Fisheries Research 117–118 (2012) 63–74

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Thirty years of using the postovulatory follicles method: Overview, problems and alternatives

Konstantinos Ganias*

School of Biology, Aristotle University of Thessaloniki, University Campus, 54 124 Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 23 July 2010
Received in revised form 6 January 2011
Accepted 6 January 2011

Keywords:
Postovulatory follicles
Daily egg production method
Spawning frequency
Biomass estimation

ABSTRACT

The postovulatory follicles' method or POFs method was introduced by Hunter and Goldberg in the start of the 80s and still remains the most popular method for estimating population spawning frequency in wild populations of multiple spawners. During these last thirty years the method has been applied to more than 50 fish stocks almost all around the world's oceans. Even though the method was initially designed for the stock of northern anchovy, *Engraulis mordax*, it has now been applied not only to other multiple spawning clupeoids (ca. 40% of total applications) but also to a large number of other fish taxa. Despite its popularity the method can be quite inaccurate when its criteria are applied to other species and populations without prior validation. Four important sources of bias in the application of the method were identified: bias related to POF staging, bias in POF ageing, sampling bias and bias in the estimation of spawning fraction. Apart from all these potential sources of bias the method is quite costly and labor-intensive because it needs large number of adult samples, much histology and many work-hours from experienced personnel. In that respect, the development of alternative methodology for estimating spawning frequency seems worthwhile.

1. Introduction

1.1. Background on spawning frequency estimates

Spawning frequency, *f*, in iteroparous fishes expresses the number of spawning events per unit time, usually per day. *f* can be estimated either as an individual parameter through interspawning interval, *ISI*, i.e. the time lag between subsequent spawning events (Wootton, 1974), or as a population parameter through the spawning fraction, *S*, i.e. the proportion of females spawning per day (Parker, 1980). Estimates of *f* are of primary importance because they are used in exploring temporal patterns in fish reproductive dynamics (Lowerre-Barbieri et al., in press), in estimating spawning biomass through the daily egg production method (DEPM) (Parker, 1980; Hunter and Goldberg, 1980; Picquelle and Stauffer, 1985) and in calculating annual egg production in indeterminate spawners (e.g. Claramunt et al., 1994; LaPlante and Schultz, 2007).

To date, most estimations of *f* in the wild are performed through the spawning fraction except from species-specific applications that provide estimates of interspawning interval such as visual census methods (Asoh, 2003; Curtis, 2007), otolith microchemical analysis (Secor and Piccoli, 2007) or cases where spawning events may be detected by means of passive acoustics (e.g. Lowerre-Barbieri et al., 2008). The most popular method for estimating spawning fraction in multiple spawning fish is the postovulatory follicle method or POF method, first introduced almost 30 years ago by John Hunter and his colleagues at the Coastal Division of the Southwest Fisheries Center (SWFC), La Jolla, CA, USA.

The POF method was developed under the framework of assessing northern anchovy, *E. mordax*, spawning biomass through the DEPM (Parker, 1980). Given that the DEPM is only valid for species with indeterminate fecundity, i.e. species where annual fecundity is not fixed at the beginning of the spawning season, the POF method has almost exclusively been applied to indeterminate spawners. According to Parker (1980) "spawning frequency can be estimated from examining the spawning condition of females: (1) females can be examined for a characteristic that indicates when spawning takes place; (2) the length of time such a characteristic remains detectable can be estimated; (3) the spawning rate remains relatively constant over the sampling interval. The spawning fraction, or frequency, is the fraction of females displaying the characteristic divided by the length of the time interval the characteristic remains detectable". Hunter and Goldberg (1980) following upon the original finding of Moser (1967) that postovulatory follicles can be seen and used to determine time of spawning in rockfish, developed criteria for ageing POFs in *E. mordax*. By doing so, females could be assigned to daily spawning cohorts and *S* was calculated as their fraction to the total number of mature females (Picquelle and Stauffer, 1985).

* Tel.: +30 2310 998760; fax: +30 2310 998279.
E-mail address: kganias@bio.auth.gr

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doi:10.1016/j.fishres.2011.01.009

The 1985 NOAA technical report on DEPM (Lasker, 1985) thereafter called as the 'DEPM manual' describes the entire methodology for estimating *S* in northern anchovy and is still considered by most authors as the key reference for the POF method. However, despite its popularity the method can be quite inaccurate when criteria described in the DEPM manual are applied to other species and populations without prior validation and when sampling schemes are not carefully designed (Stratoudakis et al., 2006). Furthermore, applications of the method are quite costly and labor-intensive since they require large number of samples from experimental fishing, much histology and many work-hours from experienced personnel. The present review attempts to summarize all or at least most of the problems and bias issues that have been accumulated during the last 30 years of its application, suggest potential solutions and improvements and also report some alternative methodologies that have been suggested for estimating *S* in wild fish populations.

