GROWTH AND REPRODUCTION OF THE BAT RAY, 
MYLIOBATIS CALIFORNICA GILL, IN CALIFORNIA

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By
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ABSTRACT

The growth and reproduction in 191 bat rays, *Myliobatis californica* Gill, from Elkhorn Slough and Monterey Bay, California was studied. Vertebral centra were removed, morphometric measurements made, and sexual condition assessed in all specimens. Among several ageing techniques tested, x-radiography and oil clearing were used most successfully to elucidate circuli (rings and bands) in the centra of the bat ray. The annual nature of band deposition was verified by comparison of modal disc widths (DW) from size frequency analysis to mean back-calculated disc widths, mineralization patterns seen in x-rays and tetracycline marking, and by correlating changes in the appearance of circuli with season and age.

Evaluation of each ageing technique based on consistency of band counts indicated that the oil clearing technique is slightly more reliable. Comparison of calculated DW to published observed maximum disc widths indicated that for males the x-radiography technique produced the most realistic growth curve, while for females the oil clearing technique was best. The Brody-Bertalanffy growth curves derived from these techniques indicated that female bat rays reach a greater size ($DW_\infty = 1587$ mm) and have a lower growth rate ($K = 0.0995$) than males ($DW_\infty = 1004$ mm, $K = 0.229$).

Reproduction in bat rays from Elkhorn Slough appears to follow a well-defined annual cycle in which mature individuals enter the slough in May to give birth and mate, and depart by late September. It is suggested that, similar to other rays (Strushaker, 1969; Smith, 1980), the bat ray's gestation period is 9 to 12 months long.
In male bat rays, three indicators of sexual maturity (presence of mature spermatozoa, clasper-DW relationship, and internal morphology) showed that onset of sexual maturity occurs at 2 to 3 years of age, at a disc width of about 622 mm. In females, presence of mature ova indicated that 50% maturity occurs at approximately 5 years of age, at a disc width of about 881 mm.
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INTRODUCTION

Utilization of elasmobranch fishes from California waters is rapidly increasing. According to Frey (1971), Ronsivalli (1978), and Fishery Market News (National Marine Fisheries Service), at least 22 elasmobranch species are presently fished commercially in California. Unfortunately, very little is known about such critical aspects of elasmobranch life histories as age structure, growth rates, and reproductive characteristics. The information that is available suggests that many elasmobranchs have relatively slow growth rates and low reproductive rates. When these features are linked to the nearly direct relationship between their stock and recruitment, they appear to be very susceptible to overfishing (Holden, 1977). Only when the critical information pertaining to life histories is made available will the effective management of this emerging fishery be possible.

The bat ray, Myliobatis californica Gill, has been the target of an annual sport fishery since at least 1950 (Herald et al., 1960), and has recently appeared in Fishery Market News. The bat ray, one of approximately 430 batoid elasmobranchs in the world (Compagno, 1977), represents the only California member of the family Myliobatidae. This species is a common inhabitant of shallow inshore waters and estuaries, ranging from Oregon to the Gulf of California (Miller and Lea, 1972).

There is little known of the ecology of the bat ray. The earliest information on its life history is provided by MacGinitie (1935), and more recently information on the diet and seasonal occurrence of bat
rays in Tomales Bay (Ridge, 1963) and Elkhorn Slough have been reported (Talent, 1973; Barry, 1981). The diet of the bat ray consists primarily of benthic infaunal invertebrates (Ridge, 1963; Talent, 1973; Karl and Obrebski, 1976; and VanBlaricom, 1977). Both Ridge (1963) and Talent (1973) indicated that adult bat rays were dominant seasonal members of the elasmobranch fauna of both Tomales Bay and Elkhorn Slough. Further evidence of their dominance is the fact that bat rays have been the most abundantly caught elasmobranch in 43 Elkhorn Slough shark derbies for which records were available since 1950. Little is known of the migratory habits of the bat ray, though they have been observed, on at least one occasion, to travel in aggregations of thousands of individuals (Odenweller, 1975). Barry (1981) reported that juvenile bat rays were common in the tidal creeks of Elkhorn Slough during spring and summer months. Thus, it seems likely that, as Herald et al. (1960) suggested, bat rays use Elkhorn Slough seasonally as a nursery ground.

The age, growth, and reproductive patterns of the bat ray are poorly understood. What is known about its reproductive biology is primarily limited to notes on the occurrence of embryos (Herald, 1953; Herald et al., 1960, Talent, 1973), and the seasonal pattern of abundance of juvenile young of the year (Barry, 1981). Size at maturity has been suggested to be 10 pounds for males and 50 pounds for females (Herald et al., 1960). Miller and Lea (1972) stated that a maximum weight of 95.25 kg (210 lbs.) and size of 1.22 m (4 ft.) pectoral wing width may be attained.

In order to adequately assess the impact of continued or increased exploitation of a fish stock it is necessary to know certain
life-history characteristics, such as growth rates and age specific fecundities. Bony fishes are commonly aged by examination of hard tissues such as otoliths, scales, or opercular bones (Williams and Bedford, 1974). The bat ray, as with other elasmobranchs, cannot be easily aged due to lack of seasonal hard tissue deposition. The structures used in teleosts are either absent or inappropriate for age determination of elasmobranchs. Alternative methods of age determination of elasmobranchs have involved use of spines (Holden and Meadows, 1962; Babel, 1967; Ketchen, 1972), tooth replacement rates (Moss, 1967; 1972), size frequency analysis (Babel, 1967; Sage et al., 1972; Jones and Geen, 1977a), and tag and recapture studies in conjunction with examination of calcified vertebrae (Holden and Vince, 1973). Recently the evidence on age determination of elasmobranchs has focused on the analysis of translucent and opaque rings in vertebral centra. Increase in the number of concentric rings with increase in specimen size has been observed in various species of sharks (Haskell, 1949; Parker and Stott, 1965; and Stevens, 1975) and skates (Ishiyama, 1951; Daiber, 1960; Richards et al., 1963; and Taylor and Holden, 1964).

A variety of chemical and physical manipulations has been used to enhance the "readability" of these rings. Paraffin and xylene impregnation methods have been used to enhance clarity of the circuli (Daiber, 1960). Staining by Alizarin Red S (LaMarka, 1966) and silver nitrate impregnation (Stevens, 1975; Harvey, 1979) has been used on the blue shark, Prionace glauca. Richards et al. (1963), working on Raja erinacea, and Smith (1980), working on Rhinoptera bonasus, used alcohol immersion and drying techniques. Finally, x-ray spectrometry for
calcium and phosphorus has been shown to yield results comparable to other methods (Jones and Geen, 1977a, b).

Various approaches can be used to validate the annual nature of circuli seen in elasmobranch vertebrae. Tetracycline derivatives are incorporated into newly calcified tissue (Frost et al., 1961; Steendijk, 1964; Frost, 1969; and Kucers and Bennet, 1965) and are thus useful as in vivo markers (Weber and Ridgeway, 1962). Tetracycline has been used to mark the centra of Raja clavata, demonstrating the annual deposition of hyaline and opaque bands. The use of tetracycline coupled with tag recapture programs offers promise as a valuable means of age validation and in the determination of growth rates.

In addition to tetracycline marking, other approaches that have been used to validate the annual nature of circuli include: (1) noting differences in appearance of circuli in embryos and young-of-the-year; (2) noting the seasonal transition in type of circuli laid down at the centra edges (Smith, 1980); and (3) noting correspondence between modal sizes and size at age as derived from each ageing technique (Weatherly, 1972; Smith, 1980).

In order to provide information essential in developing effective management plans, the objectives of this investigation were to develop and test ageing techniques useful in determining age of bat rays, to use this information to produce growth curves, and to describe the development of their reproductive capabilities, fecundity, and gestation time.
MATERIALS AND METHODS

The majority of specimens used in this study were collected from Elkhorn Slough, located midway between Monterey and Santa Cruz (Fig. 1). Elkhorn Slough consists of approximately 2,500 acres of submerged areas, tidal flats, and salt marsh and provides ideal habitat for the bat ray (Talent, 1973; Barry, 1981). Most specimens were obtained from participants in the Elkhorn Slough shark derbies held June 1 and June 15, 1980. These derbies have occurred since 1951, sponsored by the Pajaro Valley Rod and Gun Club and the Castroville Rod and Gun Club, and since 1978 also by the Salinas Izaak Walton League. These bat rays were captured during early daylight hours by hook and line.

Additional specimens from Elkhorn Slough were collected between November 1979 and September 1980 using otter trawls and gill nets. Large fish were captured with 100-ft. long nets (6- and 9-inch mesh) set perpendicular to currents along the main slough axis or in tidal channels in one of four locations (Figure 1). Nets were left out for 8 to 24 hours. Smaller individuals were collected from small tidal channels, which they commonly frequent (Barry, 1981), using a small otter trawl towed from a 16-foot Boston Whaler.

