination of parallel conversions and projections of hagfish growth data from groups maintained in the laboratory differed markedly from field data extrapolations and predictions, with very low instantaneous rate values ranging from 0.00677 to 0.07294 at ambient lab temperatures and 0.00199 to 0.01071 at 14 degrees C. (Figure 9). The group maintained at ambient surface sea water temperatures displayed positive growth albeit at a much reduced rate than both field data projections. This difference is thought to be due to sensitivity of hagfishes to subliminal disturbance and stress associated with an artificial environment despite the fact that hagfish have been maintained in our laboratory for periods in excess of two years with out apparent signs of detrimental effects. The greatest difference occurred with the group maintained at 14 degrees C., in which growth was radically reduced. This is thought to be due to the compounded effects of low temperature on the existing subliminal stressful conditions of an artificial environment. It is noteworthy that negative growth also occurred in both laboratory groups.

### 3. Age/Length Extrapolation and Prediction

A series of different absolute growth curves were constructed. Two of the growth curves were based on positive annual growth rate extrapolations using two different hypothetical lengths at hatching (25 mm and 50 mm.TL). The former was originally used on an early assumption that

newly hatched hagfish would at least as long as the longest dimension of the large mature hagfish egg which exceeds 25mm. The second starting point was utilized in conjunction with additional information suggesting that newly hatched Pacific hagfish were at least 50 mm TL. This was based on the very early embryological work of Dean (1899) who obtained the only known specimen of a Pacific hagfish embryo and Stockard (1906) who reexamined his material and made inferences relating to hatchling size which incorporated observations of late embryos completely encircling large, oblong egg mass along its longitudinal axis. Also incorporated in the use of the 50mm TL reference point was a more recent extrapolation via regression of deciduous hagfish teeth (conodonts) on body length by Kresja, Bringus and Slavkin (1990) which also approximated a 50mm hatch size.

With regard to differences in when using 50 mm vs. 25 mm as length at time zero ( $L@t=0$ ) asymptotic predicted size at extrapolated age in both cases occurred at about 420+ mm total length and 10 years. Both annual rate based size-at-age curves are similar, showing very rapid growth and asymptotic lengths close to 450mm TL and 9-12 years.(Figure 11).

Extrapolation based on positive instantaneous rates resulted in a curve similar in shape to the annual rate based curves but with an asymptotic length of 470mm TL and about 13 years (Figure 11) The similar high rates were simply due to the fact that only positive values were used. This model accommodates the view that zero and negative growth were artifacts of handling and tag stress in the field. Size-at-age predictions are summarized on Table 1 and Figures 11,12.

A fourth size-at-age series of predictions was constructed utilizing all available data (positive, negative, zero growth).on the assumption that periodic zero and negative growth are normal events. Size predictions are summarized on Table 1 and illustrated on Figure 12. Intuitively, it may be a more realistic model in that it simply did not exclude any data(Figure 12). It also accommodates the view that negative growth as described in certain other primitive jawless fishes (Dufors and Larsen,1993 Larsen,1962) is a real phenomenon partly related to the unique anatomy of hagfishes. It also recognizes the recent view that many marine teleost fishes may undergo long

Figure 11

Predicted Size-at-Age Based on Positive Annual and Instantaneous Field Growth Rates with Starting Lengths of 25, 50 mm at Time  $t = 0$

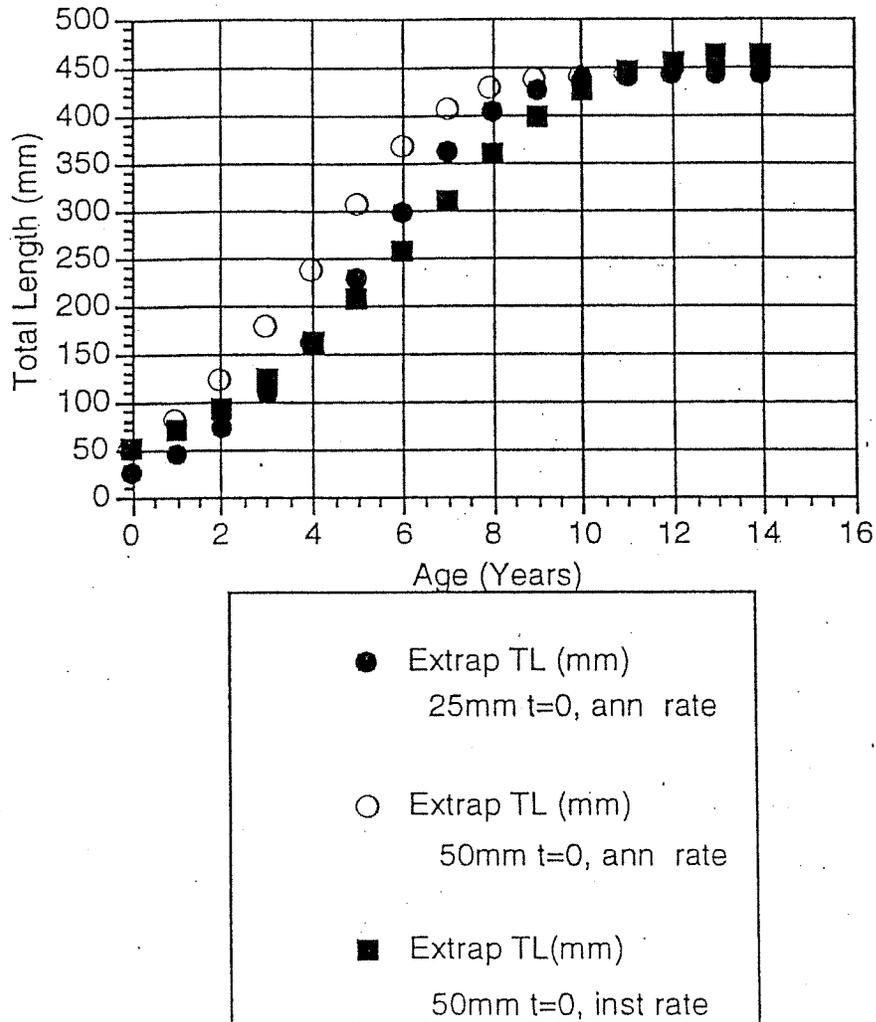
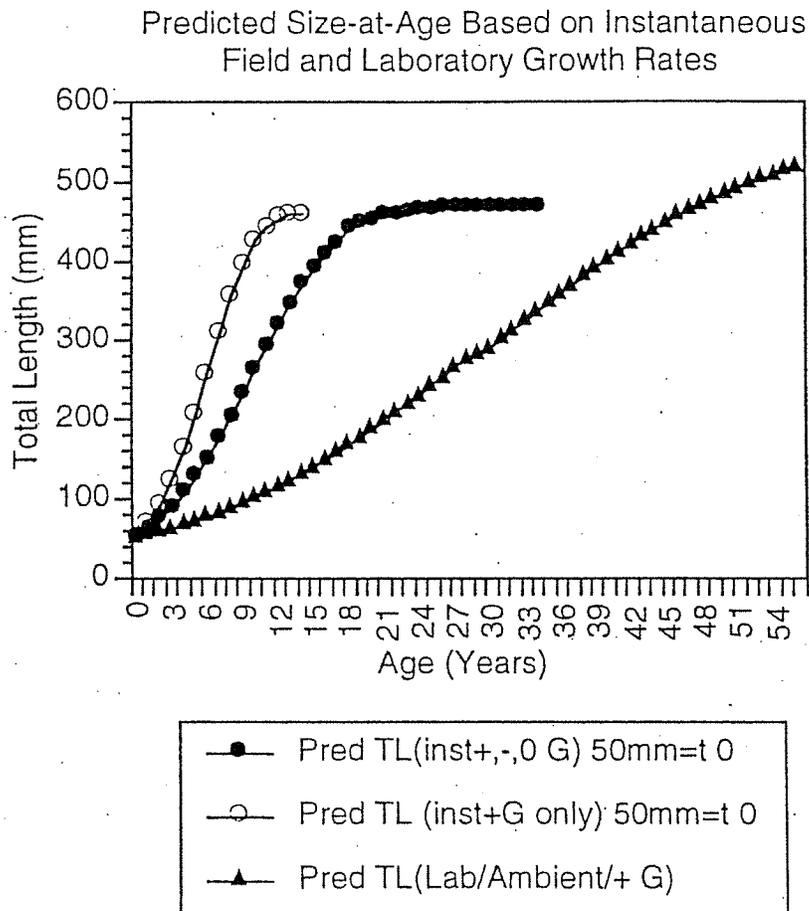


Table 1

Summary of Predicted Size-at-Age Using Annual, Instantaneous Rates  
From Field and Laboratory Groups (mm Total Length)

Extrapolated Age	Pred TL(+G Ann Rate)	Pred TL(+G Inst Rate)	Pred TL(+/-/ 0 G Inst Rate)	Pred TL(Lab+G Inst Rate)	Extrapolated Age > 25	Pred TL(+/-/ 0 G Inst Rate)	Pred TL(Lab+G Inst Rate)
0	50	50	50	50	26	466.3	252.4
1	78.5	68.2	60.8	53.8	27	467	264.1
2	119.8	92	73.6	57.9	28	467.2	275.9
3	175.9	122.5	88.4	62.2	29	467.4	281.5
4	234	160.5	106.3	66.8	30	467.5	387.7
5	304.7	205.6	126.5	71.7	31	467.58	299.6
6	365.1	255.9	149.3	76.9	32	467.64	311.4
7	404.6	307.8	174.6	82.4	33	467.7	323.5
8	426	356	202	88.3	34		335.4
9	438.4	395.6	230.9	94.5	35		346.9
10	438.9	424.2	260.7	101	36		358.2
11	441.2	442.5	290.3	107.9	37		369.3
12		453.1	318.9	115.2	38		380.2
13		458.9	345.4	122.8	39		390.8
14		458.92	369.8	130.8	40		401.1
15			390.3	139.2	41		410.9
16			407.6	147.9	42		420.6
17			421.7	157	43		429.9
18			439.9	166.4	44		438.8
19			447	176.2	45		447.4
20			452.4	186.3	46		456.6
21			456.4	196.7	47		464.4
22			459.4	207.4	48		471.9
23			461.7	218.3	49		479
24			463.3	229.5	50		485.7
25			465.7	240.9	51		492

Figure 12



periods of zero and negative growth (eg. sablefish) which results in underestimates of age (Beamish and McFarland, 1987). Such underestimates are not restricted to procedures based on growth ring analysis of hard tissues, but extends to other where other methods are utilized (eg. Purvis, 1980). In this context, it is not surprising that recent estimates now indicate that 47+% of commercially important, temperate marine species live to be between 25-50 years old. In this study, the all inclusive (+, -, zero) growth model for Pacific hagfish, growth rates were lower compared to the previous extrapolations with an asymptote at about 22 years and 460+ mm TL that extends to 33+ years and 467+ mm TL. The asymptotic size range encompasses much of the larger size categories observed in the project. Since the absolute rates of increase beyond 467 mm were very small, fish that ranged from 480 to 500+ mm TL were in excess of 50 years of age..

