AGE AND GROWTH OF THE MARKET SQUID, *LOLIGO OPALESCENS* BERRY, FROM STATOLITHS

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ABSTRACT

Growth increments have been found in statoliths. Growth increments correlate best with daily growth in juveniles and monthly growth in animals over six months of age. Monthly length at age reveals that market squid spawn at 1 to 2 years of age and most spawn when 14 to 22 lunar months of age at about 100-145 mm dorsal mantle length.

INTRODUCTION

The California squid fishery is one of the state's oldest fisheries, dating back to the 1860's when Chinese began fishing in southern Monterey Bay. The fishery has evolved over the years into an industry with annual landings averaging 22 million pounds over the period 1966-75. Today, the market squid resource of California is still considered underutilized, and considerable expansion of the fishery is likely in order to meet the world's growing protein demands.

There is relatively little known about the life history or population dynamics of the market squid. The California Department of Fish and Game with Moss Landing Marine Laboratories have undertaken a Sea Grant Project to answer some of these questions, including growth rates.

Age and growth studies on squid, as a group, are very difficult. Few species have been taken in numbers large enough to allow any age and growth analysis. *Loligo opalescens* (Fields 1965), *Illex illecebrosis* (Squires 1966), and *Loligo pealei* (Summers 1971) have been aged by analyzing modal length frequencies collected through time. All of those researchers estimated age at maturity to be two or three years. In this type of an analysis, several assumptions must be made that cast some degree of doubt on the validity of results obtained. Absolute age is not known because the age of the smallest specimens collected must be estimated. Random sampling is difficult, and sampling probably introduces bias. And finally, movements or migrations of size classes make it extremely difficult to sample the same group or size class through its life span.

Tag and recovery studies on *Todarodes pacificus* (Otsuki and Araya 1958) show that this species reaches maturity in one year, spawns, and dies. Tag and recovery studies could be conducted on *Loligo opalescens*, but it would require a method of holding specimens while experimenting with methods of tagging. The technology for a study of this nature is not available at present.

La Roe (1971) successfully cultured the loliginid squid, *Sepioteuthis sepioidea*. This species grew to 105-mm mantle length in 146 days and was sexually mature. The technology to maintain juvenile and adult squid in long-term captivity studies is not available. If squid could be raised for long-term studies, the effects of an artificial environment on growth could not be defined and comparison with wild animals would be difficult.

Either the validity of the preceding methods is difficult to define, or the methods themselves are not applicable to *Loligo opalescens*. The purpose of this study is to develop another method of aging by examining the hard body parts of squid for evidence of growth marks that could be correlated with time. The body parts considered are the gladius, beaks, and statoliths. The gladius exhibits markings that could represent growth increments. A sagittal section of the gladius reveals that growth occurs in layers which could be correlated with time. Beaks of *Moroteuthis ingens* (Clarke 1965) were found to have growth lines, but the time it took to form growth rings could not be determined. I sectioned beaks of *Loligo opalescens* and found that they were formed in layers much as the gladius was.

A few statoliths were given to Dr. Edward Brothers (National Marine Fisheries Service, La Jolla), who found that statoliths of *Loligo opalescens* had many concentric growth rings much like otoliths.

It is my opinion that the gladius, beaks, and statoliths all are suitable for age and growth studies. However, I chose the statolith because of its similarity to the otolith.

This paper is concerned with a new method of aging squid, the techniques involved, and the validity of the results.

STUDY AREA

All the specimens of market squid used in this study were collected in Monterey Bay, California (Figure 1). Squid are available in this area year round; however, sampling is difficult in the winter months.

METHODS AND MATERIALS

Sampling

The initial phase of this study was concerned with examining modal length frequency progressions in order