Measurement and dissection of specimens were carried out either at Moss Landing Marine Laboratories or at the site of collection. Each animal was sexed and weighed to the nearest 0.1 kg., and nine linear measurements were recorded (see Fig. 2).

Vertebrae were used for ageing in this study. A section of the vertebral column including the 1st through the 25th elements was
removed, labeled, placed in a twist pack bag, and frozen. Preliminary work indicated that the same number of circuli occurs consistently in all of these first 25 centra from the same specimen. Since the most proximal and distal vertebrae were more difficult to manipulate, the final counts were done using centra from the 10th through the 17th vertebrae.

Several techniques used by others to elucidate rings and to age elasmobranchs were evaluated. The methods found to be unsatisfactory for use in ageing bat rays included a modified silver nitrate staining technique as initially described by Stevens (1975) and modified by Harvey (1979), alcohol soak and drying as described by Richards et al. (1963) and Smith (1980), and staining with Alizarin Red S as described by LaMarka (1966). Two other methods worked better and were finally adopted. The first involved the examination of each centrum using a dissecting scope and transmitted light, and the second involved the use of x-radiography.

The following procedure was followed in preparation of centra for examination by either ageing technique:

1. Three to five vertebrae were removed from each defrosted vertebral column. Connective tissue and lateral portions of the neural arch were removed with scalpel and forceps.

2. Centra were then cleaned by immersion in 100% Chlorox® bleach, until extraneous tissue was dissolved. Immersion times varied between 5 and 15 minutes depending upon the size of each centrum, with larger samples requiring more time.
(3) Centra were removed from the bleach, rinsed, and then soaked in two changes of fresh water over a period of 24 hours.

(4) Centra were removed from the water, air-dried, and cut vertically, such that the resultant halves presented opposite faces of the same vertebra (Fig. 3a). Large vertebrae were cut with a small circular saw attachment for a jeweler's drill. For smaller specimens half of the centrum was ground away using aluminum oxide wheel points and jeweler's cloth. This procedure prevents circuli on opposing halves of the centra from obscuring one another during x-raying.

In the "oil clearing technique" the face of each sectioned centrum was viewed under a dissecting scope with transmitted light using fiber-optics, which provided the most intense and directable beam of light. Each centrum face was wiped with cedarwood oil, thus increasing the clarity of the circuli by eliminating superficial irregularities. The centrum face was scraped with a scalpel, thus providing a much improved view of the finer circuli.

In the "x-ray technique", vertebrae were x-rayed using a Hewlett-Packard Faxitron series X system (Model No. 43805N) with a Kodak Industrex M film (ready pack M-2), as suggested by Miller and Tucker (1979). All rays and centra were labeled as to specimen number, and three cleaned and sectioned centra from each specimen were x-rayed. The film was cut prior to use into 2.5 x 2.5 cm squares. After exposure, the film was developed according to Kodak Industrex film developing procedures.
Preliminary work revealed the presence of two conspicuous patterns of circuli in all vertebrae examined. The term "band" will be used to refer to the wider type of concentric circuli, and the term "ring" to denote the much narrower type of circuli, of which the bands are composed. Each broad band was composed of five to seven rings. In radiographs the bands appeared as alternating regions of broad light and narrow dark circuli (Fig. 3b).

As noted by Blacker (1974), the terms "hyaline" and "opaque" are often used interchangeably to describe the same zone in teleost otoliths. This confusion often arises as a result of the method of illumination. In this study opaque zones will refer to those which appear light in x-rays viewed with transmitted light. Hyaline zones will be those which appear dark in x-rays. Broad bands which appear dark in oil cleared centra appear light in x-rays. In order to avoid confusion the bands will be referred to as broad or narrow regardless of the technique being discussed.

Two to four readings were made at three-week intervals on centra prepared with both techniques. In the first reading, the number of rings and broad and narrow bands was recorded. For the second reading bands were counted again, and band diameters and centrum diameter were measured. Each band diameter was measured from the bands dorsal to the ventral peripheral margins. The centrum diameter was measured from dorsal to ventral edges (Fig. 3b). All measurements were made using an ocular micrometer (1 ocular micrometer unit = 0.1 mm), on an Olympus dissecting microscope. Any centrum or x-ray with a second reading which did not agree with the first was set aside to be read a third and fourth
time. Those centra with third and fourth counts that did not both agree with one of the first two readings were discarded. Initial comparisons using 25 specimens indicated that band diameter measurements from x-rays corresponded closely to those from oil clearing, and thereafter band diameter measurements in x-rays were discontinued.

The periodicity of band deposition in bat rays was assessed in several ways. First, the appearance of bands in embryos and young-of-the-year was noted. Second, transition from narrow to broad band at a centrum's edge was correlated with season. Third, back-calculated disc widths (DW) at age were compared to DW at earlier ages, DW at time of capture, and to modal disc widths of animals captured in Elkhorn Slough shark derbies. Fourth, the pattern of tetracycline deposition in centra of captive animals was compared to the pattern of mineralization as determined by x-rays of the same animals.

To back-calculate past body dimensions of a fish, it is assumed that the growth pattern as it appears in the hard part can be correctly interpreted, and three prerequisites need be satisfied before a body part can be used for back-calculation (Everhart et al., 1975). The hard part must remain present over the life span of the fish, the growth of the body part must be proportional to growth of the fish throughout the life span of the fish, and checks used for age and growth analysis must be annual and formed at the same approximate time each year. The first of the prerequisites was satisfied by simple examination of specimens, the second by calculating the functional mean regression coefficient for DW and centrum diameter (CD), and the third by correlating occurrence of transition from broad to narrow bands with date of collection.
A back-calculated DW at age, based on centra diameters from oil-cleared centra, was calculated using the equation:

\[ \text{DW}_n = \frac{\text{CD}_n}{\text{CD}_c} (\text{DW}_c - C) + C \]

where: \( \text{CD}_n \) and \( \text{CD}_c \) refer to the centrum diameters at age \( n \) and at time of capture, respectively; \( \text{DW}_n \) and \( \text{DW}_c \) refer to the DW's at age \( n \) and at time of capture, respectively; and \( C \) is the intercept of the functional mean regression of DW on CD, representing a correction factor (Everhart et al., 1975; Ricker, 1975).

The potential value of tetracycline as an \textit{in vivo} marking agent was evaluated using oxytetracycline, a broad base tetracycline derivative. The 15 bat rays utilized were taken from Elkhorn Slough and ranged in size from 0.21 kg (DW = 288 mm) to 58.0 kg (DW = 1333 mm). Dosages ranged from 10 to 100 mg of oxytetracycline per kilogram of body weight. Injections were administered in the animal's ventral surface, distally and slightly anterior to the cloaca (Fig. 2). All injected animals were tagged on the dorsal surface of one pectoral fin with a numbered Floy spaghetti tag (Fig. 2). These bat rays were held at either Moss Landing Marine Laboratories or Steinhart Aquarium, California Academy of Sciences, and were fed a diet consisting primarily of squid and/or smelt.
Upon death each animal was weighed and dissected under an ultra-violet light. Occurrence of fluorescences in internal organs, skeletal system, teeth, and centra was noted.

An Analysis of Covariance (ANCOVA) was used to compare functional mean (FM) regression lines which described disc width and centrum diameter (CD) relationships as calculated for males and females. Functional mean regression lines were calculated according to Ricker (1973).

Growth curves based on mean back-calculated disc widths of the two most recent age classes and empirical data from each technique were calculated using the Brody-Bertalanffy equation (Ricker, 1975):

\[
DW_t = DW_\infty \left[1 - e^{-k(t-t_0)}\right]
\]

where: \(DW_t\) = disc width at time \(t\);
\(DW_\infty\) = the theoretical maximum disc width;
\(k\) = the rate at which \(DW_\infty\) is approached; and
\(t_0\) = the age at which the bat ray would have had zero disc width had it always grown in the manner described by the equation (Ricker, 1975).

A computer program was developed for the Hewlett-Packard 9825-A programmable desk calculator to calculate parameters of the Brody-Bertalanffy growth equation according to four different methods, as described by Everhart et al. (1975), Walford (1946), Gulland (1965),
and Allen (1966). The mean square for each of the four lines was calculated to determine which method provided the best fit for the Brody-Bertalanffy growth curve.

FM regression equations describing the disc width and weight relationships were calculated and were compared using an ANCOVA.

Differences in the variances and mean DW at age between sexes and between both ageing techniques were compared using F- and t-tests, respectively, as described by Sokal and Rohlf (1969). Also, 95% confidence limits were calculated for all mean disc widths for both ageing techniques.

Size frequency data collected in the 1971 to 1973 and 1980 Elkhorn Slough Shark derbies were analyzed and compared to mean DW at age as determined by both ageing techniques.

