Application of GAMs and multinomial models to assess the spawning pattern of fishes with daily spawning synchronicity: A case study in the European anchovy (*Engraulis encrasicolus*) in the central Mediterranean Sea

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ABSTRACT

Information on the breeding behaviour of fish such as spatial segregation phenomena during spawning events is important both for understanding their reproductive ecology but also for correctly designing sampling schemes in stock assessment. The daily spawning pattern of the European anchovy, *Engraulis encrasicolus*, was assessed using a novel methodology that included the analysis of the hourly distribution of imminent and recent spawning stages using a combination of generalised additive models (GAMs) and of multinomial models for ordinal categorical data. To do so we used data from nine years of daily egg production method, DEPM, surveys, carried out in the Strait of Sicily. The spatial distribution and occurrence of actively spawning individuals were also analysed. Results showed that the actively spawning phase period (ASPP), i.e., the period from the onset of oocyte hydration to spawning ranged approximately 4.5 h between ca. 18:30 and 23:00 while a daily spawning peak was identified at 22:30. The sex ratio was skewed during the ASPP such that females exceeded males by ca. 20% at mid ASPP. During the ASPP the proportion of Day-0 females (i.e., females that spawned or would spawn the day of sampling) decreases with increasing bottom depth while outside the ASPP Day-0 spawners did not show any particular bathymetric pattern. In the study area, anchovies exhibit segregative spawning behaviour which drives spawners to select shallower waters, similar to other anchovy populations in the Mediterranean while in the Atlantic and in other clupeoid populations the opposite bathymetric trend has been observed.

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1. Introduction

Reproductive traits in marine fishes commonly occur on four different temporal scales: lifetime, annual, seasonal and diel (Lowerre-Barbieri et al., 2011). Studying the diel reproductive pattern of wild fish populations usually involves the study of their spawning biology and behaviour such as the existence of a daily spawning period or the formation and characteristics of spawning aggregations. This biological knowledge is particularly important in stock assessments because segregation phenomena, both in time and space, require special sampling schemes to cover the breeding and distribution ground of the evaluated stocks. The assessment of the daily spawning pattern is usually a difficult exercise, which can become complicated in cases where reproductive events occur on large spatial scales or in areas that are difficult to approach, such as offshore or in deep water. In such cases, an assessment of spawning behaviour might only be based on indirect observations, such as the prevalence of spawning markers in samples of adult fish (e.g., Alheit et al., 1984; Ganias et al., 2003; Ganias and Nunes, 2011), the spatiotemporal distribution of embryonic stages in ichthyoplanktonic samples (Bernal et al., 2011; Basilone et al., 2013) or the acoustic identification of spawning schools (Zwolinski et al., 2006; Walters et al., 2009; Lowerre-Barbieri et al., 2013).

The daily spawning pattern of marine multiple spawning clupeoids, especially of anchovies and sardines, has been extensively studied mainly through data collected during the application of the daily egg production method (DEPM) (Parker, 1980). Otherwise the application of DEPM in a particular area requires a good knowledge

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of the spawning behaviour of the assessed stock, such as an estimate of daily spawning time and the spatiotemporal characteristics of spawning aggregations. More specifically, the daily spawning time is important for the correct assignment of females to daily spawning cohorts in estimations of the spawning fraction (S), and in the correct ageing of eggs in estimations of egg mortality.

Populations of anchovies and sardines display daily spawning synchronicity (Stratoudakis et al., 2006); in particular, actively spawning individuals synchronise their daily reproductive rhythms to mature their gonads and spawn their gametes at specific periods of the daily cycle. In general, the daily spawning period (DSP) of these species ranges from dusk to the early morning hours. The daily spawning period of the European anchovy, *Engraulis encrasicolus*, has been identified between 22:00 and 24:00 in different areas, both in the Mediterranean (Palomera and Pertierra, 1993; Somarakis, 1999; Somarakis et al., 2004) and the Atlantic (Motos, 1996; ICES, 2004, 2010; Uriarte et al., 2012). A similar DSP has also been identified for other species of the genus *Engraulis* (for review, see Ganias et al., 2014), such as *E. capensis* (around 22:00; Melo, 1994), *E. japonicus* (21:00–02:00; Wan et al., 2008) and *E. mordax* (21:00–23:00; Hunter and Goldberg, 1980; Stauffer and Picquelle, 1985).

In general, as in the above-quoted studies on anchovy populations, the DSP estimate is based on the assessment of actively spawning individuals, i.e., the appearance and distribution along the day of females having hydrated oocytes and/or early postovulatory follicles (POFs) in their gonads, whereas in fewer cases, estimates are based on the hourly frequencies of the early embryonic stages in ichthyoplanktonic samples. A few studies also used the temporal dynamics of the oocyte hydration process. For example, Garcia et al. (1994) based their estimate of the DSP of North-western Mediterranean anchovy on the time at maximum hydration, assuming that this corresponds to the time at spawning. Lisovenko and Andrianov (1996) analysed the DSP of Black Sea anchovy based on oocyte size and gonadosomatic index frequency distributions. The greatest problem with using the hydrated stage is that it is particularly rare to encounter in adult anchovy samples (Picquelle and Stauffer, 1985; Motos, 1996).