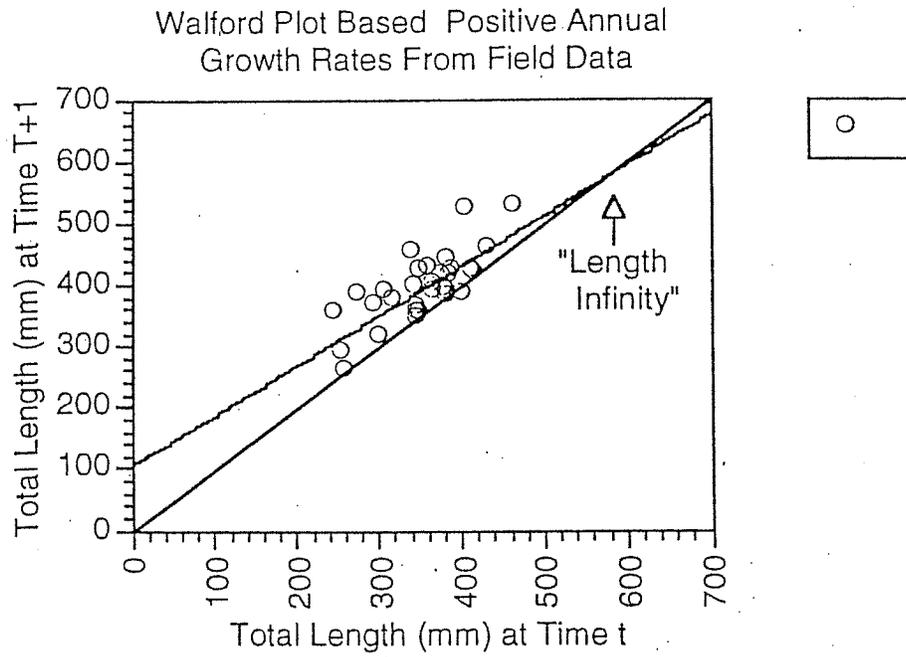
A fifth curve based on the laboratory maintained hagfish is also shown (Figure 12). In this extrapolation, a 470+ mm hagfish is almost 50 years old. While slow growth and great longevity appears to be more the norm in many temperate marine species (Beamish and McFarlane, 1987), in this instance the greatly reduced growth of hagfish in the laboratory is believed to be more of a reflection of effects of living in an artificial environment and being subjected to periodic unnatural disturbance and subliminal stress of captivity. This view is consistent with field observations and explains the great discrepancy between fish living in the natural environment and those maintained in the laboratory.

#### 4. Walford Plots

A series of Walford plots were constructed using size-at-age predictions based on instantaneous field growth rate extrapolations (Figure 13, 14, 15). The first of these utilizes predictions based only on positive annual field growth rates. This plot of predicted lengths at time  $t+1$  against predicted length at time  $t$  indicates an "L-infinity value" or hypothetical maximum size for the species of 580 mm TL. The instantaneous positive growth rates predict an L-infinity of 544 mm TL. The Walford Plot using values from size-at-age predictions based on positive, negative and zero instantaneous field growth rates indicate an "L infinity" approximating 600 mm TL (Figure

15). The largest hagfish captured in this study was 531mm TL and most closely approximates the Walford Plot based on positive instantaneous predictions. The slopes of the plots are reflective of the instantaneous growth functions in the widely used von Bertalanffy growth curves (Ricker, 1958).

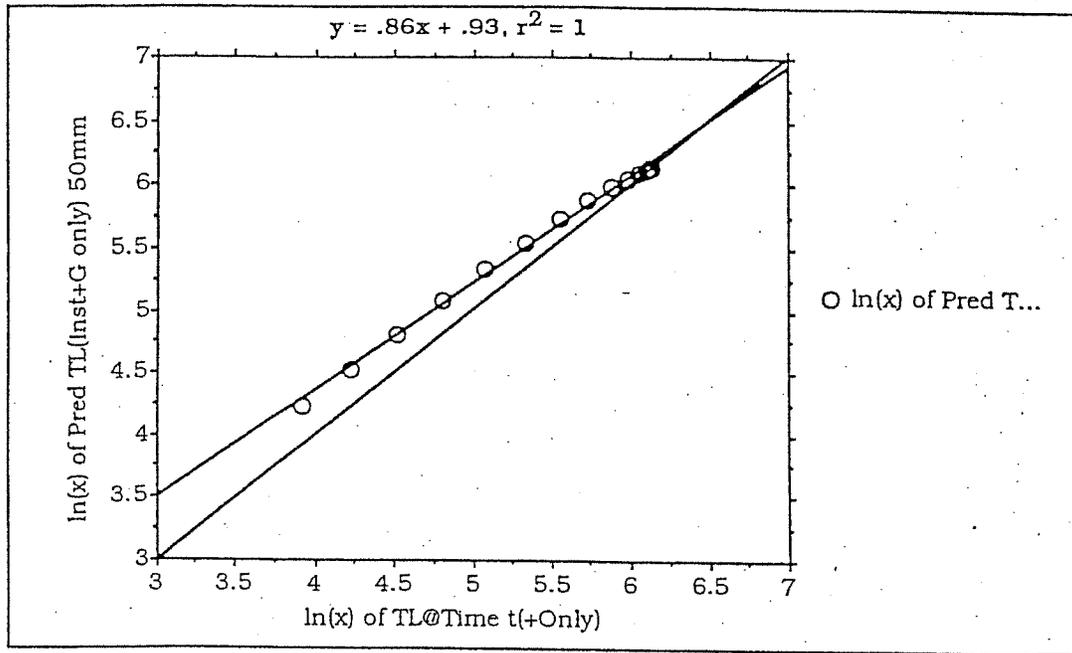
Figure 13



Linear Regression ( $r\text{-square}=0.57+$ ) used for this Walford Plot resulted in a theoretical maximum size ("Length Infinity") of 535 mm Total Length and a slope value (K) of 0.8152 which is also an approximation of the Instantaneous Growth Rate.

Figure 14

Log Transformed Walford Plot Based on Positive Growth Rates Only



Simple Regression X<sub>1</sub> : ln(x) of TL@Time t(+Only) Y<sub>1</sub> : ln(x) of Pred TL(inst...

Beta Coefficient Table

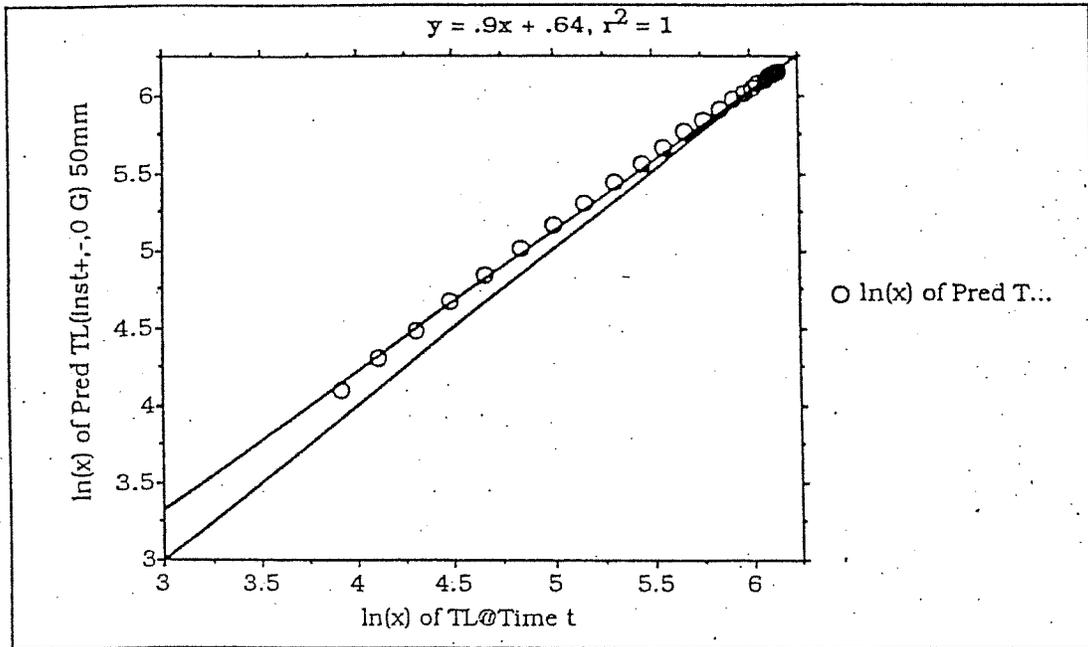
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	.93				
SLOPE	.86	.01	1	57.73	.0001

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	5.52	5.57	5.52	5.56
SLOPE	.82	.89	.83	.88

Figure 15

Log Transformed Walford Plot Based on Positive, Negative and Zero Growth Rates



Simple Regression X<sub>1</sub> : ln(x) of TL@Time t Y<sub>1</sub> : ln(x) of Pred TL(Inst+,-,0 G...

Beta Coefficient Table

Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	.64				
SLOPE	.9	.01	1	166.74	.0001

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	5.71	5.72	5.71	5.72
SLOPE	.89	.91	.89	.91

## 5. Population Size Structure

Examination of the size-class structure of the population reveals the presence of discreet isolated modal size groups. However the separation of the groups is not always apparent in all distributions encompassing the twelve sampling periods. Seasonal distributions are shown on figures 16,17,18,19. Distributions for the entire project are displayed in Appendix ii. Modal groups, when apparent, are most obvious in the smaller size categories ranging from 180 mm to about 330 mm total length and from about 400 to 500 mm total length. It is apparent that there are difficult to separate mixed sub-distributions in the middle of the overall distribution. The length-frequency distribution patterns (Figures 16-19) indicate stock recruitment occurs at about 180-200 mm total length. The occurrence of these size categories in most of the months throughout the year suggests recruitment is not confined to any single season. The implication of this is that Pacific hagfish may actually be multiple spawners and/or reproduce as a population throughout the year with a protracted spawning season. These findings would be consistent with work on reproductive biology in our earlier project and to some degree other studies on hagfish reproductive biology.

During most seasons, small and large individuals are represented in the length frequency distributions. However, it was noted in this and our previous project that occasionally the distribution may be skewed with the absence of certain size cohorts. The winter distribution (Figure 17) shows a conspicuous absence of fish above 430mm TL. This deviation from other length-frequency distributions was shown to be statistically significant in a study ancillary to this project and hypothesized to be due to prespawning shallow water migration of large males (Stephens, 1993).

An example of readily observable modal groups showing clear separation are found on Figures 20 and 21. Tentative age class assignments were made based on two instantaneous rate based size-at-age predictions (positive growth only and positive, negative and zero growth models). Of these, the size-at-age predictions based on positive growth only, appear to best approximate apparent modal size classes.