The reproductive status of each animal collected during this study was examined. The attainment of sexual maturity in male bat rays was based on three criteria. The first indicator was an abrupt change in the relationship of clasper length (Figure 2) to disc width (Babel, 1967; Struhsaker, 1969; and Smith, 1980). The second criterion was the occurrence of coiling in the vas deferens (Pratt, 1979). The final criterion used was presence of mature spermatocytes in seminal fluid, as determined by microscopic examination of smears taken from the testes, epididimis, and ampulla ductus deferens (Pratt, 1979).

Based on examination of numerous females the following criteria were used to determine the state of sexual maturity in female bat rays:

1) **im immature** - ovaries thin and of homogeneous cellular appearance
throughout the gonad. Uteri thin and flaccid and relatively indistinct from the oviducts.

2) maturing - ovary showing differentiation of ova, ova approximately 5 to 10 mm in diameter. Uteri distinct from oviducts with thickened walls.

3) mature - ovary with large yolked eggs, greater than 10 mm in diameter. Uteri well developed and rich in trophonemata.

Further evaluation of female reproductive status included measurement of the width of the uterus and oviducts. The diameter of the largest generation of ovarian eggs is a valid index of first maturity when compared with body length (Pratt, 1979), and therefore the number and diameter of all eggs was recorded. In order to evaluate seasonality the ova were grouped into four size classes or generations. The frequency of occurrence of the four size classes was recorded for all females.

All embryos were labeled, weighed, and frozen. Later examination involved measurements and ageing procedure as described for other specimens.

To insure accuracy of field counts and to provide a more comprehensive study of detailed reproductive development, the reproductive systems from 20 females ranging in DW from 516 mm to 1510 mm were removed, labeled, and frozen. The sample organs were thawed in 80% isopropyl alcohol, then transferred to 40% isopropyl alcohol for 32 to 48 hours. This procedure served to impart a tough leathery nature to the otherwise extremely fragile ova. The alcohol was drained off and
the organs returned to the freezer. Ova from frozen ovaries were then easily separated, counted, and measured.

Determination of size and age at maturity was based on specimens collected at the June 1 and June 15, 1980 shark derbies. Among these females, only those individuals whose ages agreed between the two techniques used in determining age were utilized.
RESULTS

Centra as Ageing Material

Vertebrae were present in all bat rays examined. Although difficult to prove, it seems obvious that the vertebrae are neither lost nor regenerated during the life of the fish.

There was a significant linear relationship between disc width and centrum diameter for both sexes (Fig. 4). No significant differences existed (P < .05) between the lines for males and females (Appendix A); therefore, an equation derived from both males and females was used for back-calculations. The linear relationship was described by the FM regression equation:

\[ \text{DW} = 105.9 \times \text{CD} + 65.89 \ (r = 0.98; \ n = 162). \]

The transition from narrow to broad bands correlated well with season in small bat rays, thus satisfying the third and final prerequisite for use of centra in back-calculations. A transition from narrow to broad bands was difficult to distinguish in specimens greater than approximately 600 mm DW; however, in smaller specimens which had been collected from November 1979 through December 1980, a transition was observed. The outer margins of centra from all young-of-the-year rays collected between May and January exhibited one broad band. One individual collected in January had a peripheral narrow band. Among those specimens with unobscured edges collected between May and October, 70% of their radiographs (n= 40) had light broad edges. Seasonality of
band deposition is indicated since 67% of those 21 samples collected between November and May exhibited a narrow peripheral band. The number of bands was consistent for each centrum of each vertebra and from one vertebra to another.

A graphical representation of mean back-calculated DW at age for male and female bat rays shows that the back-calculated mean DW's at each age for each year class correspond closely to back-calculated disc widths in other year classes (Figures 5 and 6, Tables 1 and 2). Among the females and, to a lesser extent among the males, there is a noticeable expression of "Lee's phenomenon", a smaller estimated size for fish of younger ages, when calculated from vertebrae of older fish, than the observed average size at the age in question (Ricker, 1975).

The narrow band counted as the first annulus was in actuality preceded by one which had apparently been formed in utero. The first narrow band which was present in nearly all centra at 0.4 to 0.5 mm radius also occurred in fetuses with disc widths greater than 240 mm. On centra from free-swimming young-of-the-year, a wide band occurred peripheral to this initial narrow band. It appears, based on the above evidence, that there is an initial narrow band formed in utero.

Tetracycline

General deposition of oxytetracycline was observed in 10 of the 15 injected bat rays. The five bat rays that had been transferred to Steinhart Aquarium were eaten by tank mates before examinations could be carried out. None of the bat rays grew during their short time in captivity. All rays exhibited the same pattern of deposition. The tissue immediately surrounding the site of injection glowed, as did the
liver, walls of the spiral valve, and neurocranium. Animals that had been injected with dosages of 100 mg/kg exhibited fluorescence in wing musculature. The eight bat rays that had lived three to eight days after injection, plus one that had lived two months had a very bright fluorescence in newly formed tooth plates. One animal that had been held three months had no fluorescence in its teeth or centra and very little in any internal organs. The intensity of fluorescence obviously varied with dosage and time in vivo. Generally, increased dosages resulted in greater intensity, while increased time in vivo allowed for diminished fluorescence in soft tissues and organs.

Similar patterns of oxytetracycline deposition appeared in the centra of all rays. The central portion of the centrum fluoresced in 9 of the 10 bat rays. Fluorescence was observed on the periphery of all centra and in all broad bands, yet was absent from the narrow bands. After the centrum face was scraped, it was apparent that, rather than the entire band, the rings which composed the broad bands, fluoresced.

**Growth Curves**

The Brody-Bertalanffy growth curves for males resulting from the two ageing techniques were considerably different (Fig. 7). The growth of the male bat rays aged using the oil clearing technique was best represented by a Brody-Bertalanffy growth curve calculated according to the Gulland (1965) method. This equation was:

\[ DW_t = 1991 \left( 1 - e^{-0.0596(t + 2.879)} \right) \]
For males aged using the x-ray technique, Allen's (1966) method provided the best fit. This equation was:

\[
DW_t = 1004 \left[ 1 - e^{-0.2294(t + 1.5803)} \right],
\]
with 95% confidence intervals for \(DW_\infty\), \(k\), and \(t_0\) being 204, 0.11142, and 0.541, respectively (Fig. 7).

The Brody-Bertalanffy growth curves for females generated by the two ageing techniques were very similar (Fig. 8), and the Allen (1966) method produced the best fit. The Brody-Bertalanffy growth equation from radiographs was:

\[
DW_t = 1566 \left[ 1 - e^{-0.099(t + 1.935)} \right],
\]
with 95% confidence intervals for \(DW_\infty\), \(k\), and \(t_0\) being 148, 0.0209, and 0.421, respectively. The Brody-Bertalanffy growth equation generated from the oil clearing technique was:

\[
DW_t = 1587 \left[ 1 - e^{-0.0995(t + 2.059)} \right],
\]
with 95% confidence intervals for \(DW_\infty\), \(k\), and \(t_0\) being 140, 0.0188, and 0.354, respectively.

The mean back-calculated disc width at age from the two most recent consecutive year classes was nearer the disc widths at age as determined by both ageing techniques for both sexes (Table 1 and 2) than those using all year classes. This back-calculated curve, for males, is intermediate in position between that of the oil clearing and x-ray derived curves (Fig. 7). The Brody-Bertalanffy growth equation obtained for males using Allen's (1966) method was:
\[ \text{DW}_t = 1517 \left[ 1 - e^{-0.834(t + 2.546)} \right], \]

with the 95% confidence intervals for \( \text{DW}_o, k, \) and \( t_0 \) being 115, 0.0994, and 1.148, respectively. The curve back-calculated for females was very similar to those determined by both ageing techniques (Fig. 8). The equation representing this curve was:

\[ \text{DW}_t = 1567 \left[ 1 - e^{-0.096(t + 2.040)} \right], \]

with the 95% confidence intervals for \( \text{DW}_o, k, \) and \( t_0 \) being 131, 0.021, and 0.673, respectively.

X-ray vs. oil clearing. The oil clearing technique provided a more consistent ageing method than did the x-ray technique. Approximately 17% of the centra examined in oil clearing (\( n = 152 \)) and 28% of the radiographs (\( n = 137 \)) were discarded based on inconsistent band counts.