1.2. Historic overview of the POF method

Prior to the POF method the only means for estimating spawning frequency was through measuring interspawning intervals in laboratory kept individuals (e.g. Wootton, 1974) or through implying the number of spawnings per breeding season from the number of oocyte modes that co-occur in the ovary. In contrast to the latter method which indicated 1–3 spawnings per year (see review of this misconception in Blaxter and Hunter, 1982) the application of the POF method to the population of northern anchovy by Hunter and Goldberg (1980) has shown that a female may produce about 20 spawning bouts per year (see also Hunter and Macewicz, 1980). This finding may be considered as a breakthrough in fisheries science not only because of the involvement of *S* in the assessment of spawning biomass through the DEPM model but also through improving our understanding on various aspects of fish reproductive biology.

Three important phases may be distinguished in the development, the propagation and the applicability of the method since the start of the 1980s. The years between 1980 and 1985 could be considered as the validation period which coincided with the effort of improving accuracy in the SSB estimates in two Pacific anchovy stocks, the northern anchovy, *E. mordax* and the Peruvian anchovy, *Engraulis ringens*; results of this effort are summarized in a number of papers inside the DEPM manual mainly in the contributions of Hunter and Macewicz (1985) and Alheit (1985) for the Californian and the Peruvian stocks respectively. A second important period may be located at the start of 1990s when staff from the SWFC helped to 'export' the method together with all the know-how of the DEPM to fisheries institutes of W Europe as a response to the need of deploying the DEPM in the assessment of the Atlantic sardine, *Sardina pilchardus* and anchovy, *Engraulis encrasicolus*, stocks, e.g. Pérez et al. (1992). From this period onwards the POF method has been systematically applied not only to these clupeoid stocks but also to several other fish stocks around the world oceans within the framework of DEPM surveys (see Table 1 in Stratoudakis et al., 2006). In many cases correct applications of the POF method are considered as the most crucial prerequisite for applying the DEPM to a fish stock (e.g. the European Hake, *Merluccius merluccius*, in the Bay of Biscay: Murua et al., 2010).

During the third period which dates back to late 1990s the method started also being applied independently of DEPM surveys to a number of fish stocks from various taxonomic groups and ecosystems. An analysis of the literature shows that the POF method has already been applied to more than 50 fish stocks all around the world's oceans (Table 1). Almost 1/3 of total applications were performed to stocks of multiple spawning clupeoids, i.e. anchovy and sardine stocks (Table 1); however, as shown in Fig. 1, during the last decade there has been an impressive increase in the number of fish families where the method has been applied. Given that the analysis was performed mostly using papers published in SCI journals the actual number of stocks and applications might be quite bigger. These findings suggest that despite difficulties and bias issues in its applications the POF method is still considered as the method of choice for estimating *S* in populations of multiple spawners with indeterminate fecundity. In addition, besides SSB estimations in several commercially important stocks, applications of the POF method have produced a series of spawning frequency values that can lead to new insights into the reproductive biology of indeterminate spawners, particularly when such values are compared between species and stocks or habitats and seasons (e.g. Alheit, 1993; Somarakis et al., 2004a; Ganias, 2008).

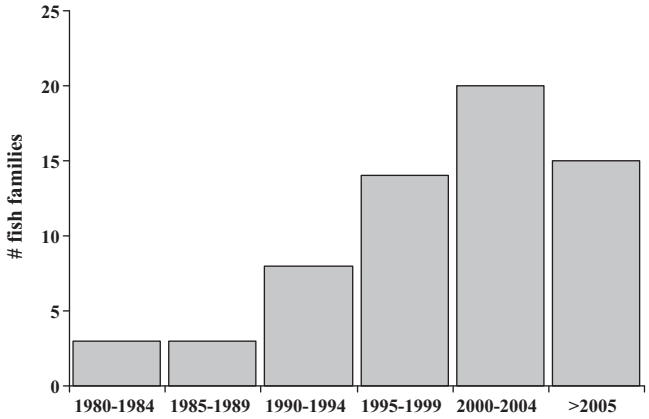

Fig. 1. Change in the number of fish families to which to the POF method has been applied during the last thirty years. The analysis was based only to papers published in SCI journals.

2. Description of the POF method

2.1. Main principles

As already mentioned, the method principally relies on the finding that POFs constitute the most reliable evidence of previous spawning activity in fish. Even if recent papers suggest that POFs may be identified and measured in preparations of ovarian whole mounts (e.g. Witthames et al., 2009) the most accurate means for assessing POFs is through histology. In that respect, estimation of *S* through the POF method is a 4 stage process consisting of:

(a) identifying and staging POFs in histological preparations,
(b) ageing POFs using POF ageing keys,
(c) assigning females to daily spawners classes, Day_i, and
(d) dividing the number of females of each Day_i class to the number of fish analyzed.

2.2. The process of POF degeneration

The postovulatory follicle consists of the follicular layers that remain in the ovary after the release of the ovum during spawning (Saidapur, 1982). Initially, the POF is a distinct structure, but it rapidly deteriorates and becomes undetectable usually within a few days (Hunter and Goldberg, 1980); however, there are cases of species that signs of POFs may persist in the ovary for quite larger periods, e.g. cod, *Gadus morhua* (Saborido-Rey and Junquera, 1998). Even if there are published descriptions of the process of POF degeneration for a number of fish species, most of these publications follow terminology and criteria provided by Hunter and Macewicz (1985) for northern anchovy. The same paper includes a