Figure 16

Sample of Length Frequency Distribution  
of Pacific Hagfish Population in the Fall

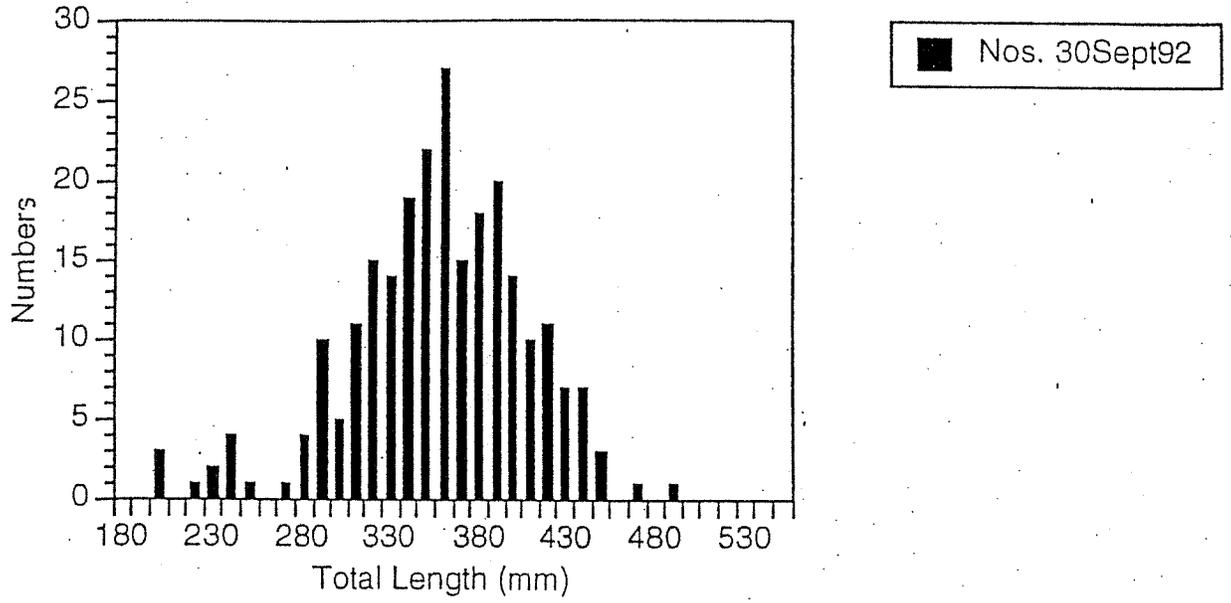


Figure 17

Sample of Length Frequency Distribution  
of the Pacific Hagfish Population in Winter

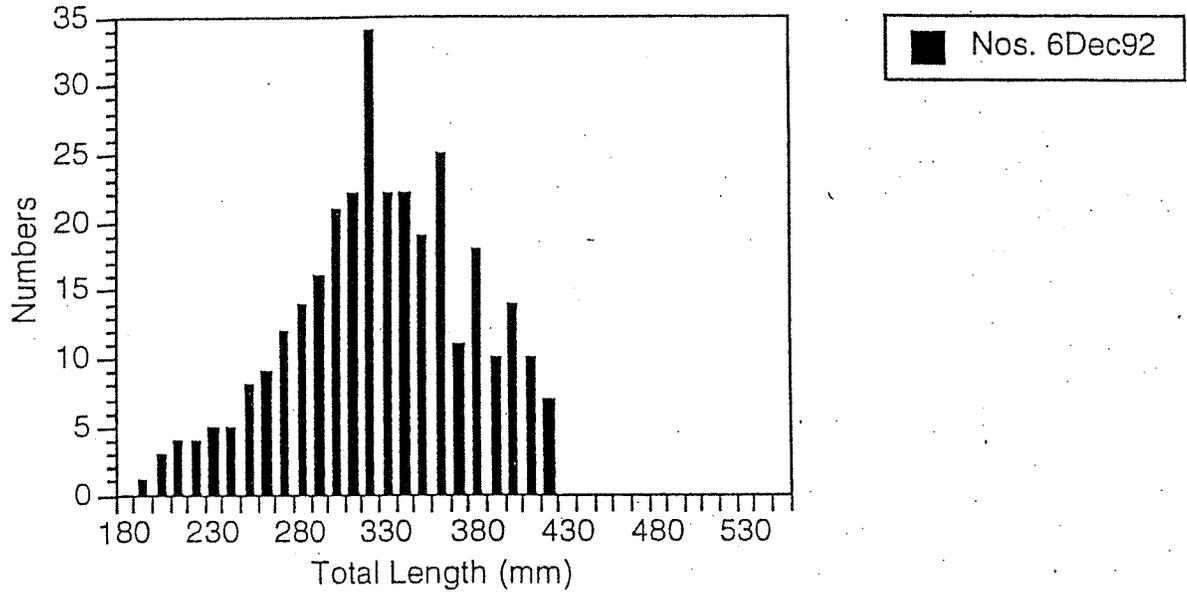


Figure 18

Sample of Length Frequency Distribution  
of the Pacific Hagfish Population in Spring

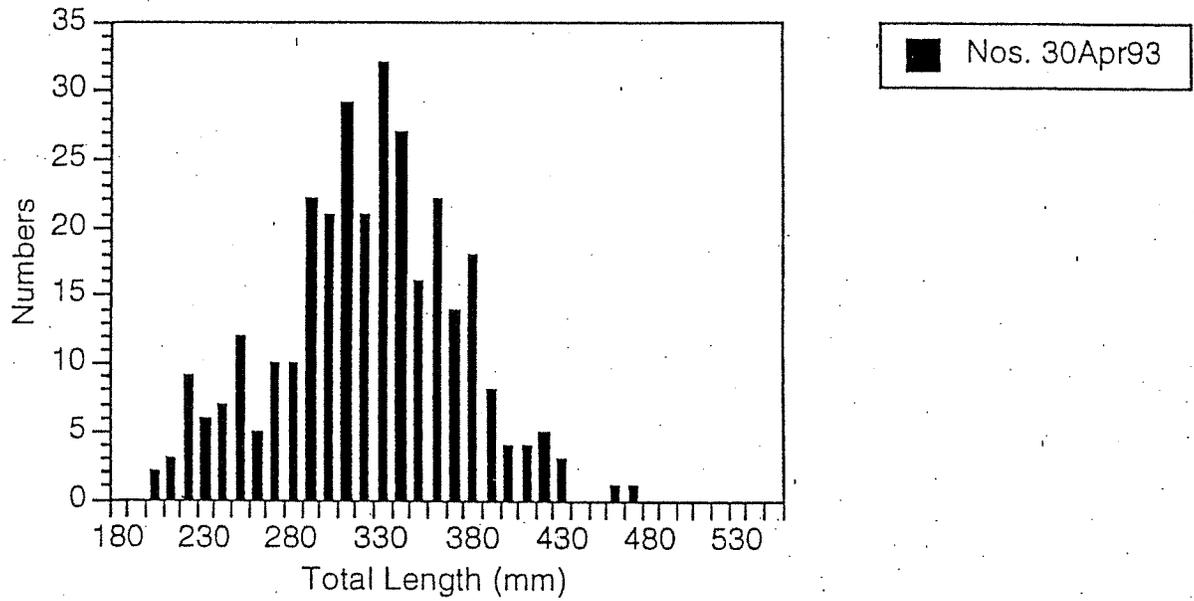


Figure 19

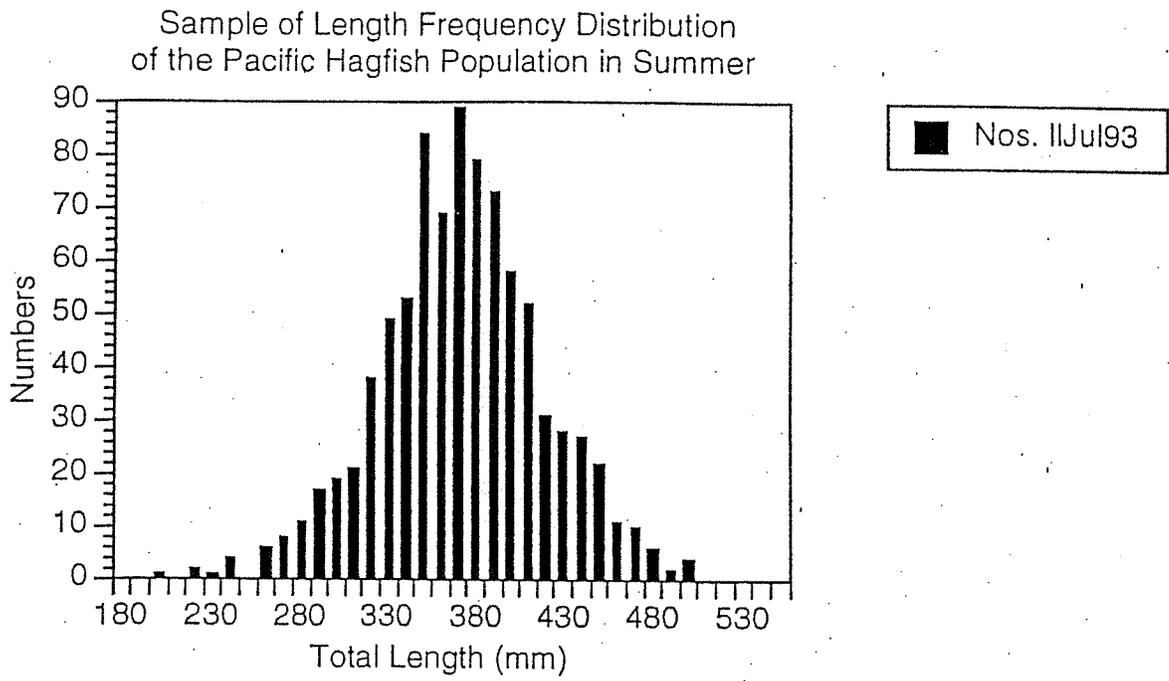


Figure 20

Fall Length Frequency Distribution of Pacific Hagfish  
and Estimated Age of Possible Modal Size Classes  
Based on Positive Instantaneous Growth Curve Predictions

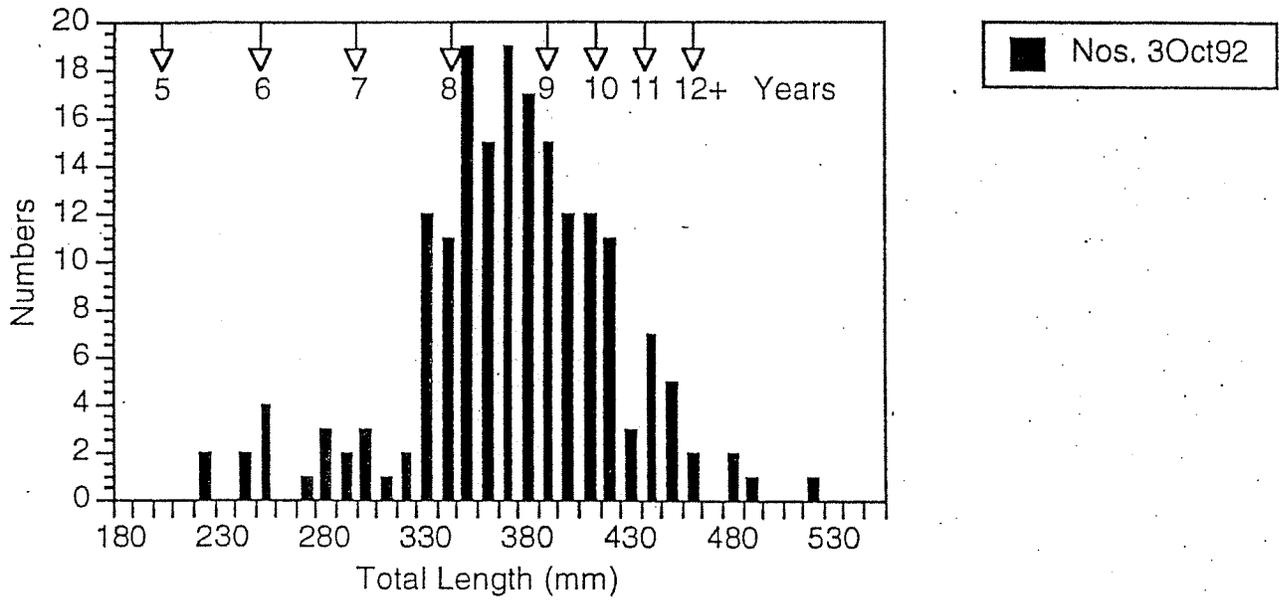
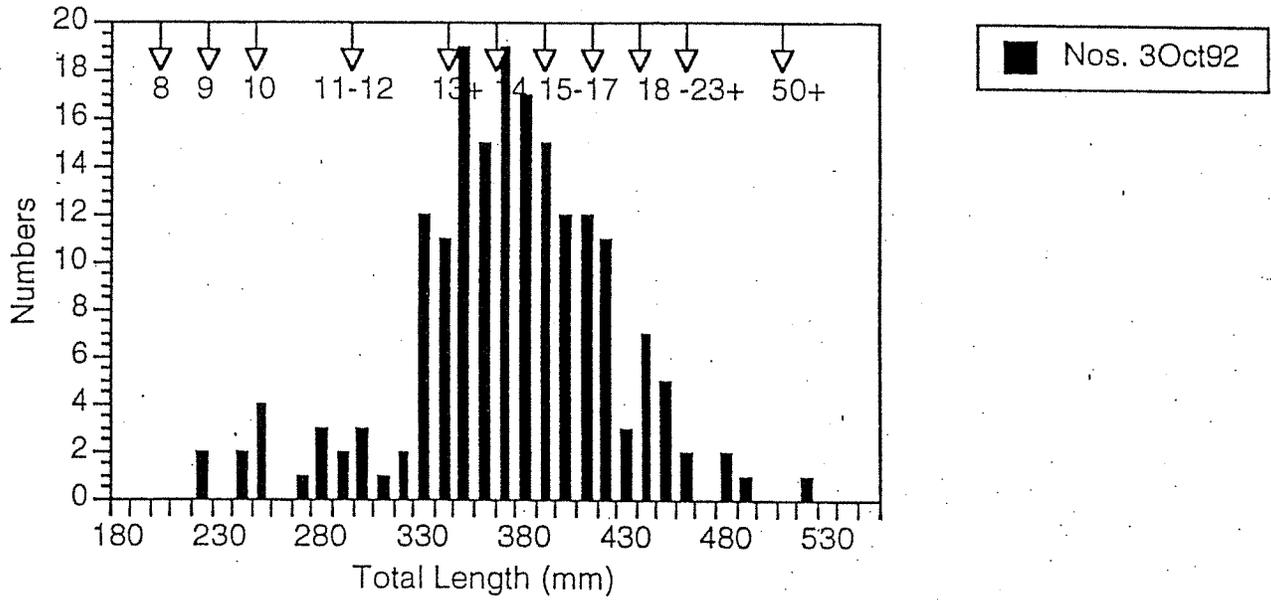


