#### *Recent Advances in Fishery Biology techniques for Biodiversity Evaluation and Conservation* **CHAPTER**

**14**

# **Cephalopod Growth in the Fisheries Context**

**GEETHA SASIKUMAR AND K.K. SAJIKUMAR** ICAR-Central Marine Fisheries Research Institute

## **Introduction**

Cephalopods comprising of squids, cuttlefish and octopus, have emerged as commercially<br>
Cimportant marine resources of the world. Cephalopod abundance has increased over the last six decades, resulting in quadrupling in landings from 1 to 4.71 million tonnes (2015). Many countries have initiated stock monitoring programs of this resource because of the increasing trends in their annual catches. The age composition and growth rate of fishery stocks are among the most important parameters for studying population biology, stock structure, life span and eventually for monitoring and managing the stocks appropriately.

Maximal sizes in cephalopods species may vary not more than a few grams weight as in *Octopus micropyrsus* to nearly 450 kg as in *Architeuthis* spp. Characterizing growth patterns in cephalopods continues to remain an important aspect in cephalopod biology and ecology even after the first published work by Verrill (1881).

Growth in an organism is the increase in size, dimension or mass. It represents the net outcome of a series of behavioral and physiological processes. The estimation of growth rates requires both measurements of size at various intervals of time (age). Existing body of knowledge concerning cephalopod growth is derived primarily from a combination of laboratory rearing studies and fisheries related field surveys of commercially important species. Estimates of cephalopod growth in the wild use various mathematical models to describe patterns of growth, while laboratory studies describe growth under captivity. Several authors have noted extreme variations in the growth of cephalopods in relation to food availability, water temperature, sex, maturity, season and hatching time. The final size attained by adult males and females may vary within a species; also, within groups of siblings reared under identical conditions, hence size may not be a reliable indicator of age in field-caught cephalopods.

Cephalopod growth is estimated by using indirect and direct methods

# **Indirect methods**:

The indirect method involves the analyses of the length frequency data. Dorsal mantle length of cephalopod samples collected from commercial landings or experimental surveys are measured. In squids and cuttlefish, mantle length (along the dorsal surface from the anterior most point to the posterior most end of the mantle) is the standard measurement. In octopus the, mantle length is measured from the midpoint between the eyes to the posterior end of the mantle along the dorsal surface. These length measurements (frequencies) are subjected to modal progression analyses, whereby the growth of a cohort is followed through most of the life-cycle. Since this method involves the identification and interpretation of the different modes, it is highly subjective and are prone to

high errors, particularly in situation where there is prolonged spawning behavior. Thus, use of indirect methods for age estimation must be regarded with caution and verification of such data using one of the direct methods is necessary.

# **Direct methods**:

The direct estimation of growth rate in cephalopods are by 1) using tag–recapture in the wild 2) direct observation of growth under captive condition 3) examination of growth rings in hardstructures such as the statolith, gladius, beak, sepion and crystalline lens.

#### **3.1 Tag–recapture of cephalopods in wild**

Releasing and recapturing tagged cephalopods is a direct method for arriving at growth rates during the time interval between tagging and recapturing from the wild. Tagging was used since 1920s in cephalopods for investigating vertical and diurnal migrations, besides collecting information on biology, physiology, ecology and stock identity of the investigated populations. Depending on the size range of cephalopods, different types of tags are used, such as Spaghetti tag, Dart tag, Tbar anchor tag, Petersen discs. There are several potential limitations in using these tags, including physical damage to the tagged animal (mantle integrity, swimming ability), tag loss, and the difficulty in using relatively large tag in juveniles. Recapture rates are variable and influenced by level and extent of fishing, tag colour, tag type and tag placement. Reporting rates for external tags can also be highly variable for a wide variety of reasons such as, fisher apathy, resentment towards research and insufficient rewards. The recapture rates of tagged squids are highly variable especially in migratory species like ommastrephids. External tagging experiments on octopus present additional challenges to those for squid and cuttlefish as their dexterity and strength allows them to easily remove external tags.