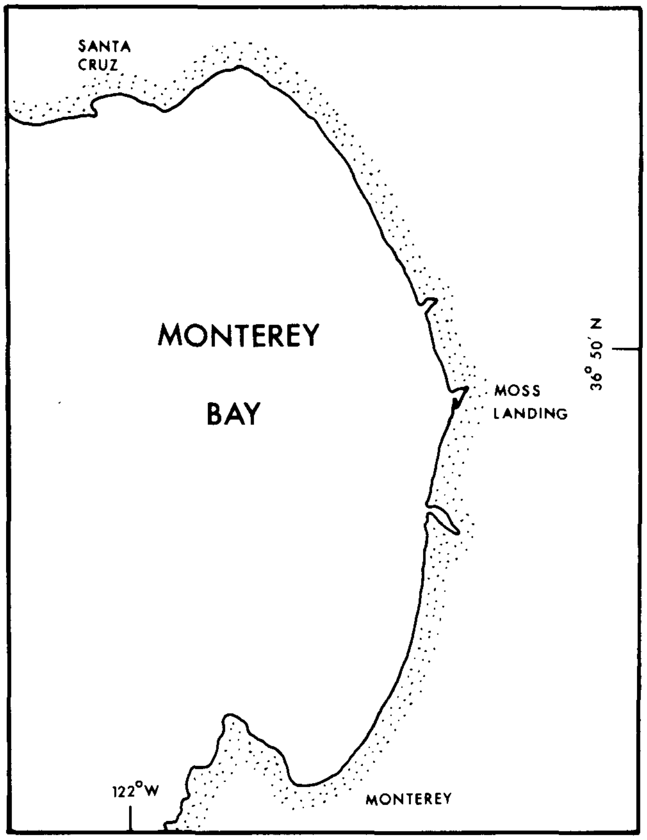

Figure 1. Study area.

to estimate growth. Length was recorded as dorsal mantle length (DML) in mm. The commercial catch was sampled for adult animals. The commercial squid fishery takes spawning adults with lampara nets and does not provide samples of immature animals.

Larval and juvenile squid were sampled as the opportunity presented itself. Most collections were by midwater trawl from research vessels. Juvenile and adult squid are also present with purse seine-caught anchovies, and their occurrence there provided an additional source of squid during the fall and winter months.

Larval squid are present nearly year round from May to at least January, but peak hatching occurs June through August. Larvae were taken during this period, and young juveniles sampled from the winter anchovy catch were considered to be from the previous summer spawning period. By the following summer the earliest-hatched squid from the previous summer were large enough to appear with spawning schools and were taken in small amounts in the squid fishery.

Sampling of juveniles may be biased since a certain size could school with anchovies. However, sampling apparently reveals growth. The validity of the growth rate will be determined from growth increments in statoliths. Statoliths were taken from squid during normal sampling operations. Collecting statoliths was biased in the sense that statoliths were taken from all sizes of squid available.

Statolith Extraction and Preparation

Statolith Extraction

A dissecting microscope with magnification up to 40-50x is necessary to dissect larval-sized squid (<10 mm total length). Larger sizes required progressively less magnification, and adults over 100 mm DML do not require a microscope for dissection.

Statoliths are located just posterior and ventral to the eyes and are removed in the following manner. Begin with the ventral side up and remove the funnel apparatus. In large squid it may be ncessary to split the mantle in order to remove the funnel. In small squid (<75 mm DML) the two statoliths will be visible, appearing as white opaque objects lying side by side under a thin layer of transparent tissue and cartilage. In larger squid it is necessary to scrape the muscle tissue away with a scalpel until the ventral side of the cranial cartilage is exposed. The two visible statoliths can be removed with forceps after cutting the statocyst in half. The statoliths are not fragile but can be broken if handled roughly.

Cleaning and Storing

Generally statoliths require no cleaning, but any adhering tissue should be removed. They can be stored indefinitely in labeled gelatin capsules.

Embedding

Statoliths must be ground in order to see growth rings, and due to their small size, handling is much easier if they are embedded. A Fullum mold (Figure 2), which is reusable and has space for 24 statoliths, is used. The statolith must be placed on its side in the bottom of the mold so that it will be parallel with the plane of grinding. Any clear, fast-drying resin may be used and is poured over the statolith, filling the mold. The hardened resin block is a permanent mount and can be stored in labeled coin envelopes.

Grinding

The embedded statoliths should be just under and parallel to the surface of the resin block. Grinding is done on carborundum paper (200-600 grit) and must be done very slowly with frequent stops to check progress under a microscope. Polishing with aluminum oxide or diamond plate will help eliminate scratches in the statolith but is not necessary to view growth rings.

Viewing

A compound microscope with substage illumination was used to check the statolith during the grinding procedure and for viewing growth rings. Magnification of 400-600X is adequate for counting growth rings. Higher magnification up to 1500X does not reveal any more detail.

Reading Criteria

Growth Increments

When viewed under transmitted light, a growth increment is defined as the interface between an inner light and outer dark band. The market squid statoliths do not reveal annual growth increments. Growth increments change as the squid becomes older and can be grouped into two distinctive patterns.