Figure 21

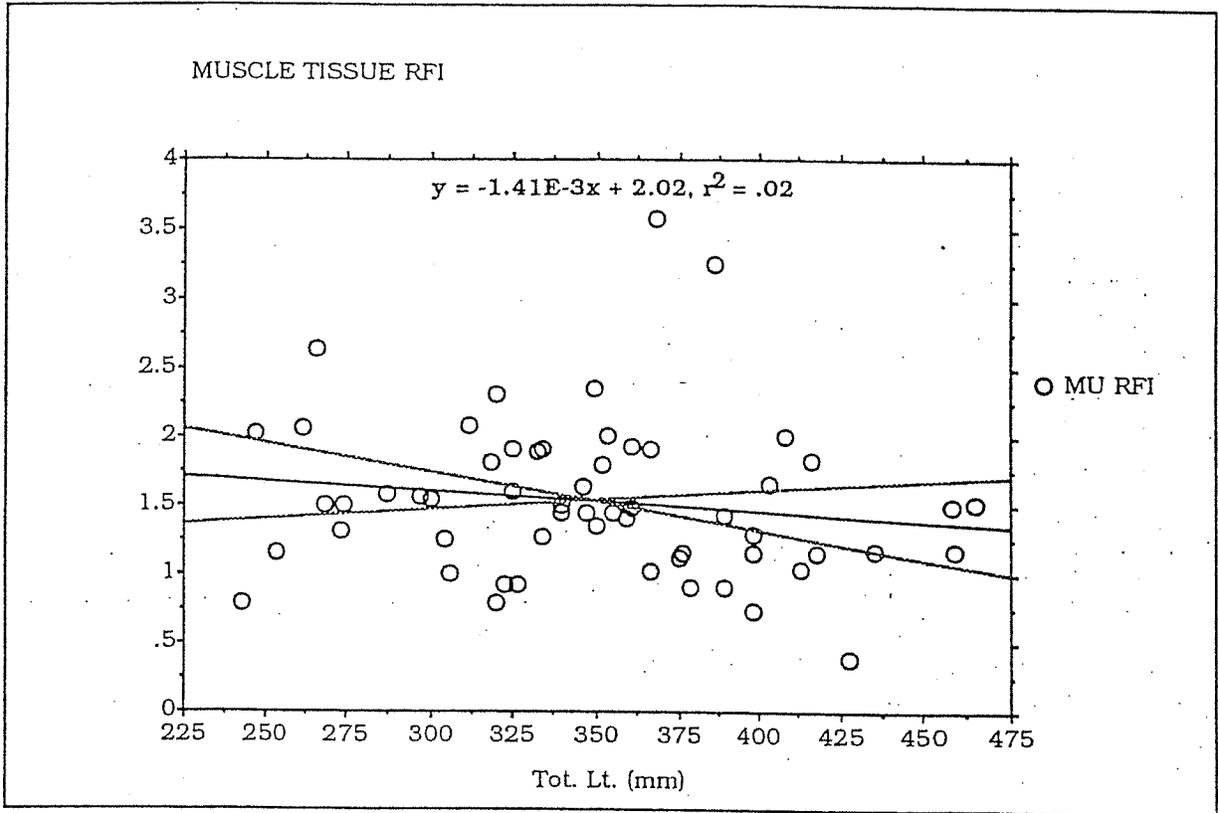
Fall Length Frequency Distribution of Pacific Hagfish and Estimated Age of Possible Modal Size Classes Based on Instantaneous Growth Curve Predictions Utilizing Positive, Negative and Zero Growth Data



## 6. FAP Analysis

Biochemical assays for FAPs varied greatly in all instances. The greatest variations occurred within samples of muscle, heart tissue extracts shown with bracketing 95% confidence intervals on Figures 22,23,24. For all practical purposes, FAP analyses of these tissues do not appear to be of immediate value in confirming hagfish growth and age relationships as pooled data for each tissue type do not show clear relationships with size. However, brain and heart tissue extracts, when separated by sexes show a definitive trend although this is confounded by the finding that trends applying to one sex do not apply to the other. In this particular instance, brain tissue FAPs show relationship to size in males but not in females while heart tissue FAPs are related to size in females but not in males (Figures 25,26). Given these initial results, it is clear that further clarification on FAP relationships by tissue type, sex and size are required before the procedure can be utilized.

Figure 22



Simple Regression X<sub>1</sub> : Tot. Lt. (mm) Y<sub>1</sub> : MU RFI

Beta Coefficient Table

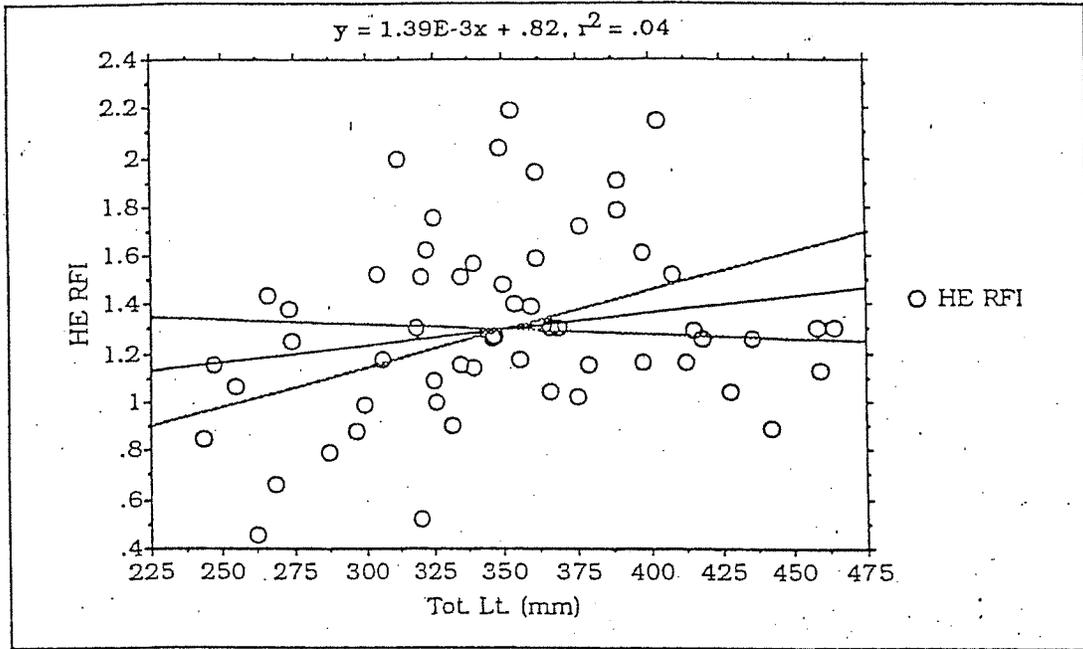
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	2.02				
SLOPE	-1.41E-3	1.38E-3	-.13	1.02	.314

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	1.38	1.68	1.41	1.66
SLOPE	-4.18E-3	1.37E-3	-3.72E-3	9.09E-4

Figure 23

Heart Tissue RFI vs Total Length



Simple Regression  $X_1$  : Tot. Lt. (mm)  $Y_1$  : HE RFI

Beta Coefficient Table

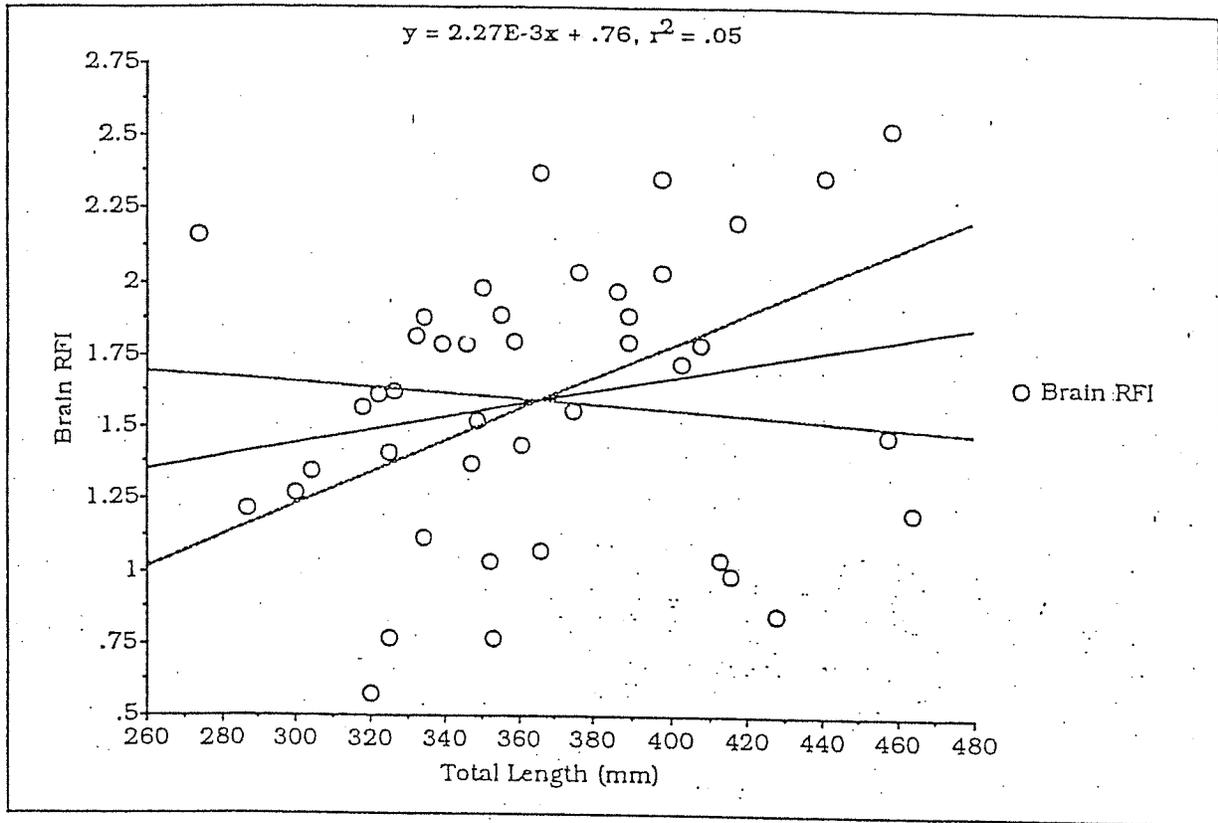
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	.82				
SLOPE	1.39E-3	9.02E-4	.2	1.54	.1296