Both ageing techniques consistently placed specimens in the same age group and produced similar mean DW's at age in both males and females. As shown in Table 3, 65.9% of a total of 132 bat rays were placed in the same age class by both techniques. Among those rays assigned to different age classes, 25.7% differed by only 1 year. The younger age classes had the highest percent agreement (Table 4). The older year classes, with such small sample sizes, are more difficult to evaluate. The only significant difference in mean DW for the males as determined by the two techniques was at age 3 years (\( t_{\text{calc}} = 2.34, t_{\text{crit}} = 2.179, \alpha = .05, \text{df} = 12 \) (Table 1). Disc width at age 10 was the only age for the females at which significantly different mean DW occurred between the two techniques (\( t_{\text{calc}} = 2.72, t_{\text{crit}} = 2.57, \alpha = .05, \text{df} = 5 \) (Table 2).
It is apparent that small differences in yearly growth increments between the two techniques resulted in very different growth curves. In males, the rates of growth from year to year vary enough to give different impressions of the overall growth patterns (Fig. 9). In fact the Brody-Bertalanffy growth curves generated by each technique yield strikingly different curves (Fig. 7). Based on the oil clearing technique the rate of growth seems fairly equal for all ages, while the line generated from the x-ray technique suggests a more sigmoid curve. The portion of the females growth curve from 0 to 7 years of age (Fig. 10) is notably similar for both techniques. From the 10th year class on, the growth pattern suggested by the two techniques becomes less congruent.

Males vs. females. Female bat rays attain a larger asymptotic DW and have a greater growth rate than do the males. Statistical comparisons between male and female DW at age as determined by the oil clearing technique indicated a significant difference at age 5 years ($t_{\text{calc}} = 3.53$, $t_{\text{crit}} = 2.365$, $\alpha = .05$, df = 7). Based on ages determined using the x-ray technique the mean disc widths of the 2 and 3 year age classes were significantly different between the sexes (two year olds: $t_{\text{calc}} = 5.17$, $t_{\text{crit}} = 2.131$, $\alpha = .05$, df = 14, three year olds: $t_{\text{calc}} = 2.84$, $t_{\text{crit}} = 2.11$, $\alpha = .05$, df = 17).

Morphometrics

Disc width was strongly correlated with all other body measurements (Appendix B) for both males and females. The disc width (DW, mm) to weight (W, g) relationship for males was significantly different than that for females ($P < .05$). The log W and log DW relationships for
males and females, respectively, were linear (Appendix B).

Reproduction

Males. Onset of sexual maturity in male bat rays appears to occur between 500 mm and 650 mm DW or around two to three years of age. Of the 61 males studied, 27 of the 28 specimens, with disc widths less than 450 mm, had straight vas deferens, and were staged as immature. Based on a moderate coiling of the vas deferens 10 of 11 males between 450 and 622 mm DW were categorized as maturing. All specimens greater than 622 mm DW were fully mature, as indicated by extreme coiling of the vas deferens. The ratio of clasper length to DW increased noticeably at a DW of 550 to 660 mm (Fig. 11). Sperm smears were taken from the ampulla ductus deferens from 30 males ranging between 275 mm and 915 mm DW, collected during the months of April, May, and June. Mature spermatozoa comprised approximately 10% of the cells present in a smear taken from an individual which measured 499 mm DW. All smears from specimens with disc widths greater than 680 mm (n = 11) had 80% to 95% of the total of all cells visible in the sperm smears composed of mature spermatozoa.

Females. Based on a June 1980 sample of 108 animals, 50% of the female bat rays were mature at a mean DW of 881 mm, or approximately 5 years of age. The reproductive status of 130 female bat rays was evaluated over the period from 28 November to 21 September 1980. The smallest maturing specimen measured 545 mm DW. All females of a size greater than 1002 mm DW or greater than 7 years of age, were found to have attained sexual maturity.
The right ovary was consistently less developed than the left. Eggs were occasionally present in the right ovary, but usually did not exceed 10 mm diameter. One female had five ova, each of 20 mm diameter in her right ovary. In all gravid females both uteri seemed to be functioning on an equivalent level.

The more detailed laboratory analysis of sizes and numbers of ova in each ovary agreed well with field estimates. Discrete size classes, or generations, of ova were readily discernible within each ovary regardless of time of year or size of individual. In the June sample, the mean number of eggs increased with age up to age 14 and then leveled off, while the trend in egg size was to increase continuously with age (Fig. 12).

Evaluations of seasonal change in size class of ova in mature females indicated a general annual periodicity. The smallest generation (< 5 mm) diminished in percent composition of total ova from winter to summer, while both intermediate size classes (10 mm and 20 mm) increased (Fig. 13). The 10 mm size class dominated other size generations of ova by number, regardless of season. The largest size class (≥ 25 mm) appeared in both winter and summer.

Evidence of ovulation was observed only in the June sample, when 6% of the females examined had ovulated. Ovulated eggs ranged in diameter from 22 mm to 28 mm. In one ovulating female (DW = 1242 mm), three ova enclosed in a shell capsule were removed from the left uterus. The clear brownish capsule which measured about 8 cm long was of a thin diaphanous material. The three bright yellow eggs were each
approximately 25 mm in diameter. Embryos were not visible upon macroscopic examination.

The general pattern of egg development appears to be a proliferation of follicular activity throughout the winter, followed by the maturation of a discrete generation of ova during the spring and summer months. The cycle culminates in mid-summer with the resorption and/or selective maturation of certain ova, subsequent to ovulation.

Increase of oviduct length and uterus width was found to be proportional to an increased disc width (Fig. 14 and Fig. 15). The increase in the range of uterus widths with increase in disc width resulted from measurements having been made on females collected during varying stages of their pregnancy.

**Embryonic Development**

It appears that bat rays mate soon after parturition. This is indicated by the presence of mature ova in four females carrying full- or near-term embryos. The most mature brood was removed from an ovulating female on 15 June. Whether it is possible for ovulation to have been induced prior to parturition by the trauma of capture is uncertain. However, it appeared that ovulation, if it had not actually occurred, was imminent.

Disc width at birth in bat rays based on results of this study is approximately 274 to 315 mm. Size at birth has been reported as 502 mm and 510 mm total length (TL) and 349 mm DW (Herald, 1953; Herald et al., 1960). Free living young-of-the-year captured in June from San Francisco Bay (Herald and Ripley, 1951) had total lengths of 435 mm and 500 mm. When the total lengths were converted to disc widths according
to the FM regression equation calculated for DW-TL relationship (Appendix B) the total lengths of 510 mm, 502 mm, 435 mm, and 400 mm become disc widths of 342 mm, 336 mm, 285 mm, and 258 mm, respectively. Mean disc widths of full-term fetuses taken from females in June of 1980 compare favorably to the above converted disc widths and to disc widths of free-living young-of-the-year collected in previous and subsequent months in Elkhorn Slough (Fig. 16). The contention that these were full-term young is further supported by agreement with mean size at age as calculated from the oil clearing technique, the x-ray technique, and back-calculation (Tables 1 and 2).
DISCUSSION

Centra as Aging Material

The vertebrae of Myliobatis californica are suitable for use in age determination since it appears that circulus formation provides a continuous record of growth. Three factors lend support to this contention. First, in elasmobranchs, growth of the calcified cartilagenous skeleton occurs by a one-way process of deposition, and there is no internal remodeling or resorption (Urist, 1961; Applegate, 1967; and Simkiss, 1974). Second, increased disc widths are accompanied by proportionate increases in centrum diameters. Third, since the banding pattern visible in x-rays occurs as a result of density differences in subject matter (Gosline, 1948), it is likely that the difference between broad and narrow bands is due to differences in mineralization occurring during different growth phases. The pattern of tetracycline absorption is additional evidence that broad bands are more heavily mineralized than narrow bands. As suggested by Jones and Geen (1977b), the pattern of mineralization might result from seasonal changes of the thermal environment and concomitant changes in growth rates.

Validation of Annuli

While it is generally accepted that the incremental growth rings found in the hard parts of northern temperate teleost species are of an annual nature (Williams and Bedford, 1974; Holden, 1977), a similar premise for elasmobranchs has yet to be verified. However, numerous authors working on elasmobranchs have postulated or assumed annual ring
formation in vertebrae (Aason, 1963; Richards et al., 1963; Taylor and Holden, 1964; Stevens, 1975). Holden (1974) analyzed data from Ishiyama (1951), Daiber (1960), and Richards et al. (1963) on various rajoid species, and concluded that circuli on the vertebrae were annual. Only in one elasmobranch species, *Raja clavata* in which tetracycline injections combined with a tag and recapture program has the annual nature of the vertebral circuli been conclusively validated (Holden and Vince, 1973).

The suggestion that the narrow bands on the centra of the bat ray represent valid yearly annuli is based on four findings:

1) free-swimming young-of-the-year and returning yearlings had one broad band which was absent in all embryos;

2) returning yearlings had a narrow band on or near the periphery of the centrum;

3) mineralization pattern in vertebrae is reasonably attributable to annual changes in environmental factors; and,

4) modal disc widths of bat rays taken during Elkhorn Slough shark derbies correspond to mean disc widths at age as derived from each ageing technique and from back-calculations.