Juvenile market squid form uniform, regularly spaced growth increments (Figure 3). Up to about 150 of these small, regularly spaced growth increments can be seen in juvenile market squid. These are considered to be near-daily growth increments. At about six months of age, growth increments change and become irregular in size and spacing, with large, prominent growth increments separated by usually five or six smaller ones (Figure 4). The larger growth increments are considered to form at monthly intervals. The validity of daily and monthly growth increments will be discussed later.

Counting Growth Increments

Two types of growth rings are visible in statoliths. An ocular micrometer is useful in counting the uniformly spaced rings in juvenile squid (Figure 3). At 450X with a 10-mm ocular micrometer, the growth ring widths nearly equal the graduations on the ocular micrometer. These first uniform rings are best seen from the nucleus to the posterior margin of the statolith (Figure 5). The larger rings formed later in life can be counted without any special techniques. On adult squid it is difficult to see the first uniformly spaced rings, and counting begins with the first larger ring formed, which is believed to be formed at about six months of age. The growth rings formed later in life are best seen on the rostrum (Figure 5).

RESULTS

Sampling of commercial squid landings, anchovy catches, and midwater trawling from 1972-1975 provided length-frequency data that indicated the market squid lives a maximum of two years (Figure 6). The spring brood grows rapidly during the summer months and is ready to spawn the following summer. The fall brood grows slowly until the next summer, when growth accelerates, and will spawn the next fall or spring.

The spawning season extends from April to December in Monterey Bay, but peaks in May-June and November-December. The long spawning season clouds length-frequency data because squid of all sizes are present at all times of the year and it is difficult to follow each brood as it matures.

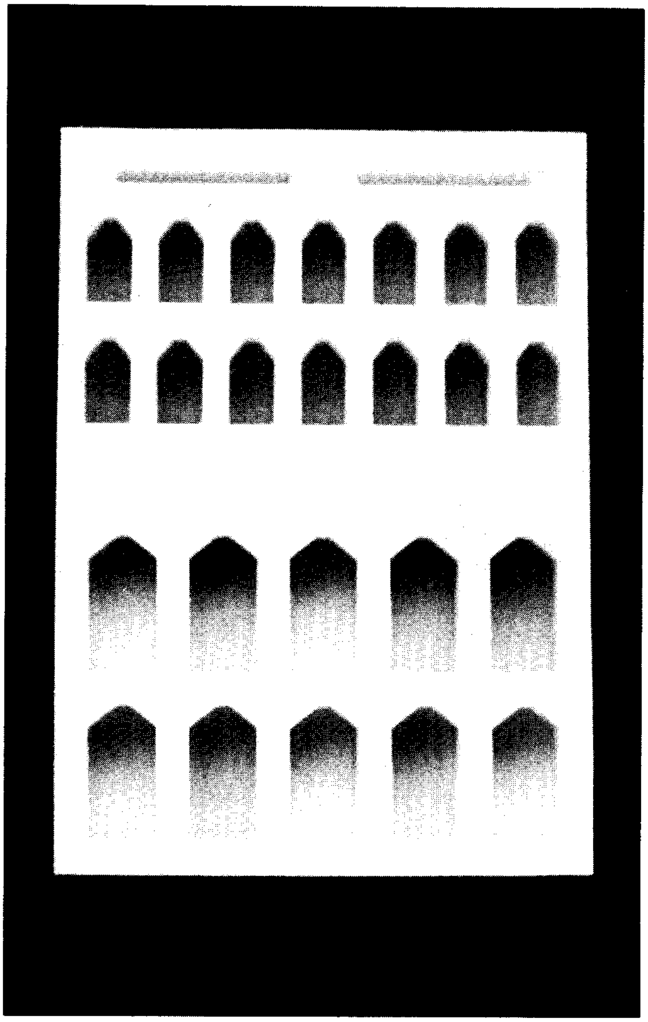

Figure 2. Fullum mold with space for 24 statoliths.

Fields (1965) analyzed modal length frequencies of market squid from Monterey Bay and concluded that most market squid spawn at three years of age but that some animals mature at 1, 2, and 4 years of age. His basic hypothesis is that the growth rate is constant and about 4 mm/month. This is unrealistic, since growth should be rapid initially and gradually slow as age progresses. Fields' results are at best hypothetical, and their validity has not been determined.

Fields and I disagree, but growth increments found in statoliths support my results that the market squid spawns at between 1 and 2 years of age.