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	1.2	1.4	1.22	1.38
SLOPE	-4.20E-4	3.20E-3	-1.21E-4	2.90E-3

Figure 24

Brain Tissue RFI vs Total Length



Simple Regression X<sub>1</sub> : Total Length (mm) Y<sub>1</sub> : Brain RFI

Beta Coefficient Table

Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	.76				
SLOPE	2.27E-3	1.59E-3	.22	1.43	.1598

Confidence Intervals Table

Variable:	95% Lower:	95% Upper:	90% Lower:	90% Upper:
MEAN (X,Y)	1.44	1.74	1.47	1.72
SLOPE	-9.35E-4	.01	-3.99E-4	4.94E-3

Figure 25

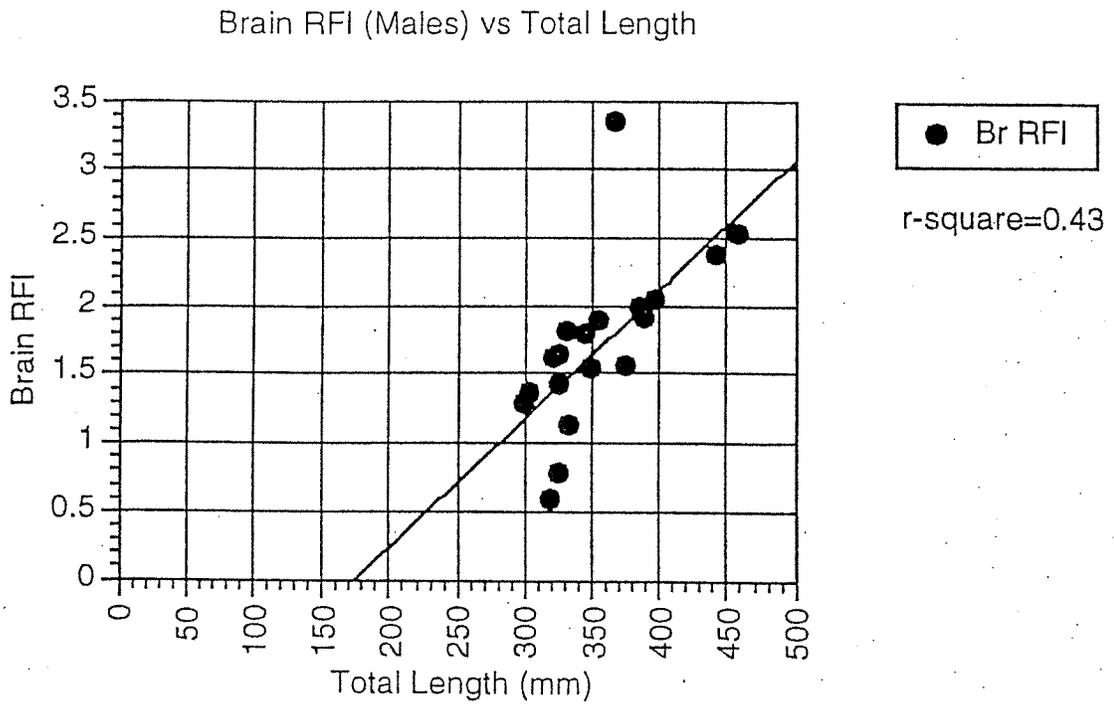
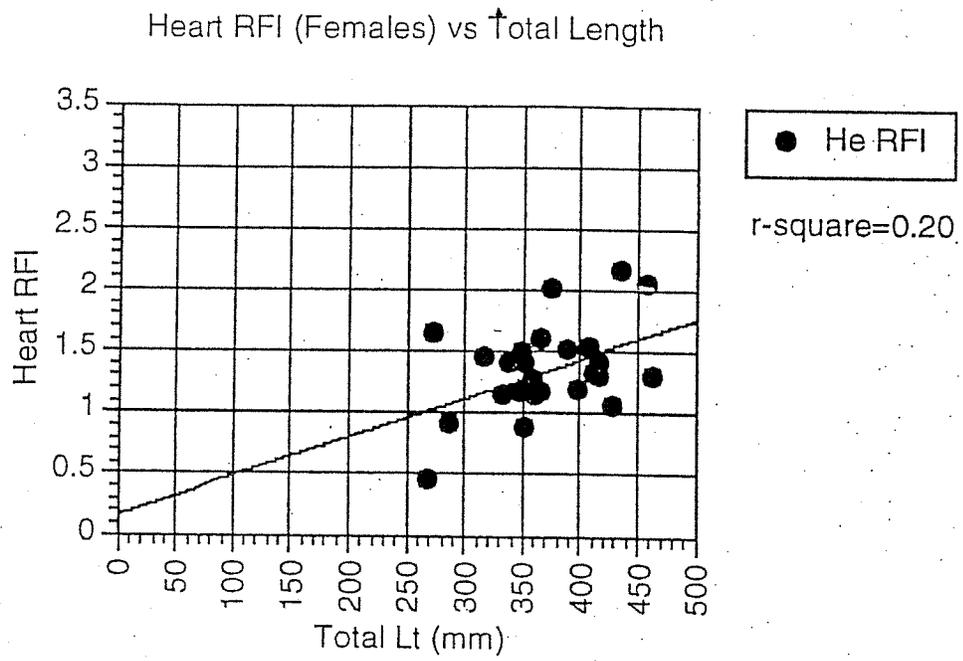


Figure 26



## B. Problems Encountered Resulting in Less Than Satisfactory Results

### 1. The Species:

- a. The unique anatomical characteristics of hagfish resulted in the enigmatic problem of consistent observations of negative or zero growth in field and laboratory observations. This might be perceived as diluting the robustness of the data collected because it was unusual and did not fit traditional or conventional fish growth patterns. This also necessitated the generation of multiple growth curve extrapolations.
- b. The absence of very small specimens throughout the entire project represented a major problem which remained unresolved despite occasional efforts to resolve the problem with mesh outer coverings on a few traps.
- c. Specific information on post-hatch and juvenile Pacific hagfish were not available to fill data gaps.

### 2. Field and Laboratory Work

- a. Occasional logistical problems with scheduling of the chartered fishing vessel due to factors such as weather or boat maintenance and repair..
- b. Academic work loads and schedule conflicts of both principal investigator and graduate research assistants often resulted in fractionated research time.
- c. Periodic laboratory sea water system breakdowns and apparatus malfunctions may have had an affect on the laboratory growth experiments.

## VI Evaluation

### A. Original Project Goals

1. Original project objectives were to:
  - a. obtain growth data direct measurements of hagfish in the field and in laboratory experiments
  - b. construct overall growth curves based on annual and instantaneous rates derived from absolute growth data
  - c. predict size-at-age indirectly, using extrapolated growth curves
  - d. describe general growth pattern(s) of hagfish
  - e. conduct biochemical assays for FAPs and identify trends which might serve as a means of confirming growth and age relationships in hagfish
  - f. obtain a large volume of size measurements throughout the calendar year and analyze composite length-frequency distributions for identifiable modal size groups.
  - g. assign tentative ages(s) to modal size groups when possible
2. Quantifiable or measurable goals were achieved in this project although the traditional ideal of confirmation of size-at-age relationships by three methods was not possible in this particular case, due to paucity of pre-existing relevant information and the unique attributes of hagfishes. Quantifiable goals attained in the project included the following:
  - a. absolute growth increments, absolute growth rates and absolute annual growth increment

- b. annual and instantaneous growth rates
  - c. extrapolated overall annual and instantaneous growth curves
  - d. annual and instantaneous rate based predictions
  - e. predicted size-at-age for hagfish
  - f. hypothetical general growth curve for hagfish
  - g. FAP levels for different tissue types and between sexes for an array of size ranges
  - h. length-frequency distribution patterns throughout the calendar year
  - i. identification of discreet modal size groups at different times of the year
  - j. tentative assignment of age to definable modal size groups in the population
3. Some modification in project protocols and outcomes did occur due to unforeseen aspects of weather, scheduling logistics as well as idiosyncrasies of incoming data during the course of the study. The most important of these was:
- a. establishment of an alternative size measurement to verify and confirm occurrence and consistency of unusual negative and zero growth observations
  - b. generation additional growth-age scenarios to accommodate unusual growth patterns and accommodate item B-1a mentioned above.
  - c. Statistical data transformations or data treatment as appropriate

4. The original goals were attained as originally planned with appropriate reservations for certain aspects described previously (eg. FAP analyses).

## B. Specific Accomplishments

### 1. Product or Service:

- a. absolute growth data for adult hagfish
- b. determined annual and instantaneous growth rates under different data handling protocols
- c. generated predictive growth curve based on item B-1b above
- d. described several possible growth patterns based on items B-1b and B-1c above
- e. predicted a range of size-at-age values based on B-1b, B-1c, B-1d above
- f. determined presence of discreet modal size groups in population length-frequency analyses
- g. tentative age assignments to separable modal size classes in population length-frequency analyses based on B-1e above
- h. ancillary studies on symbiotic organisms on mucus and epidermal tissues was conducted and histological and photographic evidence of a possible new species of Turbellarian (Platyhelminthes) has been obtained.
- i. ancillary studies on relationship of size class distribution variations to reproductive behavior

### 2. Interrelationship of Product or Service.

- a. items a-g above are integrally related as the growth models. Ultimate size-at-age predictions

and tentative age assignments to certain modal size groups were derived from sequential quantitative conversion of the original absolute growth observations

3. Relationship of Product and Services to Original Goals:

- a. items B1a-h immediately above are consistent with original goals of project

4. Value of Product or Service

- a. there is no direct calculatable economic value to the product at this point as the fishery is in hiatus and interest has moved to a different region focusing on a different group of hagfish
- b. there is applied value in the product in the context of future development of the fishery as previously unavailable information on aspects of hagfish biology relevant to fisheries conservation and management has been obtained
- c. There is scientific value in the product as previously unknown information on aspects of Pacific hagfish have been obtained and may be applicable to other species of hagfishes

C. Benefits to the Fishing Industry

1. Industry Access to Product or Services

- a. All components of the commercial fishing community including the appropriate scientific agencies and individuals will have access to the product through this report and subsequent publications and related on-going and future research.

2. Industry Use of the Product

- a. Use of the product by industry will not be immediate and depends of further development of commercial hagfish harvesting on either or both coasts of the U.S.

3. Future Use of the Product by Industry

- a. Future use of the product (scientific information) by the commercial fishing industry would occur provided there is a continued demand for hagfish skins by the "eel-skin" industry. The commercial hagfish fishing industry and appropriate regulatory agencies would need the product to insure long term availability of the resource

4. Use of Product (Scientific Information) by Others

- a. The product obtained through this research will be utilized by the scientific community as it provides previously undescribed information on Pacific hagfish. The information may serve as the bases for working hypotheses for future research as well as provide insights relevant to other hagfish species.