The bat rays that remain in Elkhorn Slough through the winter and those which move into coastal waters encounter seasonally predictable fluctuations in the environment, which might in some part be responsible for the formation of discrete circuli in the centra. The periodic deposition of calcium salts resulting in the formation of discrete rings and bands may be attributed to several factors including changes in temperature, salinity, light, and diet (Simkiss, 1974; Stevens, 1975).
Jones and Geen (1977b) using x-ray spectrometry found periodic peaks of phosphorus and calcium in the vertebral centra of *Squalus acanthias* which they attributed to the presence of annual rings which formed as a result of seasonal metabolic changes. During the months of May and June, when the bat ray population density peaks in Elkhorn Slough (Talent, 1973), the temperature gradient ranges from 22°C to 14°C, beginning from the upper slough waters and extending to nearshore coastal waters (Nybakken et al., 1977). Temperatures off the California coast generally range from a winter temperature of 15°C to about a 5°C increase in summer (Thurman, 1975). In addition to the seasonally varying physiographic factors encountered by bat rays as they leave the slough, the changes in epifaunal community assemblages probably necessitate some dietary adjustment. Thus, changes in diet and/or changes in temperature might help account for an annual pattern of circuli deposition represented by one narrow and one broad band.

The correspondence between modal disc width from size frequency analysis and back-calculated disc widths (Fig. 17), though not perfectly aligned, does lend support to the premise that bands in the centra of the bat ray represent annual increments of growth. Holden (1974) pointed out that the Peterson Method of size frequency analysis is of little use in elasmobranch age determination because they typically have very slow growth rates. However, if modal disc widths correspond to mean disc widths at age as derived from a valid method of back-calculation then this can be taken as additional evidence of the validity of marks on the vertebrae (Weatherly, 1972). The modes in the frequency distributions of disc widths in the bat rays caught in Elkhorn
Slough shark derbies (Fig. 17) were not particularly distinct. The first mode, at approximately 320 mm to 350 mm DW, probably represents young-of-the-year, born in early spring. The modal disc width is greater than the mean back-calculated disc width at age zero (Tables 1 and 2) because disc width is calculated from the centrum center to first narrow band, to yield size at birth, whereas the modal disc width includes young-of-the-year which are several months of age. The second mode, at approximately 400 mm DW reflects the first year age class. The less distinct third mode, at approximately 464 mm DW, represents the 2 year age class. The discrepancies between modal values for 1 and 2 year old rays and corresponding back-calculated values (Tables 1 and 2) may be ascribed to overlap of age groups due to unequal individual growth rates as described by Everhart et al. (1975).

Evaluation of Ageing Techniques

The oil clearing technique used to age bat rays is similar to some otolith ageing techniques but has not been used to elucidate circuli in vertebrae prior to this study. Radiography in ichthyology has been used in morphological, taxonomic, and physiological research (Gosline, 1948; Bartlett and Haedrich, 1966; and Miller and Tucker, 1979). Only Aason (1963) has previously used x-rays to clarify rings in an elasmobranch species. The x-ray technique is more complex, but it should be noted that the total time from preparation through final reading did not differ substantially between the two techniques.

Each technique's suitability as an ageing method can be evaluated by the consistency within each technique and the deviation between the results of the two techniques. Such an evaluation is possible since the
bands viewed in the oil cleared centra were the same bands viewed in the x-rays.

Consistency within technique. Assuming that all factors were consistent and provided clear and distinct x-ray negatives, then the x-ray technique should have resulted in band counts as consistent as those of the oil clearing technique. However, the somewhat greater proportion of x-rays discarded (28%) compared to oil cleared centra counts that were discarded (17%) implies less consistency within the former technique. This is in part due to the variability in the quality of x-rays obtained. The factors known to vary and thereby contribute to the inconsistency in the clarity of the x-rays include time and intensity of exposure to x-rays, quality of the film, and condition (i.e., age) of developing fluid and fixative.

Deviation between techniques. Numerous authors have commented on the increased difficulty in interpreting growth marks on hard parts used in ageing with increased size and age of fish (Bonham et al., 1949; Ketchen, 1975; Holden and Meadow, 1962; Six and Horton, 1977; and Smith, 1980, among others). A similar phenomenon occurred in ageing the bat ray. The greater agreement of ages of smaller size classes (Table 4) reflects the decline in interpretability of circuli with age. Therefore, in evaluating the two techniques, one should place more emphasis on comparisons among the younger age classes.

The oil clearing technique usually produced different sizes at given ages and greater asymptotic disc widths for both sexes (Fig. 9 and 10). The reason that the two ageing techniques gave different age estimates is not readily apparent. Increased variability in estimation
of mean disc width at greater ages is evident in the 95% confidence intervals from each technique (Fig. 9 and 10; Tables 1 and 2). Using a criterion that the ageing technique that exhibits the wider confidence intervals within each age class might be less reliable as a predictive tool, for both male and female bat rays the x-ray technique was more reliable. Although one technique may appear more reliable than the other, either technique could be expected to yield similar results, as evidenced by the close agreement between female growth curves (Fig. 8) and also by the finding that differences in disc widths as estimated by each technique were insignificantly different in all but one age class for both sexes. On the other hand the potential for markedly varying results depending upon technique is illustrated by the incongruent growth curves derived for male bat rays (Fig. 7). The difference between curves representing growth in males is a consequence, not as much from having different mean disc widths at age, but of having different incremental growth patterns between year classes. The oil clearing pattern indicated a fairly constant rate of growth through the last age class. Whereas the x-ray technique's pattern of growth increments indicated a decreasing rate of growth through the last age class. Thus, the growth curves derived to accommodate these different rates of change were quite different.

Back Calculation

Lee's phenomenon accounts for the difference between the growth curve derived from the two most recent year classes and the curve derived utilizing all year classes. The weighted mean back-calculated disc widths from each age group's two most recent year classes more
closely paralleled mean disc width at age as derived from each ageing technique, than did weighted mean back-calculated disc width at age when calculated from all year classes. This is because after about the fourth year class in females, and the second year class in males, the weighted back-calculated values from older fish tended to be less than the preceding years estimated back-calculated disc width. This, the occurrence of Lee's phenomenon, may be attributed to natural selection in which differential size specific mortality occurred. Typically it results from either greater mortality of larger individuals or greater natural mortality of faster growing individuals (Ricker, 1975). Size selection by a fishery may also account for, or contribute to the mortality pressure resulting in Lee's phenomenon. Selection for larger members of a year class over 30 years of shark derbies in Elkhorn Slough may conceivably be the vehicle for just such a mortality pattern.

Growth Curves

The growth curves derived from the two ageing techniques and back-calculated disc widths for male bat rays were considerably different. The x-ray ageing technique appeared to provide the most realistic estimate of asymptotic disc width. Herald et al. (1960) gave 22 lbs (9.98 kg) as the "usual maximum weight" observed in males, with the exception of only one 37 lb. (16.78 kg) individual, caught in 17 Elkhorn Slough shark derbies. The largest male captured by Ridge (1963) in Tomales Bay weighed 23 lbs. (10.4 kg), while the largest male listed by a National Marine Fisheries Service study (Sue Smith, pers. comm.) weighed 22 lbs. (9.98 kg). Herald (1972) gave 24 lbs. (10.9 kg) as the maximum size measured in any male bat ray. The largest male encountered
in this study had a disc width of 915 mm and weighed 26.5 lbs (12.51 kg). Given an average maximum weight of 12.1 kg, the corresponding disc width calculated from the weight-disc width relation: \( W = 8.2 \times 10^{-9} DW^{3.096} \), is approximately 916 mm. Holden (1974) states that the maximum observed length of an elasmobranch species provides a good estimate of \( L_\infty \). Based on this assumption it is suggested that the x-ray technique which produced a disc width (1004 mm) nearest to the observed maximum disc width, provides the most realistic growth curve. The curve derived from mean back-calculated disc widths is most similar to the oil clearing curve because it is derived from measurements taken from oil cleared centra. The lower \( K \) and \( DW_\infty \) values for the back calculation-based curve are a consequence of Lee's phenomenon.

The growth curves for female bat rays derived from both ageing techniques underestimate the maximum observed disc width of 1664 mm. However, the oil ageing technique provides the closest \( DW_\infty \) and thus may provide the most representative growth curve for female bat rays. Maximum observed weight for female bat rays is reported to be 160 lbs (72.6 kg) (Herald, 1972). However, one individual weighing 209 lbs (94.8 kg) has been noted (Herald et. al., 1960). Using the weight-DW relation \( (DW = 4.7 \times 10^{-9} DW^{3.199}) \), derived for females in this study, a weight of 94.8 kg becomes a disc width of 1664 mm. The "usual observed" weight of 72 kg becomes a disc width of 1530 mm. The oil clearing technique produced a DW of 1587 mm which is nearest to the observed maximum disc width and is therefore likely to represent the most realistic growth curve.
Holden (1974) theorized that growth rates in elasmobranchs can be described by values of $K$ from 0.1 to 0.2 for sharks and from 0.2 to 0.3 for batoidea. The growth coefficient for female bat rays of 0.1 derived from the best fit growth curve is of questionable agreement with the predicted value. Smith (1980) working on *Rhinoptera bonasus* calculated $K$ values of 0.149 and 0.215 for females and males, respectively. These growth coefficients are also somewhat inconsistent with Holden's (1974) predicted values.