#### **3.2 Growth under captive conditions**

Captive rearing of cephalopods provides information on life cycle characteristics, including growth, feeding and behaviour. Information on cephalopod growth can be derived by recording changes in length and weight at intermittent intervals. Growth in cephalopods is highly variable, as it is affected by various biotic and abiotic factors. The advantage of captive rearing is that growth and life cycles can be measured and monitored under known conditions; environmental factors can be controlled or monitored. This provides sound information on individual growth rate at different ontogenetic stages.

The major disadvantage of captive rearing is that the laboratory conditions are unnatural or artificial. The impact of predation on behavior and feeding is removed, while population density compared to the natural levels is increased due to crowding. Indeed, most of the available information on octopod growth over their life cycles have come from laboratory rearing. Several problems limit a wide application of this method. Not many cephalopod species have been successfully reared in captivity for the entire life cycle, mostly due to a considerably high mortality rate at the early stages.

In spite of these negative constraints, studies performed on reared animals helped to understand growth performance in several species and were used to validate the time interval necessary for the formation of growth increments in statoliths and gladii.

#### **3.3 Examination of growth increments in hard-structures**

Several studies on cephalopods globally have estimated age based on interpreting periodic growth increments in hard-structures over the lifetime of the animal. The criteria to be fulfilled for successful age estimation of hard-parts include:

- 1. The hard-structures must contain interpretable increment structures that are sufficiently clear to facilitate precise interpretation
- 2. Increments can be correlated with a regular and determinable time scale
- 3. The formation of increments continues at a measurable rate throughout life; and
- 4. The increments are permanent and not resorbed during remobilization of hard tissue.

There are a number of hard-structures in cephalopods which grow throughout, and may be considered as archives of their life cycle. The statoliths, gladii, beaks, stylets and eye lenses are found to bear periodic growth increments within their microstructures and are used for growth estimations.

# **3.3.1 Statoliths**

The statoliths are paired calcareous concentrations within the statocyst of the cephalopod cranium. In cephalopods, statocysts detects gravity, angular accelerations and low-frequency sound. The teuthoid and sepioid statoliths are composed of calcium carbonate in aragonite form, intergrown with thin matrix of organic compounds, which consist mainly of high-molecular proteins.

Statoliths grow continuously throughout their life and are capable of recording life history events useful for stock assessment. In most cephalopods they form concentric rings visible under a light microscope, originating from a periodically changing amounts of organic material incorporated into the aragonite crystal. The better increment visibility is observed with increased incorporation of organic material in statoliths. The shape of the statolith varies markedly between species.

The statolith preparation and processing consist of extraction, cleaning and preservation, preparation of statoliths for age estimation, interpretation of growth increments and image analysis.

- **Extraction:** The squid head is cut between the muchal cartilage and dorsal proximal Vridge, without causing any damage to the liver initially. The statocysts are then exposed by surgically dissecting the severed squid head in the frontal plane. The statocysts are dissected transversely and the statoliths are extracted manually using forceps.
- **Cleaning and preservation:** The tissue fragments attached to the statoliths after dissection are removed prior to mounting for proper observation. The extracted statoliths are cleaned carefully using mounting needle and fine forceps. They are preserved in vials containing ethanol.
- **Preparation of statoliths for age estimation:** Statoliths are mounted on glass slides using thermoplastic glue for grinding. After drying, the statoliths are ground using lapping films. The ground statoliths are observed under binocular microscope continuously for completely grinding the opaque area.
- **Interpretation of growth increments:** Statolith samples for each major category of size and state of maturity are set aside for age reading. Growth rings are counted using the binocular microscope by changing the focal plane under higher magnifications. Highresolution photomicrographs are difficult to produce, because not all growth increments occur in the same focal plane under high power. Therefore, images are captured continuously for creating a montage of light micrographs for further reference.
- **Image analysis:** Image analysis systems are not commonly used for statolith studies. However, the use of electronic cursor is easier and more accurate than visual counting. Measurements of statolith radius and increment widths are recorded using software. Image enhancement using the software improved the visibility of the increments.
- **Information from optical analysis:** The growth rings in statolith microstructures in many squid species are validated as 'one growth increment-one day' using chemical marking. Maintaining squids in closed culture system for validation of growth is difficult for certain species, therefore, given the similarity of growth increments in validated species with those in invalidated closely related species, the validity of 'one growth ring, one day' hypothesis is generally accepted. The total number of growth increments within the statolith microstructure represents the age of an individual squid in days. More than 52 species of commercial squids of the world oceans have been aged.