D. Benefits Received By the Fishing Industry

1. Economic Benefits

- a. No direct or calculable economic benefits can be demonstrated at this point. The commercial fishery on the Pacific Coast of the U.S. is in hiatus as commercial fishers shifted to other species for a number apparent, but unconfirmed reasons including disagreements with foreign buyers on prices, skin quality and more lucrative markets.
- b. Presently, attention has shifted to the Northeastern U.S. as representatives of the "eel-skin"

industry as well as fisheries agencies and commercial fishing interests are investigating the feasibility of the harvesting the Atlantic species of hagfish (Myxine glutinosa). However no direct economic benefits relating to the information in this project are demonstrable for other hagfish species at this time.

## VII Conclusions

### A. Conclusions From The Work:

#### 1. Growth of Pacific Hagfish:

- a. smaller (younger) hagfish grow faster than larger hagfish
- b. hagfish show wide variations in growth rates at all size categories directly observed in the project
- c. hagfish consistently show negative and zero growth in the field which may be attributable to any or a combination of the following:
  1. hagfish undergo periodic reduction in growth to the extent of displaying negative growth
  2. hagfish can reduce linear size by resorption of notochordal tissue and muscle tissue
  3. hagfish are easily stressed by handling and tagging procedures which manifests itself in reduced or negative growth
- d. hagfish growth rates are reduced when maintained in captivity
- e. hagfish growth is reduced at lower temperatures
- f. growth curve extrapolations based on only positive growth result in faster calculated growth rates than those utilizing positive, negative and zero growth observations.

## 2. Age of Pacific Hagfish

- a. On the basis of positive growth data only, hagfish at the lower size range (180-200mm TL) sampled in this study are estimated to be 4-5 years old while those at the higher ranges (450-500+ mmTL) are estimated to be 14-15+ years old. Fish (females) attaining reproductive viability (325mm TL) are estimated to be 7-8 years old.
- b. On the basis of combined positive, negative and zero growth data, hagfish at the lower end of the size range sampled in this project (180-200mm TL) are estimated to be about 7-8 years old, while those on the higher end (450-500+mm TL) are 19-20+ years old. Fish at sexual maturity (females) are estimated to be 12-13 years old.

## 3. Population Size Class and Age Structure

- a. definitive modal size (age) groups can be detected in length-frequency analyses made throughout the year.
- b. definitive modal size (age) groups are most evident among the lower (180-200mm TL) and higher (425mm+ TL) size classes.
- c. clear separation of distinct modal size (age) groups in the mid-size classes is very difficult and cannot be made with any useful degree of certainty
- d. growth rate derived age predictions applied to length-frequency distributions display closer agreement using positive instantaneous growth data
- e. recruitment to the hagfish stock appears to be spread over a period of the year as the smaller

distinct modal size (age) classes appear through most months surveyed in this project.

4. Fluorescent Age Pigments

- a. FAP concentrations vary widely between tissue types (muscle, Heart, Brain)
- b. Brain and Heart tissue Relative Fluorescence Indices of FAP shows the least variability and demonstrable trends with size when separated by sex.
- c. A relationship between brain tissue FAPs and size is found for males but not for females.
- d. A relationship between heart tissue FAPs and size is found for females but not for males.

5. Other Ancillary Projects and Findings

- a. Pacific hagfish harbor an abundance of a symbiotic flatworm (Platyhelminthes). Direct microscope observation, photographs and histological slides suggest an ectoparasitic relationship.
- b. Seasonal vertical movements of hagfishes has been preliminarily described (Stephens, 1993) and hypothesized to be related to pre-spawning events

B. Project Solution of Original Problem

1. Determination of Growth Rates and Description of Growth Patterns

- a. Growth rates were determined along separate scenarios based to different data treatment assumptions.

- b. Several general growth curves and patterns were generated based on extrapolations and prediction of B1-a above.

## 2. Prediction of Size-at-Age

- a. predictions and tentative assignments of size-at-age were made based on separate scenarios mentioned above (item B1-a)
- b. size-at-age predictions were assessed to separable modal size-class cohorts detected in analyses of population length-frequency distributions

## 3. Detection of Distinct Modal Size Class Cohorts in the Natural Population

- a. distinct modal size classes were detected in the lower and higher size ranges of the sampled population
- b. tentative age assignments were made when possible

## 4. Fluorescent Age Pigment Analysis

- a. FAP analysis was carried out for the three tissue types as originally described.
- b. FAP analysis was ultimately deemed of only limited utility for purposes of this investigation

## C. Further Work

### A. Future Work to Be Conducted

- 1. Further analysis of population length-frequency distributions using more sophisticated mathematical approaches such as Maximum Likelihood Analyses for Mixed Distributions

2. Continued updating and refinement of data and analyses from this project as new information becomes available
3. Analyses of data from these project in relation to other fishery questions not addressed by the current study (eg. trends in Catch Unit Effort)

#### B. Future Work That Should Be Conducted

1. Examination of possible use of proported statoliths of the hagfish inner ear
2. Microhabitat location, capture and study of post-hatch and juvenile hagfish
3. Elucidation of location, behavior and mechanisms associated with hagfish reproduction
4. Physiological studies of hagfish as it relates to metabolism and growth in a partially anoxic microhabitat
5. Parallel studies on North Atlantic species of hagfish currently being considered a potential new commercial species

## VII Literature Cited

- Adam and Strahan. 1963. Notes on the habitat, aquarium maintenance and experimental use of hagfishes. In: The biology of the Myxine (A. Brodal and R. Fange, eds.) Oslo Universitetsforlaget.
- Agha, M. and M.T. Ibrahim. 1984. Maximum likelihood estimations of mixtures of distributions. Appl. Stat., 33:327-332.
- Beamish R. and G. McFarland 1987. Current trends in age determination methodology. In: Age and growth of fish. (R. Summerfelt and G. Hall, eds.) pp15-42. Iowa State Univ Press, Ames, Iowa. 544pp.
- Bond, C. 1979. Biology of Fishes. W.B. Saunders Co., Philadelphia. 514pp.
- Brooks, A. and M. Love. 1990. Marine Fisheries Impacts Workshop. Office of Environ. Protection, Secretary for Environ. Affairs, State of California. San Pedro, Dec, 1990, Santa Barbara, May, 1991
- Calliet., G.C. 1991. The biology and fisheries potential of hagfish. Calif. Sea Grant Final Rept., R/F-129. Moss Landing Marine Laboratories, Moss Landing, CA.
- Cassie, R.M. 1954. Some uses of probability paper in the analysis of size frequency distributions. Aust. J. Mar. Freshw. Res., 5:513-520
- Clark, W.G. 1981. Least squares estimates of age from length composition. Can. J. Fish. and Aquatic Sci., 38:297-307.
- Crossland, C.J., G. Denby, B.F. Phillips and R. Brown. 1988. The use of fluorescent pigments (lipofuscins) for ageing western rock lobster (Panulirus cygnus) and scampi (Metanephrops andamanicus): a preliminary assessment. CSIRO Mar. Lab. Rept., 195:1-21.

- Dean, B. 1899. On the embryology of Bdellostoma stoutii. A general accounting of development from egg and segmentation to hatching. Festschr. Carl von Kupfor. Jena, 221-276.
- Dowdy, A. and S Wearden. 1991. Statistics for research. 2nd ed. John Wiley and Sons. New York. 629pp.
- Ettershank, G. 1983. Methodology for age determination of Antarctic krill using the age pigment lipofuscins. Biomass Handbook 26.
- Fernholm, D. 1974. Diurnal variation in the behavior of hagfish Eptatretus burgeri. Mar. Biol., 27:352-356.
- Gorbman, 1990. Sex differentiation in the hagfish Eptatretus stoutii. Gen. Comp. Endocrin., 77:309-323.
- Gorbman, A., H. Kobayashi, Y. Honma and M. Matsuyama. 1990. The hagfish fishery of Japan. Fisheries., 15(4):12-18.
- Hardisty, M.W. 1979. Biology of cyclostomes. Cambridge University Press. Cambridge, U.K. 428pp.
- Hardisty, M.W. and I.C. Potter. 1982. Biology of lampreys. Vol. 4B Academic Press., New York. 475pp.
- Hill, K.T. 1992. Fluorescent age pigment accumulation as a determinant of chronological age in aquatic organisms. Ph D. dissertation, University of Hawaii.
- Hill, K.T. and C. Womersley. 1991. Critical aspects of fluorescent age-pigment methodologies: modification for accurate analysis and age assessments in aquatic organisms. Mar. Biol., 109:1-11.
- Hirsche, H. and K. Anger. 1987. The accumulation of age pigments during larval development of the spider crab Hyas araneus (Decapoda: Majiidae). Comp. Biochem. Physiol., 88B:777-782.
- Johnson, E. 1992. Aspects of the biology of the Pacific (Eptatretus stoutii) and black (E. deani) hagfishes from Monterey Bay, California. M.S. Thesis, Moss Landing Marine Laboratories. CA.

- Kato, S. 1990. Report on the biology of Pacific hagfish, Eptatretus stoutii and the development of its fishery in California. Tiburon, CA. NMFS, Tech. Rep.
- Krebs, C. 1978. Ecology. Harper and Row. New York, Philadelphia
- Krebs, C. 1989. Ecological Methodology. Harper and Row. New York, Philadelphia. 654 pp.
- Kresja, R.J., P. Bringus Jr. and H.C. Slavkin. 1990. The cyclostome model: an interpretation of conodont element structure and function based on cyclostome tooth morphology, function and life history. Courier Forsch.-Inst. Senckenberg, 118:473-492.
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- \_\_\_\_\_. 1989. Hagfish tooth development and replacement. Amer. Zoo., 29.
- Larsen, L. O. 1993. Growth, reproduction and death in lampreys and eels. In: *Fishes ecophysiology*. (Rankin, J and F. Jensen, eds.) Fish Fisheries Ser., 9. Chapman Hall, London, New York, Tokyo. 21pp.
- Larsen, L.O. 1962. Weight and length of the river lamprey. *Nature.*, Lond. 194:1043
- McDonald, P.D.M. 1987. Analysis of Length frequency distributions. In: *Age and growth of fish*. (R. Summerfelt and G. Hall, eds.) Iowa State Univ. Press., Ames, Iowa. 544pp.
- Melvin, E. and S. Osborn. 1992. Development of the west coast fishery for Pacific hagfish. Final Report, Saltonstall-Kennedy Grant NA90AA-SK142. Natl. Mar. Fish Serv., NOAA
- Mullin, M.M. and E. Brooks. 1988. Extractable lipofuscins in larval marine fish. *Fish. Bull.*, U.S. 86:407-415.
- Nakamura, R. 1991. A survey of the Pacific hagfish resource off the central California coast. Final Rept., Sec. Env. Affairs, Office of Environ. Protection, Marine Fisheries Impact programs, A-800-84. 63pp.