Based on the above evidence and a consideration of batoid life history it is suggested that Holden's (1974) proposed $K$ values inadequately describe batoid growth. Holden's (1974) predicted growth coefficients for batoids were based on growth data from several genera of rajoids. Though very similar in body form, the quite different reproductive modes and taxonomic relationships of myliobatoids (stingrays, butterfly, eagle, cownosed, and devil rays) and rajoids (skates) might account for a dissimilarity in growth rates. Rajoids are a sister group to myliobatoids, each being one of five groups within the batoid superorder (Compagno, 1973 and 1977). The evolutionary time over which these two groups have evolved has produced two very different reproductive strategies. While rajoids are oviparous, myliobatoids exhibit aplacental viviparity (= ovoviviparous). Though the taxonomic relationships between rajoids and myliobatoids may be closer than either is to the other three elasmobranch superorders, the reproductive strategies of each is more similar to other elasmobranchs than to each other. Factors influencing evolution of viviparity and retention of oviparity include not only phylogenetic position but also geographical
distribution, habitat (benthic vs. pelagic), feeding ecology, adult size, egg/embryo size, osmoregulation, and general reproductive strategy of viviparity (Wourms, 1977). That these factors are different enough for skates and rays to have evolved such divergent modes of reproduction indicate that Holden (1974) may have erroneously assumed that these two groups should have growth rates similar to each other yet distinctly different from other elasmobranchs.

**Tetracycline**

The appearance of tetracycline in organ and skeletal systems of the bat ray is probably due to its ability to bind to large protein molecules of hard or soft tissue in the presence of a metallic cation (Norton et al., 1968). The initial reports of Milch et al. (1957, 1958) on the phenomenon of *in vivo* fixation of tetracycline antibiotics was followed with work by Weber and Ridgeway (1962) and Kobayashi et al. (1964) on several teleost species, and by Holden and Vince (1973) on *R. clavata*. These more recent reports indicated tetracycline had potential as a marking technique for growth studies in fish. As noted by others (Weber and Ridgeway, 1962; Frost, 1969) and in this study, tetracycline possesses some advantages, such as simplicity, effectiveness, and economy, over other marking techniques.

Bat rays injected with oxytetracycline exhibited a similar pattern of fluorescence as was reported by Holden and Vince (1973) in *R. clavata* injected with tetracycline hydrochloride. In both species the antibiotic was deposited in all calcified tissue present at the time of injection. Neither species exhibited the sharply defined zone marking the time of injection which has been reported to occur in teleosts.
(Weber and Ridgeway, 1962; Kobayashi et al., 1964). Teleosts absorb tetracycline only at growing surfaces of bone, whereas elasmobranchs absorb the drug throughout the calcified cartilage present at the time of injection. The reason a different depositional pattern occurs in the elasmobranchs compared to the teleosts may lie in the differences inherent in each group's skeletal and metabolic systems as described by Simkiss (1974).

The similarities in tetracycline deposition in the centra of _R. clavata_ and _M. californica_ are more striking than the differences, which were actually quite small. The major difference between the depositional patterns of tetracycline in _M. californica_ and _R. clavata_ was that in the centra of _R. clavata_ there was an unmarked zone peripheral to the tetracycline-labeled inner portion of the centrum. This is obviously because enough time elapsed after injection and prior to recapture to allow the formation of unmarked rings in _R. clavata_. Apparently in the bat rays injected and held in captivity no growth occurred. Therefore it is not surprising that unmarked bands failed to appear peripheral to marked areas. The presence of fluorescence in the broad bands of injected bat rays indicates that part of the difference between broad and narrow bands in the centra is due to greater calcification of broad bands. It follows that the occurrence of fluorescence in the pattern of alternating rings which comprise the bands is also the result of differential mineral deposition in the rings. Holden and Vince (1973) suggested that tetracycline deposition in opaque bands implies greater calcification than elsewhere in the
centra. The same conclusion can be drawn based on examination of the x-rays of bat ray centra.

Reproduction

Males. All three indicators of reproductive maturity employed in this study (presence of mature sperm, the clasper disc width relationship, and internal morphology) indicate that onset of sexual maturity occurs at about two to three years of age, at a disc width of 622 mm, and at a weight of approximately 3.7 kg (8.2 lbs), which is 62% of the asymptotic DW. The size at which 100% maturity is attained (680 mm) is approximately 68% of the asymptotic DW. This is comparable to Herald's (1972) suggestion that male bat rays mature at 10 lbs (4.5 kg). This is also similar to Urolophus halleri in which 100% maturity is attained at 59% of the asymptotic disc width (Babel, 1967). Smith (1980) reports the R. bonasis males begin to mature at approximately four years of age and that 100% maturity is attained at 84% of DW<sub>∞</sub>. Similarly, Dasyatis centroura attain 100% maturity at about 86% of DW<sub>∞</sub> (Struhsaker, 1969). In male elasmobranchs changes in relative size and hardness and development of the claspers is the most frequent method for determining sexual maturity (Pratt, 1979). A species usually exhibits either an abrupt or a gradual transition in clasper body size relation at maturity (Pratt, 1979). Bat ray males resemble several batoid species as discussed by Babel (1967), Struhsaker (1969), and Smith (1980), in that they exhibit an abrupt transition in clasper-DW relationship upon sexual maturity. Presumably this abrupt change reflects the increased growth of two to six additional cartilaginous
elements which develop at the distal end of the clasper (myxopterygium) (Bigelow and Schroeder, 1953).

**Females.** Age and size at sexual maturity of female bat rays as determined in this study are in keeping with Holden's (1974) suggestion that the mean length at maturity for female elasmobranchs approximates 60% to 90% of the asymptotic length and that 50% maturity occurs at 5 to 6 years of age for female bat rays. My data established that 50% maturity of female bat rays is attained at approximately 5 years of age at a disc width of 881 mm, which represents 56% of the asymptotic disc width. This is comparable to *Manta birostris* and *Mobula hypostoma* which attain 50% maturity at approximately 66% and 88% of their respective asymptotic disc widths (Holden, 1974). The bat ray reaches 100% maturity at 63% of the asymptotic disc width compared to *U. hallieri* which reaches 100% maturity at 47% DW (Babel, 1967), *Dasyatis centroura* at 80% (Struhsaker, 1969), and *R. bonasus* at 81% (Smith, 1980).

It may be speculated that the discrepancy between the sizes at maturity given in the literature and those determined in this study have resulted from either a difference in criteria upon which designation of "maturity" was founded, or that size at maturity may have truly changed between 1951 and 1980. Based on examination of female bat rays caught in shark derbies between 1951 and 1959, Herald et al. (1960) gives 52 lbs (23.6 kg) as size at sexual maturity. The disc width corresponding to this weight, calculated according to the weight-disc width relation $W = 4.7 \times 10^{-9}DW^{3.199}$, is 1077 mm. Information collected during this study indicates the Herald et al. (1960) may have overestimated size at maturity.
Similar to other viviparous rays in which the right ovary and oviduct undergo varying degrees of reduction or loss (Wourms, 1977), the bat ray has a nearly non-functional right ovary accompanying fully functioning right and left uteri. The extreme condition in which both right ovary and uterus are non-functional has been reported to occur in _Pteryomylarus bovina_, _Aptomylarus niewhofii_ (Babel, 1967), _Dasyatis centroura_ (Struhsaker, 1969), _Urolophus paucimaculatus_ (Drummond, 1978) and _R. bonasus_ (Smith, 1980). Babel (1967) characterized _U. halleri_ as having a non-functional right ovary and a right uterus which functioned at a slightly reduced capacity. Though not detected in this study the possibility exists that a similar functional asymmetry may occur in the uteri of bat rays.

Female bat rays produce ova in discrete size classes, or generations, similar to the condition described by Babel (1967) for _U. halleri_ and by Pratt (1979) for _Prionace glauca_. However, whereas _U. halleri_ and _P. glauca_ produce two to three generations of eggs, bat rays carried four to five size classes of ova. An increased number and size of eggs with increased size which was noted for bat rays has also been reported for several other elasmobranch species (Bable, 1967, Ketchen, 1972, 1975; Holden, 1974; and Pratt, 1979). Since the number of embryos is ultimately limited by space, which is in turn determined by the size of the female, the number of eggs ovulated should also have some upper limit. To exceed this limit would be a waste of reproductive energy. Though number of eggs might be limited, a continual increase in size of the eggs would facilitate nourishment to embryos. It is suggested that maximum efficiency of reproductive energy is achieved by limiting the
number of eggs produced, while simultaneously allowing eggs size to increase.