I have aged 100 squid from statoliths. Age was assigned in months, based on the number of near-daily growth increments (Figure 3) and the number of proposed monthly increments (Figure 4). The maximum age recorded was 25 lunar months or almost 2 years. A growth curve was developed (Figure 6) which shows growth is rapid from spring to summer and slows during the winter. Squid mature at about 100 mm DML or at 14 months of

Figure 3. Small, regularly spaced growth rings formed during juvenile life stages.

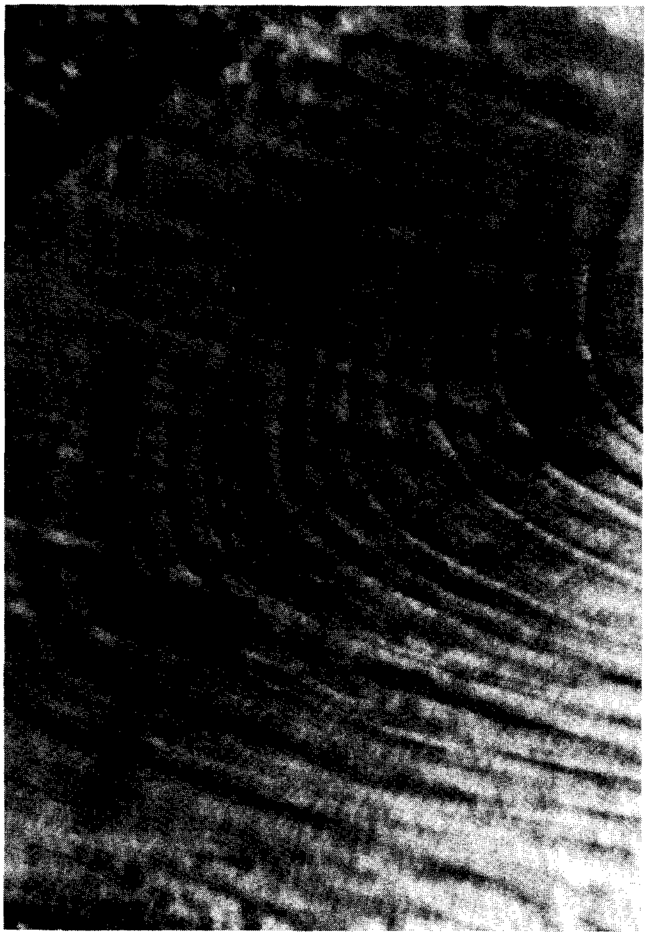

Figure 4. Large, irregularly spaced growth rings formed during adult life stages.

age. During the spawning season the size range for most market squid is from 100 to 145 mm DML when they are 14-22 lunar months old. Squid up to 200 mm DML have lived through two growing periods (summers) before they spawn.

Validity

Conclusive evidence that the majority of market squid spawn at 1 to 1½ years of age is very elusive. However, the validity was tested by two independent methods, and both methods provide a measure of validity to my results.

Rearing

Ann Hurley, at Scripps Institution of Oceanography, attempted to raise market squid and was kind enough to provide me with squid of known age that could be aged from the statoliths. Unfortunately, she was unable to raise squid past 2 months of age. The growth rate of these squid is suspect because they were held in a near-starvation state due to the difficulty in providing proper food organisms. The squid that lived 2 months were only 4-5 DML or about 2 mm larger than when they hatched. I

Figure 5. Ventral view of hypothetical statolith from an adult squid, as it appears under transmitted light. Anterior (A), posterior (P), median (M), and lateral (L) surfaces are labeled. Areas where daily and monthly growth increments are best viewed are indicated.

would expect a squid 2 months old to be 15-20 mm DML. The statoliths from Hurley's squid did have small, uniformly spaced growth increments. Growth increments were possible to count on six specimens, and counts were

