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Review

METHODS FOR AGE AND GROWTH DETERMINATION IN CEPHALOPODS

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Summary

Different methods used in age determination of cephalopods and their importance for assessment and management purposes for this group of marine organisms are described in the paper. Scientific investigations have proved that indirect methods (length frequency data) are not reliable enough and should always be accompanied with one direct method, although these methods are very demanding and time consuming. Today, statoliths are the most frequently used direct method for age estimation. These hard structures are proved to be very useful tool in age determination in squids (Teuthida). On the other hand, there are no standard age determination techniques for cuttlefishes and octopuses (Sepiida and Octopoda) and for these groups of cephalopods scientists are still searching for methods suitable for routine use.

Key words: cephalopods, age and growth determination, direct and indirect methods

INTRODUCTION

Cephalopods represent one of the most important commercial marine resources. In recent decades they have been recognized as an alternative to more traditional marine resources and their importance for human consumption is likely to continue to increase in the future (Caddy and Rodhouse, 1998; Piatkowsky *et al*., 2001). In the last 30 years annual world catches of cephalopods increased from around 1 to over 3.5 million tonnes (FAO, 2006). The increased relative abundance of cephalopods reflects, at least in one part, reduction in competition and predation owing to diminished stocks of some finfish and marine mammals (Bravo de Laguna, 1989). In recent decades increasing market demands all over the world and very efficient fishing techniques, have put strong pressure on some of the most important cephalopod

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stocks. These stocks showed wide annual fluctuations due to many different reasons, some of which are still poorly understood (Krstulović Šifner *et al*., 2005). Due to the short life cycles and variable growth rates of most cephalopods, stocks may be highly variable, both highly susceptible to recruitment over–fishing and, conversely, capable of rapid recovery (Pierce and Guerra, 1994). Increasing annual catches in most cephalopod fisheries have prompted many countries to initiate programs for monitoring stocks and forecasting their abundance. Instability of stocks, together with the increasing commercial importance, underlined the need for better understanding of the biology, ecology and population dynamics of cephalopods, in order to obtain predictive fisheries management information (Jereb *et al*., 1991).

Age composition and growth rates are extremely important population parameters for studying population dynamics, stock structure, and monitoring fishery performance. Methods of estimating cephalopod growth can be divided in two basic groups: direct and indirect methods.

Indirect methods

Indirect methods for estimation of cephalopod growth are based on the analysis of the length–frequency data obtained from experimental surveys or commercial landings. With this method a growth curve can be constructed by connecting modal or mean length values for successive time intervals. Verrill (1882) first used this modal analysis method for estimating growth rates of cephalopods. Indirect methods have been found useful only in cases where one or several well–defined and short term spawning events take place in the investigated area with no changes in population structure due to migration, and that situation is very rare in practice. In most cases the spawning of the species extends throughout the year with peaks lasting 1–2 months. Several generations may occur in one area simultaneously and in such cases the general growth curve obtained by connecting modal or mean length values for successive time intervals will not reflect the growth rates of each or any generation. Another problem is the interpretation of length frequency modes. Growth curves may vary considerably in shape depending on the interpretation of modal sizes. Therefore, use of length frequency data to estimate the rates of squid growth should be treated with caution, and verification of these data using one of the direct methods is necessary (Jereb *et al*., 1991).

Direct methods

These methods for estimating cephalopod age and growth rate include: 1) direct observation of cephalopod growth in captivity, 2) tagging and recapturing in the wild, 3) examination of growth–recording hard structures.

1) **Direct observations of cephalopod growth in aquarium** started in 1960ies with the development of mariculture (Itami *et al*., 1963, Choe,

1966). In controlled aquarium conditions it is possible to measure the variations in size over periodic time intervals. In this way length and weight of live animals gives a good picture of individual and group growth rates at different ontogenetic stages, and the influence of abiotic and biotic factors on growth. There are some problems concerning this method as: very high mortality of young cephalopods in aquarium conditions; very low survival rate of most of oceanic squid species and pelagic octopus larvae and doubt if the obtained growth curves will be similar to those of wild animals (Boletzky and Hanlon, 1983; Hanlon, 1987). Nevertheless, studies of the cephalopod growth in captivity are more valuable than those obtained from indirect methods and in several species they were used for validation of growth rates obtained from other age determining methods (Jackson, 1994; Durholtz *et al*., 2002).

2) **The tagging and recapturing of cephalopods** is a method that avoids the main disadvantages of the first method and enables the precise assess of cephalopod growth rates in their natural environment in time between the release and recapture. The first tagging and marking experiments of cephalopods were conducted on pelagic species starting in 1927 with Soeda (1950), who studied the patterns for the establishment of migration models of *Todarodes pacificus*. Since that time tagging–recapture experiments have been made on several cephalopod species using different kinds of tags (mechanical, chemical, electronic). The main disadvantage of this method is the fact that most cephalopods are active migrants and the recapture of the tagged animals is rare, in many cases with only few individuals out of thousands tagged. The other problem is that mechanical and chemical tags used for tagging very often interfere with daily activities of cephalopods, cause infection and stress and make them an easy prey for predators. However, the data available on growth rates from tagging experiments still provide an estimate of the growth of some cephalopod species under natural conditions.