- Oguri, M. 1986. On the yellowish-brown pigment granules in interrenal cells of anglerfish and leopard sharks. *Bull. Jap. Soc. Sci. Fish.*, 52(5):801-804.
- Patzner, R.A. 1978. Cyclical changes in the ovary of the hagfish Eptatretus burgeri. *Cyclostomata. Acta Zool.*, 59:57-61.
- Purvis, H.A. 1980. Effects of temperature on metamorphosis and the age and length of metamorphosis in sea lamprey (Petromyzon marinus) in Great Lakes. *Can. J. Fish. Aquat. Sci.*, 37:1827-1834.
- Rankin, J.C. and F.B. Jensen (eds). 1993. Fish ecophysiology. Fish Fisheries Series 9. Chapman Hall. London, New York. 421pp.
- Ricker, W.E. 1958. Handbook of computations for biological statistics of fish populations. *Fish. Res. Bd. Can.*, Bull. No. 119.
- Ryan, T., Joiner, B. and S. Ryan. 1976. Minitab student handbook. Duxbury Press, N. Scituate, Massachusetts. 341pp.
- Sheehy, M.R.J. 1989. Crustacean brain lipofuscin: an examination of the morphological pigment in freshwater crayfish, Cherax cuspidatus. *J. Crust. Biol.*, 9(3):387-391.
- Stephens, B. 1993. The effect of sexual development on the distribution of the Pacific hagfish, Eptatretus stoutii. *Western Soc. of Natural.*, Ann Mtg. Newport, Oregon, Feb. 1993.
- Stockard, C.R. 1906. The development of the mouth and gills of Bdellostoma stouti. *Amer. J. Anatomy.* 5:481-517.
- Summerfelt, R.C. and G.E. Hall (eds) 1987. Age and growth of fish. Iowa State University Press, Ames, Iowa 544pp.
- Tsuneki, K., M. O uji and H. Saito. 1983. Seasonal migration and gonadal changes in the hagfish Eptatretus burgeri. *Jap. J. Ichthyol.*, 29(4):429-440.
- Vernet, M., Hunter, J. and Vetter, R.D. 1988. Accumulation of age pigments (lipofuscins) in two cold-water fishes. *Fish. Bull., U.S.*, 86:401-407.

Weatherley, A.H. 1972. Growth and ecology of fish populations.  
Academic Press., New York. 293pp.

Wisner, R.L. and C.B. McMillan. 1990. Three new species of  
hagfishes, genus Eptratretus (Cyclostomata, Myxinidae), from  
the Pacific coast of North America, with new data on *E. deani*  
and *E. stoutii*. Fish. Bull., 88:787-804.

Worthington, J. 1905. Contribution to our knowledge of  
Myxinoids. Amer. Naturalist., 39(465):625-663.

## VIII Appendices

Appendix i

## Appendix i

### ASSAY OF HAGFISH FAP PROCEDURES

#### I. STORAGE OF TISSUES

1. Live hagfish (*Eptatretus stoutii*) stored temporarily in salt water holding tank @ 14°C.
2. To prevent changes in fluorescence during tissue storage, the tissues (brain, heart, and muscle) were immersed in liquid nitrogen and stored in epindorf vials in a deep freezer @ -70°C until need be.

#### II. PREPARATION OF TISSUES FOR EXTRACTION

1. To obtain the most accurate sample weight, tissues were lyophilized on a Labconco Freeze Dryer 5 @ -60°C with a vacuum of 5-7 microns Hg for a minimum time of 16 hrs.  
*\*Note: Duration of lyophilization was tested in order to develop a standard run time. It was found that duration beyond 16 hrs. had no significant effect on the accuracy of the sample weight.*
2. Dry tissues were then weighed on a Mettler H80 electronic microbalance to the nearest tenth of a milligram and placed in 15 ml glass centrifuge tubes.
3. Tissues were macerated @ room temperature for 4 min. in an all-glass homogenizer using at least 1 ml of CHCl<sub>3</sub> per 10 mg dry tissue. The initial meniscus should be marked because approximately one-third of the solvent typically evaporates during homogenization. The homogenate should be restored back to its original volume.
4. An equal volume of a solution containing 100 mM MgCl in GDW and methanol at a ration of 3:1 was added to the homogenized tissues and vortex-mixed for 2 min.
5. Samples were centrifuged in an IEC International Refrigerated Centrifuge Model B-20 @ 0-5°C for 20 min. @ RCF = 3000 x g. The lower CHCl<sub>3</sub> layer was immediately removed and assayed for FAP content.

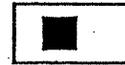
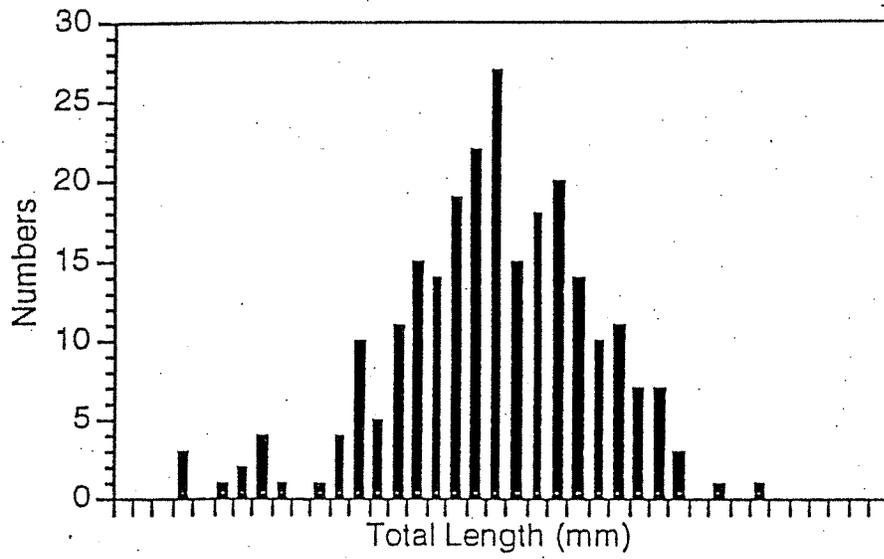
### III. ASSAY OF FAP CONTENT

1. Chloroform extracts were transferred into a ice bath to maintain a constant temperature before they were assayed.
2. Chloroform extracts were placed in matched quartz-silicate microcuvettes with a 4 mm pathlength and assayed in a Turner Model 430 spectrofluorometer using a xenon light source. Excitation and emission settings were 360 nm and 450 nm respectively.
3. Quinine sulfate at a concentration of 0.1 mg/l 1N H<sub>2</sub>SO<sub>4</sub> was used as a fluorescence standard.
4. Sample FAP concentrations are expressed as either relative fluorescence intensity (RFI) or as whole-organ percentage fluorescence (% FL).

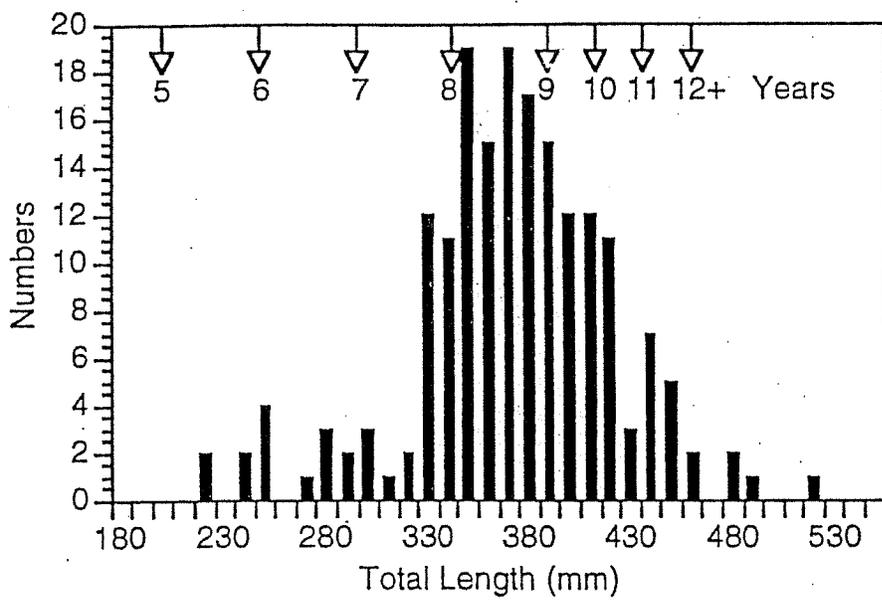
$$RFI = \frac{\text{sample luminescence}}{\text{standard luminescence}} \times \frac{\text{solvent volume (ml)}}{\text{sample dry weight (mg)}} \times 100$$

$$\% FL = \frac{\text{sample luminescence}}{\text{standard luminescence}} \times \text{solvent volume (ml)} \times 100$$