The effects of growth, mortality and recruitment act in combination on the population size frequency, and separating them requires independent information for at least two of these variables. The information on the date of capture, spawning and probable recruitment time are used for the interpretation and analysis of the increment data.

## **3.3.2 Gladius**

The gladius is the internal shells of squid (suborders Oegopsida and Myopsida) and bobtail squid (order Sepiolida). Typically, it consists of inner, intermediate, and outer shell layers, but there are variations with respect to the number of layers in some families. These layers grow periodically and the increments or the striae are used in age estimation. Gladius processing for age estimation can be divided into four stages: extraction, preservation, sample preparation and reading. The intermediate layer is the most promising gladius layer for ageing studies.

## **3.3.3 Stylets**

Statolith and gladius analyses of octopus species are unsuitable for ageing. The increment analysis in the hard rod-like vestigial shells or the stylets are used for ageing octopus. However, stylet increment analysis is not suitable for all octopus species because of variation in stylet structure and increment readability.

## **3.3.4 Beaks**

The beaks are basically composed of a chitin–protein complex. Growth process takes place from the posterior border of the beak, where the most recent chitinized and hydrated material is deposited. Growth increments in cephalopod beaks were reported for the first time in the 1960s for the squid *Onykia ingens* using the inner surface of lateral walls. Beak increments have been used for age estimation in squid species in which daily deposition was confirmed by comparing with statolith-determined ages. Beak microstructure increment analysis is affected by processes such as feeding that wear down the beak, resulting in inaccurate estimates.

## **3.3.5 Sepion**

Most attempts to age cuttlefish have concentrated on the cuttlebone. This structure functions as a dorsal backbone providing both support and buoyancy control. It consists of a thin, hard, calcified, dorsal shield and a ventral porous phragmocene comprised of numerous narrow chambers, delineated by chitinous septa. The cuttlefish controls its buoyancy by moving gas or liquid into or out of the chambers as required. As the cuttlefish grows, further septa are laid down at the anterior end. Early studies concluded that the periodicity of chamber formation was daily, however, recent studies found it was related to growth rate rather than chronological age. The growth rate of cephalopods is strongly influenced by temperature and food availability and thus subject to seasonal fluctuations. The width of individual chambers also varies with growth rate.

#### **3.3.6 Crystalline lens**

Few attempts have been made for tentative ageing of cephalopods with unreadable statoliths, like in octopus, from their crystalline eye lenses. They grow continuously throughout life by the addition of concentric layers of fibre cells to their outer surface. The stained histological sections of lenses are observed for growth rings after decalcification and dehydration.

#### **Summary**

Determination of both age and growth are critical to understand the life history of harvested species and to model the dynamics of their populations, both of which are essential for assessment and management purposes. Successful age estimates have been achieved for many squid species by counting validated concentric daily increments found in statoliths. Recent years have seen the emergence of extensive studies of myopsid squid growth of the family Loliginidae. This has greatly advanced our understanding of their life histories. Growth data have accumulated from both statolithbased field studies and culture work. Validation studies on loliginids continue to support that statolith increments are laid down daily.

Ageing cuttlefish from statoliths has been less successful. In cuttlefish, the growth increments have proven difficult to distinguish due to the irregular and concentric deposition of the aragonite crystals, which result in a strong radial appearance, and the lower percentage of organic matter, which results in weak dark rings. Statoliths of octopods contain randomly arranged statoconia, without any visible increments. This technique has failed to provide results for octopus due to the lack of growth rings and the morphology of octopus statoliths not possessing the same landmarks as those of squid and cuttlefish, which minimizes increment visualization. Stylets, however, do have concentric rings and have been validated for age estimation using *Octopus pallidus* of known age reared in captivity. At present there is no generally applicable method of age and growth determination for all cephalopods and several techniques are in their infancy necessitating continued research in finer refinements and validation.

# **Reference**

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