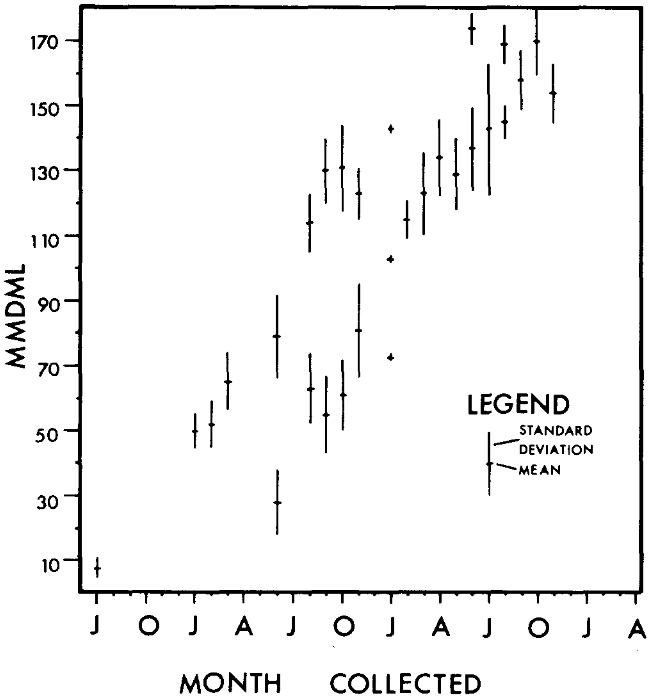

Figure 6. Modal size (mm dorsal mantle length) collected through time from 1972-1975.

TABLE 1
Number Growth Rings in Statoliths from Squid of Known Age.

Specimen	Age in days	Growth rings in statoliths
1	67-70	28
2	67-70	32
3	28-31	30
4	43-44	15
5	54-55	35
6	55-56	38

made without knowledge of age. One squid's age in days agreed exactly with the number of growth increments. However, the remaining five squid had significantly less growth increments than the days of age (Table 1); this could be due to the near-starvation situation. Hurley's study shows that growth increments are formed rapidly and can be formed at a daily rate. It is probable that a wild squid would grow much more rapidly and would form growth increments at a uniform, near-daily rate. This provides a measure of validity for designating the rate of formation of the first 150 growth increments as daily.

Time Series Samples and Estimated Growth Rate

The second method of validation was to compare my lunar-month growth rate (Figure 7) with the length modal

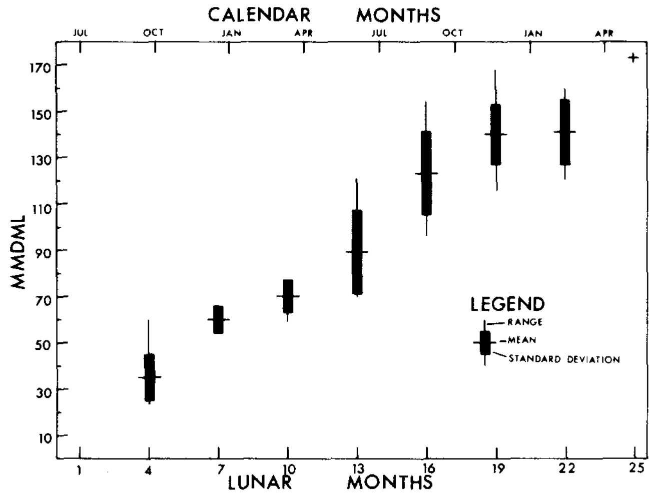

Figure 7. Growth rate of market squid (mm dorsal mantle length) from statoliths.

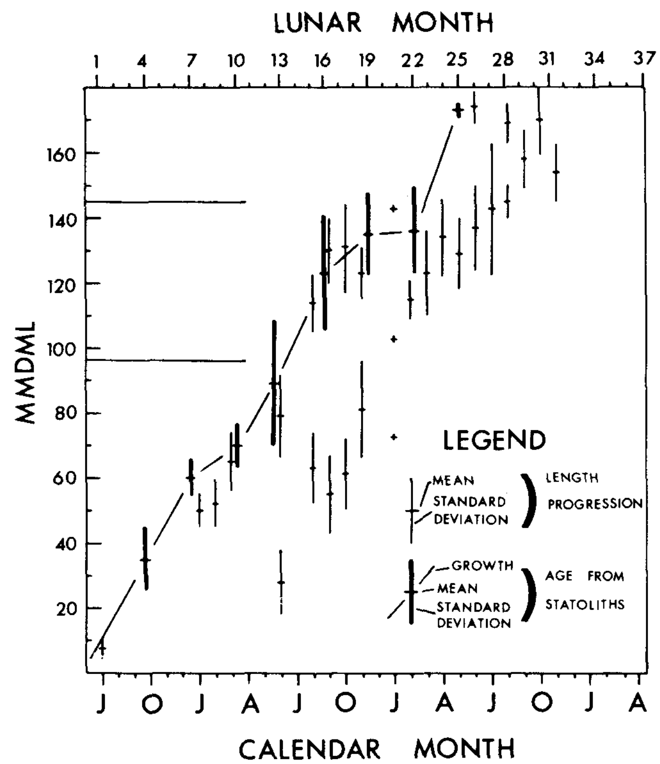

Figure 8. Market squid growth rates from statoliths compared with modal length (mm dorsal mantle length) progressions.