Reproductive Cycle

Wourms (1977) has postulated the existence of three basic types of reproductive cycles: 1) reproduction throughout the year; 2) partially defined annual cycle with one or two peaks; and 3) a well-defined annual cycle or biennial cycle. Among the batoids *U. halleri*, *D. centroura*, *U. paucispinous*, *R. javanica*, and *R. bonasus* exhibit well defined annual breeding cycles (Babel, 1967; Struhsaker, 1969; Drummond, 1978; James, 1962, 1970; Smith, 1980). Several pieces of information support the idea that the bat ray is a summer breeder with a well defined annual reproductive cycle. For example, there is a distinct peak in density of rays in sloughs and estuaries during the summer months, at which time reproductive females comprise the major proportion of the population (MacGinitie, 1935, Herald et al., 1960; Ridge, 1963; Talent, 1973). Full-term fetuses and neonates have not been reported to occur other than during summer months. Also, the pattern of seasonal development of ova implies annual ovulation. And finally, during this study ovulation was observed only in summer-caught females.

Gestation

The same factors which indicate that the reproductive cycle of the bat ray is of an annual nature might also be used as evidence that a gestation period of approximately 9 to 12 months is most likely for this species. These factors include the fact that the only known occurrences of ovulating females are during mid-summer, full-term fetuses and neonates are also known to occur only during the summer months, and rays
occur seasonally in sloughs and estuaries. In opposition to Holden's (1974) statement that gestation in rays lasts one year, Wourms (1977) has suggested that gestation in aplacental viviparous rays is short relative to other elasmobranchs, being on the order of two to four months. It appears that a short gestation period does exist in several species of rays, such as *P. bovina*, *U. halleri*, and *D. violacea* (Babel, 1967; Wourms, 1977). The following gestation periods have been found or suggested: for *P. bovina*—four months (Babel, 1967); *D. violacea*—two months (Ranzi, 1934 in Wourms, 1977); *D. say*—five to six months (Hess, 1959 in Smith, 1980); *Gymnura altavela*—five to six months (Daiber and Booth, 1960); *G. micrura*—"several months" (Daiber and Booth, 1960); *U. halleri*—three months (Babel, 1967); and *U. paucimaculatus*—about seven months (Drummond, 1978). Smith (1980) proposed that two possible schedules were likely for *R. bonasus*, either 9 to 11 months or 5 to 6 months. He concluded that a 5 to 6 month gestation period was most likely. On the other hand, at least one batoid, *D. centroura*, has a gestation period of 9 to 11 months (Struhsaker, 1969). Examination of mature specimens from the winter months and additional information on wintering habits will be necessary before the speculation that a gestation time of 9 to 12 months can be verified for the bat ray.

**Future Fishery - Recommendations**

*Myliobatis californica* appear to possess little, if any, capacity to increase fecundity unless a change in age at maturity is possible. Holden (1972) discussed the possibilities of sustainable fisheries for sharks, skates, and rays, and concluded that the relationship between stock and recruitment in the unexploited phase is nearly linear for all
elasmobranchs. In an analysis of the fishery on *Squalus acanthias*, Holden (1968) showed that the female portions of the dogfish population required protection in order for recruitment to be unaffected. If a sustained fishery for bat rays were to develop, guidelines might have to be established to prevent over-exploitation. This would require information on the size of the bat ray population. It is suggested that if regulation becomes necessary, fishing in sloughs and estuaries for bat rays be postponed until after mid-July. This would allow for parturition in early summer months, thus insuring at least partial recruitment. Also if size limits are to be established, they should be set at minimum disc widths of 900 mm for females and 500 mm for males. This would provide non-reproductive individuals an opportunity to mature and reproduce, and thus contribute to the population.


TABLE 1
Mean disc width for each age class as determined by back-calculated disc widths, oil clearing ageing technique and x-ray ageing technique for male *Myliobatis californica*.

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>t:</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>16</td>
<td></td>
<td>267</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td></td>
<td>268</td>
<td>367</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td></td>
<td>298</td>
<td>398</td>
<td>468</td>
<td></td>
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<tr>
<td>3</td>
<td>7</td>
<td></td>
<td>274</td>
<td>367</td>
<td>464</td>
<td>554</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td></td>
<td>268</td>
<td>368</td>
<td>455</td>
<td>595</td>
<td>672</td>
<td>740</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td></td>
<td>260</td>
<td>361</td>
<td>454</td>
<td>525</td>
<td>607</td>
<td>740</td>
<td></td>
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<tr>
<td>6</td>
<td>5</td>
<td></td>
<td>245</td>
<td>333</td>
<td>440</td>
<td>516</td>
<td>609</td>
<td>690</td>
<td>766</td>
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</table>

Weighted DW

<p>| | | | | | | | | | |</p>
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<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Back-calculated Disc Width (mm at successive bands)</td>
<td>277</td>
<td>364</td>
<td>456</td>
<td>553</td>
<td>634</td>
<td>712</td>
<td>766</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Weighted DW; 2 successive age classes

<p>| | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300</td>
<td>377</td>
<td>465</td>
<td>574</td>
<td>646</td>
<td>712</td>
<td>766</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DW oil clearing

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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>± 95% confidence limits</td>
<td>28</td>
<td>40</td>
<td>184</td>
<td>65</td>
<td>70</td>
<td>142</td>
<td>58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DW x-rays

<p>| | | | | | | | | | |</p>
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>± 95% confidence limits</td>
<td>27</td>
<td>30</td>
<td>17</td>
<td>28</td>
<td>68</td>
<td>--</td>
<td>57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*Significant difference in DW between the two ageing techniques, p < .05.*)
TABLE 2.
Mean disc width for each age class as determined by back-calculated disc widths, oil clearing technique and x-ray ageing technique, for female Myliobatis californica

| Age | N  | t:  | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|-----|----|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 0   | 11 | 280 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 1   | 12 | 268 | 300 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 2   | 14 | 289 | 388 | 464 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 3   | 14 | 285 | 387 | 489 | 596 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 4   | 16 | 271 | 384 | 500 | 608 | 653 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 5   | 15 | 280 | 393 | 503 | 622 | 757 | 863 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 6   | 15 | 262 | 358 | 462 | 566 | 688 | 795 | 879 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 7   | 14 | 275 | 378 | 479 | 559 | 658 | 765 | 863 | 954 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 8   | 16 | 255 | 351 | 434 | 530 | 617 | 717 | 819 | 916 | 1006 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 9   | 17 | 252 | 325 | 391 | 484 | 604 | 670 | 730 | 819 | 916 | 1006 |    |    |    |    |    |    |    |    |    |    |    |    |
| 10  | 16 | 308 | 373 | 436 | 476 | 569 | 648 | 789 | 858 | 946 | 1044 | 1147 |    |    |    |    |    |    |    |    |    |    |    |
| 11  | 15 | 297 | 387 | 456 | 537 | 627 | 727 | 829 | 879 | 955 | 1037 | 1079 | 1168 |    |    |    |    |    |    |    |    |    |
| 12  | 14 | 287 | 379 | 463 | 557 | 628 | 723 | 802 | 881 | 951 | 1025 | 1081 | 1127 | 1175 |    |    |    |    |    |    |    |
| 13  | 11 | 287 | 360 | 431 | 538 | 622 | 713 | 805 | 883 | 954 | 1025 | 1076 | 1128 | 1179 | 1218 |    |    |    |    |    |    |
| 14  | 11 | 279 | 364 | 441 | 543 | 612 | 747 | 799 | 833 | 884 | 952 | 1004 | 1054 | 1122 | 1174 | 1242 |    |    |    |    |
| 16  | 12 | 294 | 382 | 463 | 512 | 594 | 689 | 756 | 808 | 866 | 923 | 1001 | 1042 | 1081 | 1131 | 1161 | 1229 | 1277 |    |    |    |
| 23  | 1  | 269 | 350 | 405 | 507 | 575 | 660 | 745 | 796 | 847 | 915 | 949 | 1000 | 1051 | 1085 | 1120 | 1170 | 1220 | 1272 | 1306 | 1343 | 1391 | 1425 | 1459 | 1490 | 1510 |

Weighted DW

| Age | N  | t:  | 275 | 329 | 459 | 550 | 639 | 726 | 808 | 881 | 954 | 1012 | 1044 | 1098 | 1130 | 1148 | 1171 | 1209 | 1258 | 1272 | 1306 | 1343 | 1391 | 1425 | 1459 | 1510 |
|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

Weighted DW; 2 successive age classes

| Age | N  | t:  | 274 | 381 | 476 | 600 | 696 | 838 | 869 | 897 | 974 | 1034 | 1060 | 1148 | 1176 | 1196 | 1188 | 1209 | 1258 | 1272 | 1306 | 1343 | 1391 | 1425 | 1459 | 1510 |

DW - oil clearing

| Age | N  | t:  | 314 | 424 | 467 | 605 | 716 | 880 | 894 | 997 | 1025 | 1034 | 1147 | 1168 | 1143 | 1229 | 1242 | --  | 1260 | 1300 | --  | --  | --  | --  | --  | --  | 1510 |

± 95% conf. limits

| Age | N  | t:  | 23  | 18  | 28  | 32  | 80  | 46  | 109 | 50  | 39  | 37  | 56  | 5   | 46  | 22  | --  | --  | --  | --  | --  | --  | --  | --  | --  | 1510 |

DW x-rays

| Age | N  | t:  | 289 | 405 | 455 | 602 | 664 | 863 | 908 | 976 | 980 | 1029 | 1043 | 1115 | 1150 | 1199 | 1240 | 1262 | 1300 | --  | --  | --  | --  | --  | --  | --  | 1510 |

± 95% conf. limits

| Age | N  | t:  | 18  | 25  | 21  | 46  | 72  | 57  | 71  | 17  | --  | 24  | 49  | 29  | 102 | 22  | --  | --  | --  | --  | --  | --  | --  | --  | --  | 1510 |
Table 3. Discrepancies between assigned ages as determined by oil clearing and x-ray ageing techniques.