3) **The study of cephalopod growth based on hard structures** started relatively late (Young, 1960). Almost all hard parts of cephalopods, as statolith, gladius, sepion, mandible, vestigial shell, beaks and crystalline lens, have growth increments and some of them have been used in age determination:

   a) *Gladius* is an age registering structure that may be used for age or growth determination in squids. As it is composed of three shell layers it has three scales for age determination. Up to now, it has been used for age determination in several squid species, and in some of them the daily nature of increments has been proved (*Kondakovia longimana*, *Illex argentinus*), while for the others, additional studies have to be carried out to confirm if the increments are daily formed (Arkhipkin and Bizikov, 1991).

b) *Sepion* was used for the age and growth estimation of cuttlefish by counting all septa in sepion. However, it was found that in the sepion of *Sepia officinalis* formation rate depends on temperature, therefore the cuttlefish age can be correctly assessed only if the animals grew under a known constant temperature (Richard, 1969).

c) *Beaks* and *vestigial shells* have shown promise for age determination in Octopoda. In this group of cephalopods direct methods used in cuttlefishes and squids have not been found useful as aging tools (Sousa–Reis and Fernandes, 2002). Several experiments have been carried out and scientists succeeded to obtain images that allow the counting of the rings caused by a cyclical deposition in the vestigial shells and in beaks. However, the assumption of one ring equalling one day remains to be validated for both hard structures (Semmens *et al.*, 2004)

d) Throughout the years it has been proved that for majority of cephalopod species the most useful structure for age determination are *statoliths*. Statoliths are paired calcareous »stones«, usually less than 2 mm in length, located in two adjacent cavities, the statocysts, within the cartilage of the skull situated ventro–posteriorly to the brain. Each statolith lies against a ciliated area at or near the anterior end of the statocyst, and is oriented with its long axis approximately in the dorso–ventral plane of the cephalopod. Statoliths were observed and described for the first time a century ago and in early seventies they were identified as possible »time recording structures« and became the subject of intensive research. There are several methods used for extraction, storage, mounting and grinding, i. e. for preparation of statoliths for reading of growth rings and their use depend mainly on the shape and size of statoliths of the analysed species (Jereb *et al.*, 1991). When statoliths are properly prepared it is possible to see under the microscope their microstructure that reflects periodical formation. In this way a number of concentric rings called growth rings or growth increments can be seen (Figs. 1 and 2). Magnifications used for counting increments, generally fall within the range of 250 and 690 x (Lipinski, 1978; Hurley and Beck, 1979; Spratt, 1978; Rosenberg *et al.*, 1981; Jackson, 1989). Growth rings are composed of the paired dark and light growth layers produced during one day. It was experimentally confirmed for several species that in statoliths growth increments form daily (Hixon and Villoch, 1984; Hurley *et al.*, 1979, 1983, 1985; Jackson, 1990, 1994; Arkhipkin, 2004, Durholtz *et al.*, 2002 and others), i. e. age of each species in terms of days was considered as equal to the total number of minor daily growth increments in their statoliths. This hypothesis was validated in natural environment just in one case for the species *Loligo vulgaris reynaudii* (Lipinski *et al.*, 1998).