progressions that were collected previously (Figure 6). If length at age is superimposed on length frequencies (Figure 8), the results are very encouraging. The estimated growth rate fits the length modal progressions quite well. My growth rate is for summer-spawned squid, but squid also spawn in the fall. If my growth curve is shifted six months to the right, very little adjustment is needed for it to pass through length modes not accounted for by the growth curve for summer-spawned squid and could represent growth of fall-spawned squid.

The fact that the majority of squid spawn in the spring and early summer could mask a probable different growth rate for fall-spawned squid. Assuming the squid I have aged are all early-summer spawners, the resulting growth rate agrees well with monthly length frequencies and lends another measure of validity to my results.

Growth Increment Formation

Squid are strongly phototaxic and are known to feed during daylight hours. This is the mechanism that causes the formation of daily growth increments. Contrasting growth increments are formed during daily feeding and resting actvities. At about 6 months of age, a change takes place in the type and rate of growth-increment formation. This could represent a transition phase in the squid's life history. Regardless of age, this size of squid is frequently taken mixed with anchovy schools, which have a strong diurnal movement pattern, and indicates that this size of squid is beginning to migrate into the deeper adult habitat. Why the growth-increment formation becomes irregular is not well understood. Prominent large growth increments are formed periodically, which have up to 5 or 6 smaller increments between them (Figur 4). Spawning adults have 6 to 18 of these prominent growth increments. This number of adult growth increments correlates best with lunar periods. A prominent growth increment could be formed during heavy feeding activity associated with full moon. Prey organisms with a diurnal pattern would be more available on moonlight nights. At any rate, the large prominent growth increments are considered to represent monthly growth.

DISCUSSION

Market squid reach a relatively small size (100-200 mm DML) by the time they spawn, and they spawn once and die. This knowledge coupled with the knowledge that other species of squid (*Todarodes pacificus* and *Sepioteuthis sepioidea*) reach adult size in one year or less, implies that market squid could grow at a rapid rate until spawning occurs. The modal length progressions and growth rings on statoliths support the hypothesis that market squid grow rapidly and are capable of reaching adult size in about 14 lunar months.

Upwelling normally begins about April in central California, and the added nutrients in nearshore areas cause plankton blooms during the summer. Plankton are the primary food source of young squid.

Market squid spawned early in the summer (April-May) will grow rapidly during the summer growing season and are capable of reaching adult size in about one year. As spawning continues from June through September, newly hatched squid will have progressively less time available in the growing season. This will have the effect of slowing the growth rate. During the next summer (growing season) this late-summer brood will increase in size and probably spawn from October to December at an age of 14-19 lunar months.

Late-fall-spawned squid (October-December) will grow slowly until late spring, when growth will accelerate. But it is likely that they will be beyond the plankton-feeding stage and will feed on small fishes or newly hatched squid. This will cause them to expend more energy capturing food items and could cause a slower growth rate. Some fall-spawned squid may reach adult size in one year, but I suspect most fall spawners must live through part of a second growing season before they spawn. The majority of squid spawned in October to December would most likely return to spawn in their second summer when about 18 to 22 lunar months old.

Squid have been aged up to nearly 2 years old, and those represent slow-growing squid that have lived through part of their second growing season. It appears that spring and fall spawning activities do not represent separate populations. An individual squid, depending on its growth rate, could return to spawn anytime between 1 and 2 years of age. The fall spawning activity is probably composed of squid that deviate from the normally expected growth rate; that is, they grow fast and spawn at an early age or grow slow and spawn at an older age.

CONCLUSION

Fields (1965) concluded that the market squid spawns at between 1 and 4 years of age and that most spawn at 3 years of age. The validity of Fields' conclusions was never determined.

Daily and monthly growth increments found in statoliths now support my results, which indicate that the market squid is capable of spawning at 1 year of age and that all will spawn during their second year of life.

A life span of 1 year has serious management implications. Spawning biomass can fluctuate dramatically from year to year because good recruitment is necessary each year to maintain the spawning population. A method of estimating recruitment should be developed. If fishing is not controlled during a year when poor recruitment is evident, spawning stocks could be reduced to a dangerous level.

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