<table>
<thead>
<tr>
<th>Deviation (yearly increments) of x-ray band counts from oil band counts.</th>
<th>0</th>
<th>+1</th>
<th>-1</th>
<th>+2</th>
<th>-2</th>
<th>+3</th>
<th>-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of centra observed showing that increment of discrepancy.</td>
<td>87</td>
<td>23</td>
<td>11</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Percent of the total bat rays aged (n = 132).</td>
<td>65.9</td>
<td>17.4</td>
<td>8.3</td>
<td>3.0</td>
<td>3.0</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>Cumulative percent of the total bat rays aged.</td>
<td>65.9</td>
<td>82.8</td>
<td>91.1</td>
<td>94.1</td>
<td>97.1</td>
<td>100</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 4. Percent agreement = number of *Myliobatis californica* assigned the same age by both techniques/number of *M. californica* assigned that age by the oil clearing ageing technique. N = number of individuals aged using the oil clearing ageing technique.

<table>
<thead>
<tr>
<th>Year Class</th>
<th>Percent Agreement</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>92</td>
<td>25</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>93</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
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<tr>
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<tr>
<td>6</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>34</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 1. Four major collecting sites of *Myliobatis californica* from Elkhorn Slough. X: collection site.
Figure 2. Measurements recorded for *Myliobatis californica*. S.A. = snout to anus; H.L. = head length; A.M.P = anterior margin pectoral; D.W. = disc width; S.E. = snout to eye; B.L. = body length; T.L. = total length; S.D. = snout to dorsal; C.L. = clasper length; T = tagging location; X = site of oxytetracycline injection.
Figure 3. a) Sectioning orientation. b) Measurements of centra used in ageing *M. californica*. C.D. = centrum diameter. B.D. = band diameter.
a.)

b.) "rings"

"bands"
Figure 4. The centrum diameters (CD) and disc width (DW) relation for male and female Myliobatis californica. Functional mean regression equation: $DW = 105.898(\text{CD}) + 65.887$, $n = 1.62$, $r = 97.9$. 
Figure 5. The mean back calculated disc width at age for male *M. californica* (n = 50). The heavy solid line connects the mean disc width at age based on two most recent age classes. Vertical lines connect mean size at earlier ages of each age class (lower axis). Horizontal dashed lines connect mean sizes of all age classes within one back calculated age class (right axis).
Figure 6. The mean back calculated disc width at age for female *M. californica* (n=104). The heavy solid line connects mean disc widths at age based on the most recent age classes. Vertical lines connect mean size at earlier ages of each age class (lower axis). Horizontal dashed lines connect mean sizes of all age classes within one back calculated age class (right axis).
Figure 7. Brody-Bertalanffy growth curves for male *Myliobatis californica*. Oil clearing: $DW_\infty = 1991; k = 0.0596; t_0 = -2.879$. Mean back calculated disc width at two most recent consecutive ages: $DW_\infty = 1517; k = 0.0834; t_0 = -2.55$. X-ray ageing: $DW_\infty = 1004; k = 0.2294; t_0 = -1.580$. 
Brody-Bertalanffy Parameters

<table>
<thead>
<tr>
<th>ageing technique</th>
<th>D W₀</th>
<th>K</th>
<th>$t_0$</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>oil clearing (-)</td>
<td>1991</td>
<td>0.059</td>
<td>-2.880</td>
<td>60</td>
</tr>
<tr>
<td>x-radiography (·)</td>
<td>1004</td>
<td>0.229</td>
<td>-1.580</td>
<td>60</td>
</tr>
<tr>
<td>mean back calc. DW.</td>
<td>1517</td>
<td>0.0834</td>
<td>-2.55</td>
<td>60</td>
</tr>
</tbody>
</table>

DISC WIDTH (mm)

size at birth
274-315mm
DW

100% maturity at 622mm
DW

AGE (number of bands)
Figure 8. Brody-Bertalanffy growth curves for female *Myliobatis californica*. Lines above based on:

A: oil clearing ageing technique: $D_{\infty} = 1587; k = 0.0995; t_0 = -2.059$.

B: mean back calculated DW, at two most recent ages: $D_{\infty} = 1567; k = 0.096; t_0 = -2.040$.

C: x-ray ageing technique: $D_{\infty} = 1566; k = 0.099; t_0 = -1.935$
Brody-Bertalanffy Parameters

<table>
<thead>
<tr>
<th>ageing technique</th>
<th>DW&lt;sub&gt;∞&lt;/sub&gt;</th>
<th>K</th>
<th>t&lt;sub&gt;0&lt;/sub&gt;</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>oil clearing (-)</td>
<td>1587</td>
<td>0.0995</td>
<td>-2.059</td>
<td>104</td>
</tr>
<tr>
<td>x-radiography (·)</td>
<td>1566</td>
<td>0.0990</td>
<td>-1.935</td>
<td>93</td>
</tr>
<tr>
<td>mean back calc. D.W.</td>
<td>1567</td>
<td>0.096</td>
<td>-2.040</td>
<td>104</td>
</tr>
</tbody>
</table>

size at birth
274-315mm DW

50% maturity
at 881mm DW

AGE (number of bands)
Figure 9. Mean disc width at age for male *Myliobatis californica*.
Horizontal line = mean disc width
Vertical line = 95% confidence interval
Dashed line = oil clearing technique
Solid line = X-ray technique
Figure 10. Mean disc width at age for female *Myliobatis californica*.
Horizontal line = mean disc width (mm)
Vertical line = 95% confidence interval
Dashed line = oil clearing technique
Solid line = X-ray technique
Figure 11. Disc width and clasper length relationship for *Mliobatis californica*.
- single data point
- two data points
- three data points
Figure 14.  a) Mean number of ova collected from mature Myliobatis californica June 1980, n = 28. Vertical bar = ±1 S.E.
b) Mean diameter of ova collected from mature Myliobatis californica June 1980, n = 28.
Figure 13. Change in size composition of ova in mature females with season for 39 Myliobatis californica. Winter: Dec., Jan., Feb., n = 4; Spring: Mar., Apr., May, n = 2; Summer: June, July, Aug., n = 33.
MEAN OVUM DIAMETER (mm)

WINTER  SPRING  SUMMER

PERCENT OF TOTAL OVA PER SEASON

60 50 40 30 20 10 0

39.5 39.5

9.6 29.0 3.0

58.4
Figure 14. Change in oviduct length with age in *Myliobatis californica*, collected June 1980 (n = 42).
Figure 15. Change in uterus width with age in *Myliobatis californica*, collected June 1980 (n = 41).
Figure 16. Change in disc width of *Myliobatis californica* embryos and young of the year, by month.
- = embryo
■ = young of year
⊙ = 1 year of age
\{mean\} range
Figure 17. Size frequency for male and female *Myliobatis californica* (250-490 mm DW) from eight Elkhorn Slough Shark Derbies held during 1971, 1972, 1973, and 1980 and mean disc widths ± 95% confidence limits as determined by oil clearing technique (thin line) and x-ray ageing technique (heavy line); for females 0, 1, and 2 year age class.
APPENDIX A

Analysis of Covariance, critical and calculated values for linear morphometric relations of Myliobatis californica. $W =$ weight (kg); $DW =$ disc width (mm); $TL =$ total length (mm); $AMP =$ anterior margin pectoral (mm); $SA =$ snout to anus (mm). $HL =$ head length (mm); $SE =$ snout to eye (mm); $CD =$ centra diameter (mm); $a =$ intercept; $b =$ slope.

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<th>Female</th>
<th>Pooled</th>
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<th>Female</th>
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Pooled
Slope: $df_{num} = 3; f_{α} = .05$. Intercept = $df_{num} = 2; F_{α} = .05 = 3.0$.

Pairwise
Slope: $df_{num} = 1; f_{α} = 3.84$. Intercept = $df_{num} = 00; F_{α} = .05 = 1.96$.  

APPENDIX B

Functional Mean Regression Equation Parameters Calculated For Morphometric Relations of *Myllobatis californica*

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