Ganias (2008) showed that the formation of spawning aggregations, in which imminent female spawners separate spatially from the remaining population, taking with them a large proportion of males, is a generic characteristic of anchovies and sardines. However, systematic sampling of these spawning aggregations in DEPM surveys leads to many samples with increased proportions of imminent and/or very recent spawners and consequently to overestimates of S and biased sex ratios (Ganias, 2012; Ganias et al., 2014). Alternatively, collections of imminent spawners that are obtained from such samples might offer a large number of specimens for batch fecundity estimates through the hydrated oocytes method (Hunter and Macewicz, 1985).

The European anchovy, which represents one of the main resources of commercial fishery (Schwartzlose et al., 1999; Lleonart and Maynou, 2003), is a pelagic spawner that releases several egg batches in a protracted spawning season that ranges between April to October, with a peak during summer (Basilone et al., 2006).

Despite the aforementioned published data on the daily spawning period of various anchovy populations and the evidence that many of these populations form spawning aggregations, the exact spatio-temporal arrangement of actively spawning individuals has never been assessed. In particular, the question of whether ephemeral spawning aggregations of anchovies occur horizontally, with depth, or are due to differential catch rates of gravid females by the sampler (Alheit, 1985), still remains undetermined. In this study, we used data collected from nine annual DEPM surveys (from 1998 to 2001 and from 2005 to 2009) conducted in the Straits of Sicily (Somarakis et al., 2004) to assess anchovy spawning stock
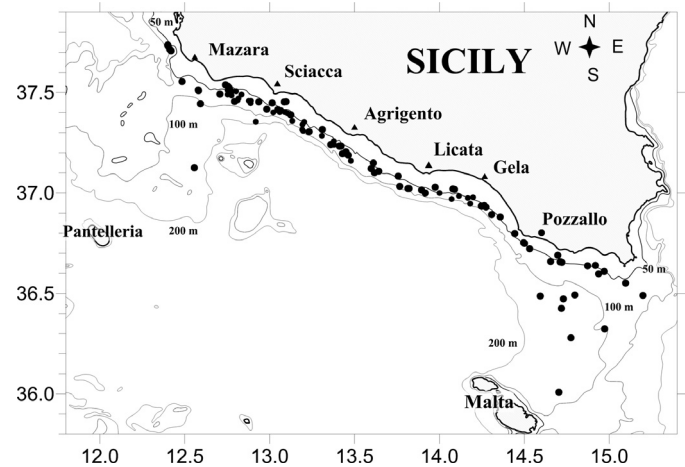

Fig. 1. Locations of European anchovy, *Engraulis encrasicolus*, sampling stations performed using research vessels (*RV*: black circles) in the Sicilian continental shelf.

biomass (SSB) to investigate its diel spawning pattern and the characteristics of its spawning aggregations.

2. Materials and methods

2.1. Sampling

Adult European anchovy, *E. encrasicolus*, were sampled in the Straits of Sicily, within the framework of nine combined DEPM and echo-acoustic surveys held between 1998–2001 and between 2005–2009 for the estimation of the spawning biomass of the local anchovy stock (Figs. 1 and 2, Table 1). Samples were collected during the anchovy peak spawning months (June–August; Garcia Lafuente et al., 2002; Basilone et al., 2006), by research vessels (RV) equipped with a midwater Pelagic Trawl (PT); vertical opening of 8 m, cod-end mesh size of 18 mm, operated at 4.0 knots. For each fishing-set, information on position, bottom depth and local time of capture was available. The use of local time in the present paper has been preferred to GMT for simplifying the further comparison among stocks inhabiting different regions worldwide. The bottom depth was obtained from a scientific acoustic sounder (SIMRAD-EK, 500 and 60), whereas haul depth was determined using a SIMRAD-ITI trawl monitoring system. On average, samples consisted of a random collection of 653 ± 200 g of anchovy, each
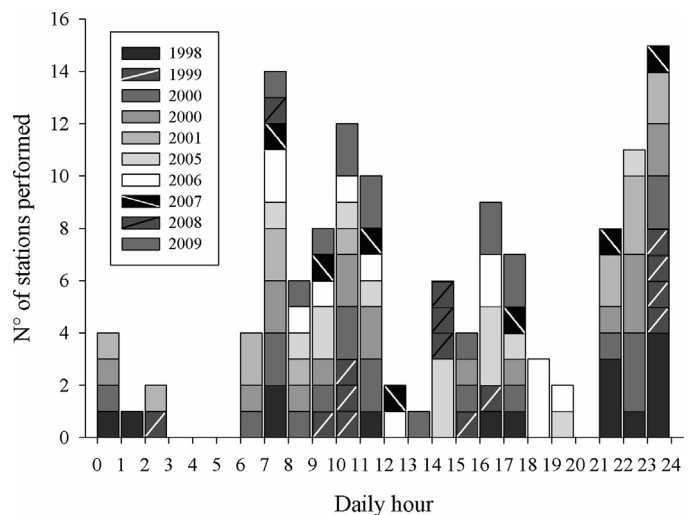

Fig. 2. Number of fishing hauls per hours (sampling time) per year.