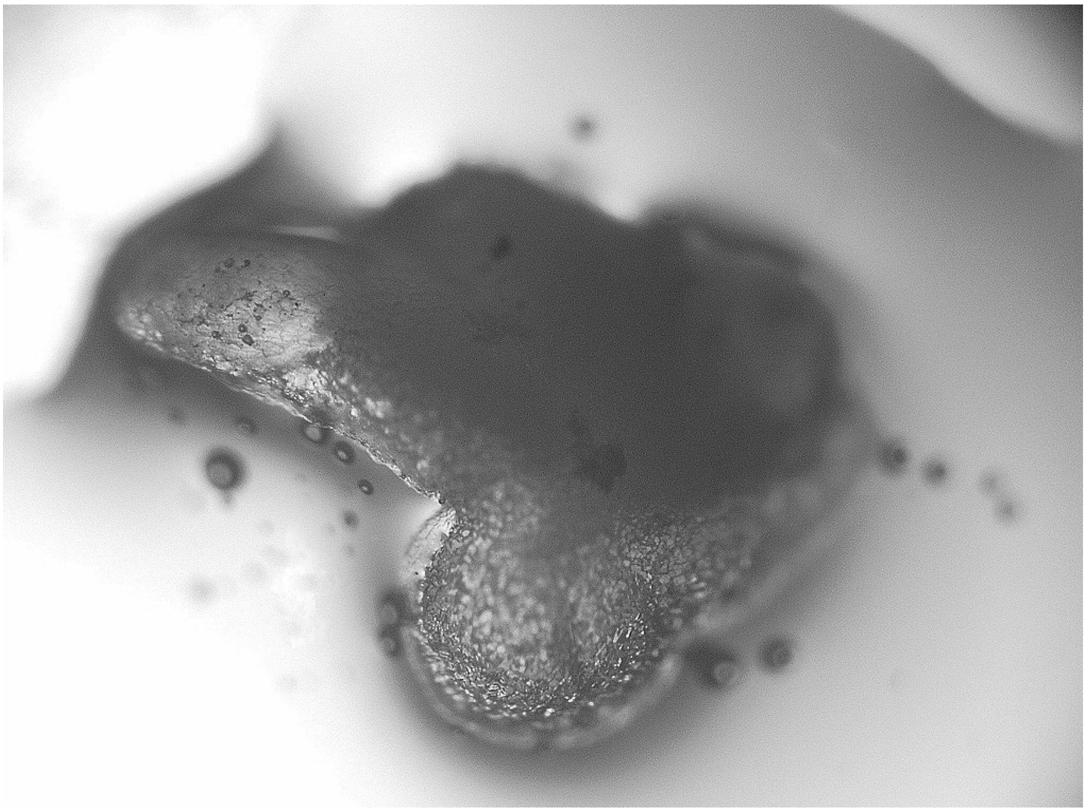

Figure 1. Statolith on a microscope slide under the transmitting light microscope before grinding (species Loligo vulgaris*, total magnification 80X)*
Slika 1. Statolit na predmetnom stakalcu pod mikroskopom s prolaznim svjetlom prije brušenja (vrsta Loligo vulgaris*, ukupno povećanje 80 puta)*

Although statoliths are present in all cephalopod species, their structure and size is very variable. Statoliths have been proved to be very useful tool in age determination in squids while in octopods they are without readable growth rings and therefore useless for age determination, so for this group of cephalopods alternative methods are used (Semmens *et al*., 2004). The use of statoliths in age determination revealed very high growth rates and short life spans of squids, ranging between 6 months and 1.5 years depending on species and area of investigation (Arkhipkin *et al*., 2000). Basically, results obtained using statoliths show much higher growth rates compared to growth rates calculated using length–frequency data. Therefore, direct methods have often estimated much lower squid's age than those by length frequency data analysis (Jackson, 1994).

Figure 2. Ground surface of statolith with concentric growth increments under the transmitting light microscope (species Illex coindetii*, total magnification 400X)*
Slika 2. Izbrušena površina statolita s koncentričnim krugovima pod mikroskopom s prolaznim svjetlom (vrsta Illex coindetii*, ukupno povećanje 400 puta)*

CONCLUSIONS

The determination of age and growth of cephalopods is of great importance as it gives the most reliable data about the growth rates and it consequently allows the proper assessment of commercially important cephalopod stocks and their management.

Although there are several methods for growth and age estimation most of them have some very serious disadvantages. As previously mentioned, indirect methods, i. e. the length–frequency data have to be used with caution because there are several problems in connection to reliability of statistical separation of complex length–frequency data into »microcohorts«, as a result of extended periods of spawning and recruitment observed in some cephalopods. Therefore, the direct methods, although very time consuming, are much more reliable and should be used for correct estimations. Today, most frequently used direct method is statolith reading in squids, that allows the insight not only in individual age and species life–span, but in many different aspects of life history as growth rates, ontogenetic shifts, number of mating events, environmental conditions, population structure, systematic, population dynamics, etc. Further progress in development of techniques to accurately determine the age of octopods and cuttlefishes is needed as for these groups there are no standard techniques yet. Nowadays further efforts are set to improve age determination methods, evaluating precision and consistency between researchers in order to make obtained results useful and comparable.

Sažetak

METODE ODREĐIVANJA DOBI I RASTA GLAVONOŽACA

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U radu su opisane različite metode određivanja dobi i rasta glavonožaca, kao i njihovo značenje za procjenu stanja i upravljanje zalihama gospodarski zanimljivih vrsta. Tijekom vremena istraživanja su pokazala da samo indirektne metode (dužinske učestalosti) nisu dovoljno pouzdane te da je najsigurniji način određivanja dobi kombiniranje indirektnih i direktnih metoda, unatoč tomu što direktne metode zahtijevaju mnogo uloženog rada i vremena. Od direktnih metoda danas se za očitavanje dobi najčešće primjenjuju statoliti, a napose dobri rezultati postignuti su kod lignji (*Teuthida*). Za sipe i hobotnice (*Sepiida* i *Octopoda*) ne postoje standardne tehnike za određivanje dobi i još uvijek se traga za metodama koje bi se rutinski primjenjivale za ove skupine glavonožaca.

Ključne riječi: glavonošci, određivanje dobi i rasta, direktne i indirektne metode

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