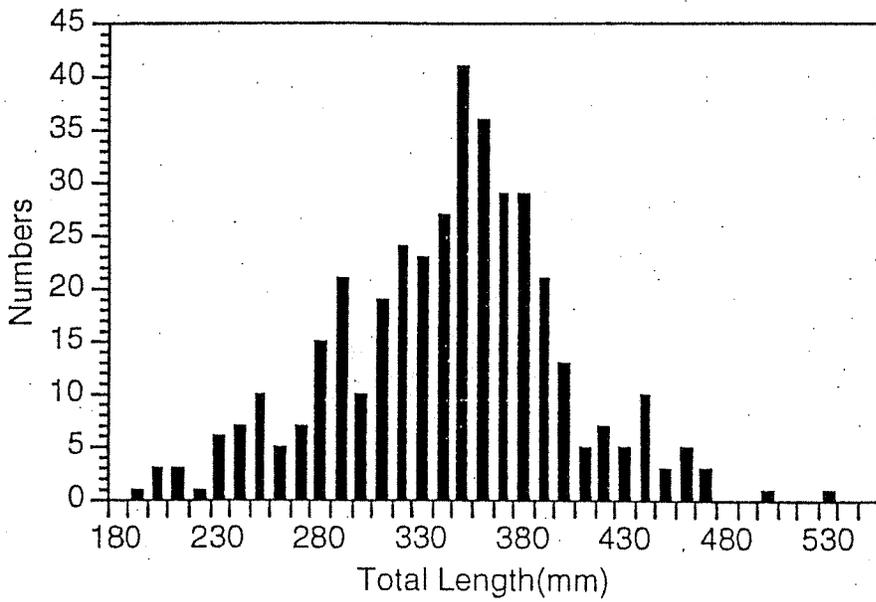
Appendix ii



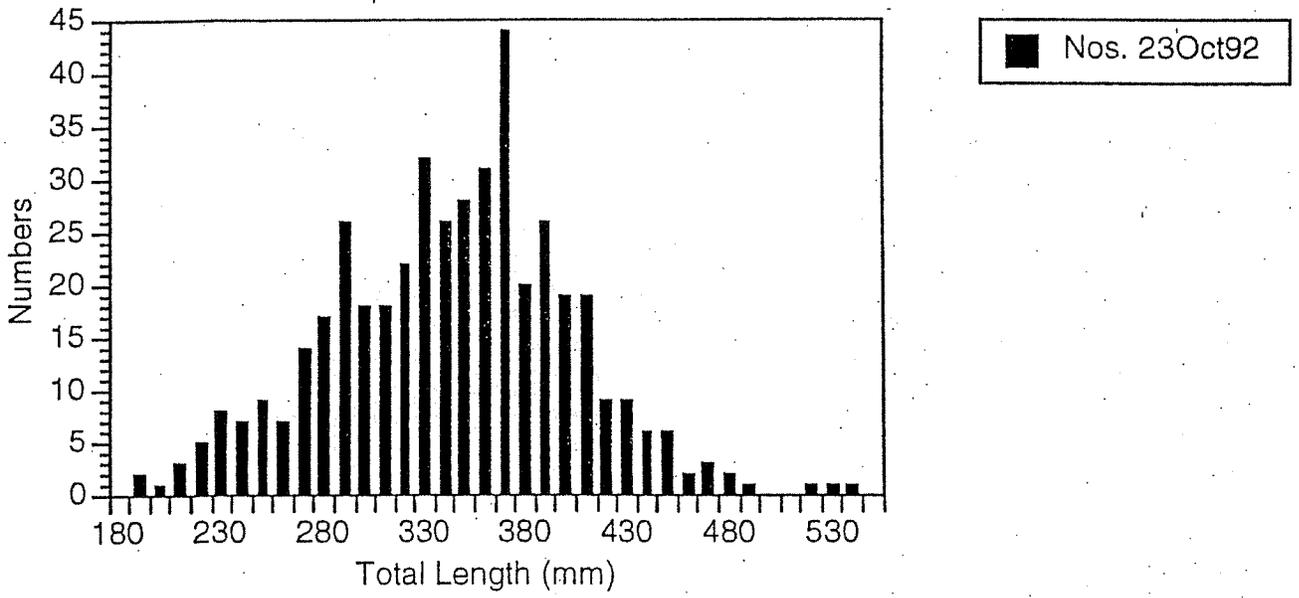
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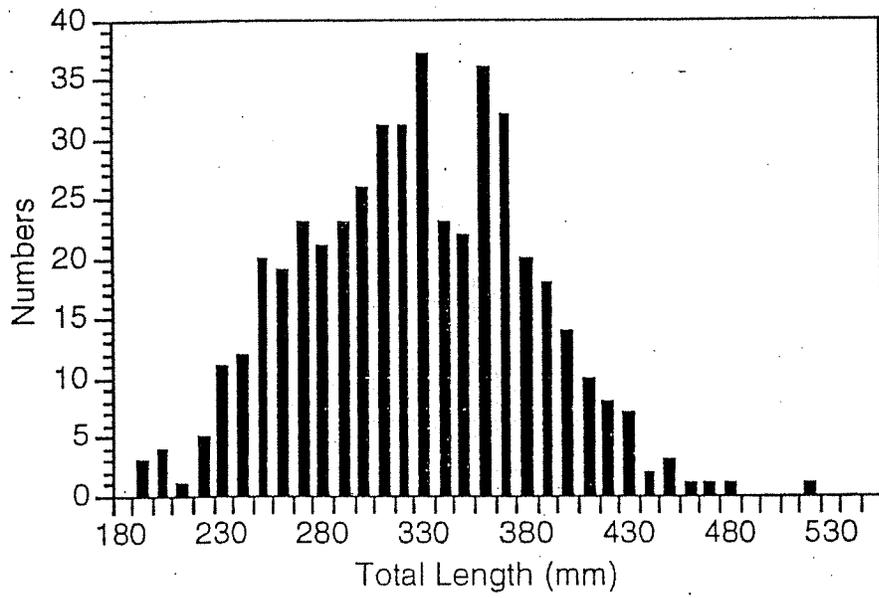


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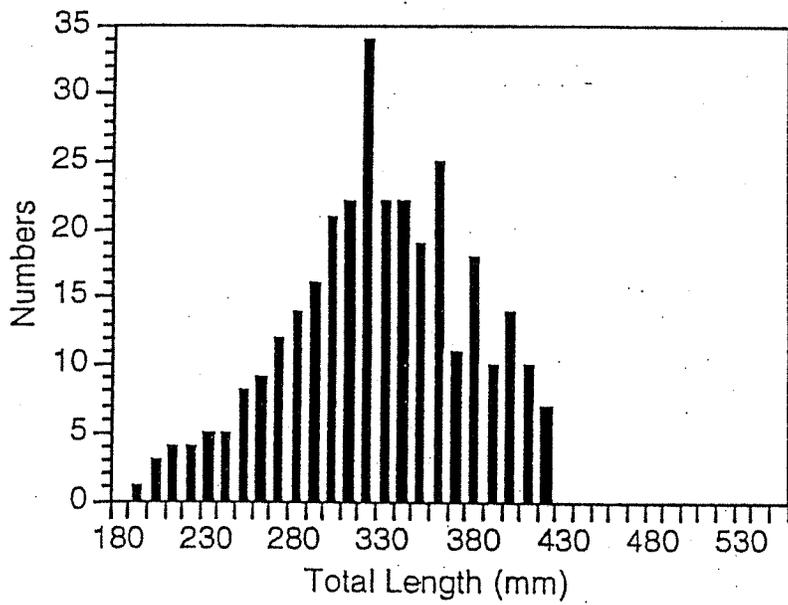


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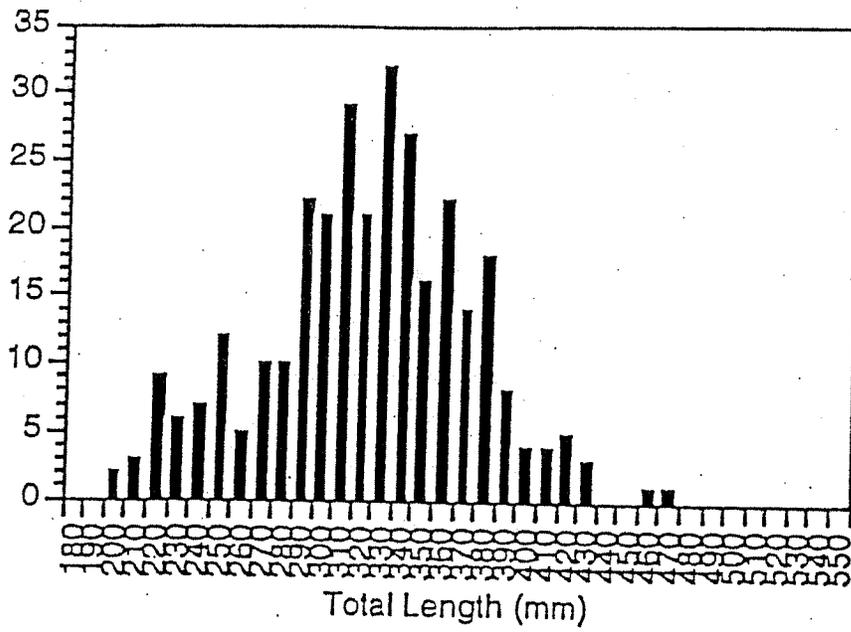




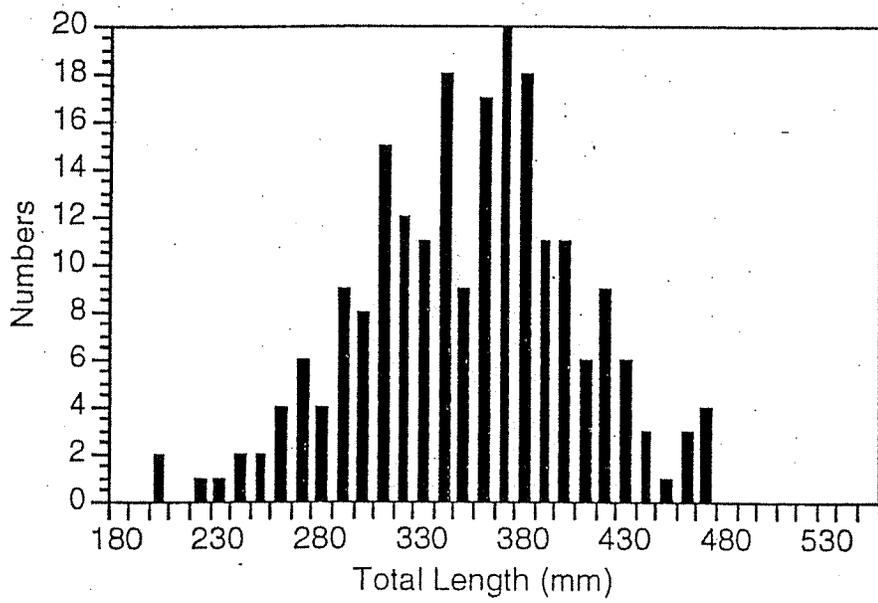
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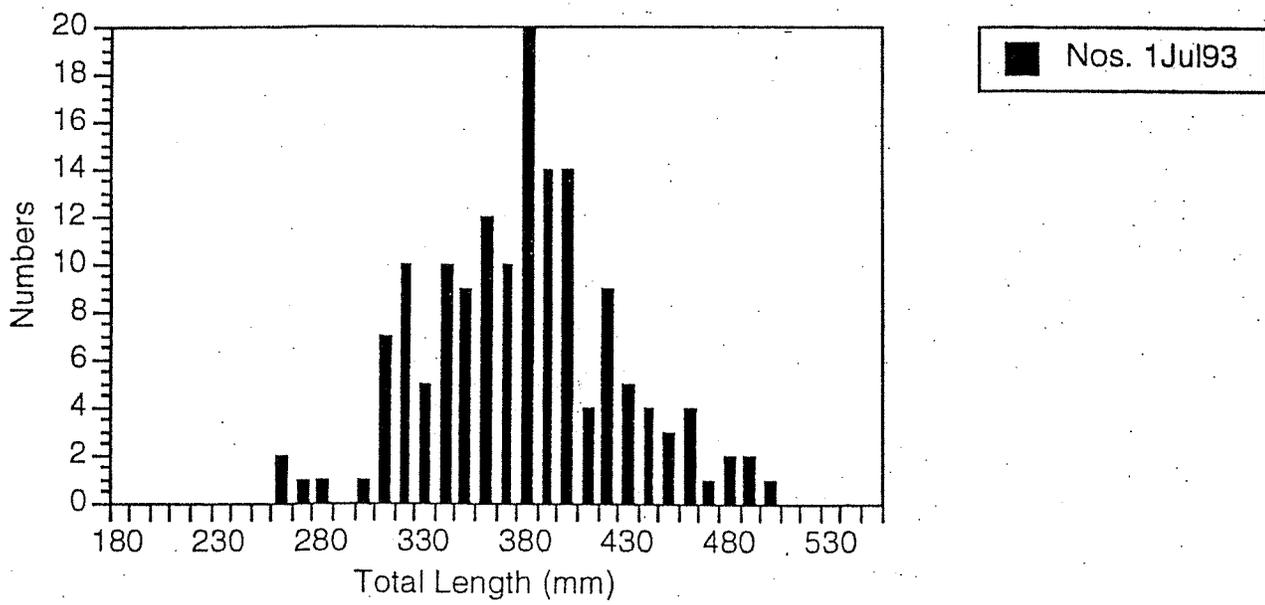
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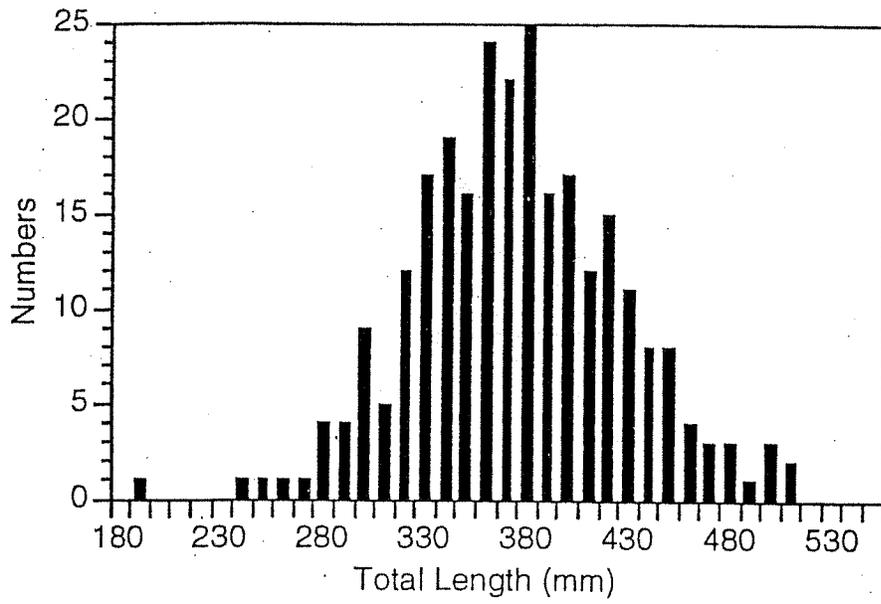


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