BOCADEVA 0720

Anchovy DEPM survey in the Gulf of Cadiz

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1.   Introduction

The Daily Egg Production Method (DEPM) to estimate the Anchovy Spawning Stock Biomass (SSB) in the Gulf of Cádiz (ICES, Subdivision 9.a South) is conducted by Spain (Instituto Español de Oceanografía, IEO) every three years, since 2005.

BOCADEVA 0720 is the sixth survey of the historical DEPM series for anchovy in the Gulf of Cádiz and was delivered on board R/V *Ramón Margalef* (IEO) from the 9th to the 17th of July 2020. The surveyed area extended from Strait of Gibraltar to Cape San Vicente (Spanish and Portuguese waters in the Gulf of Cadiz). Plankton samples, along a grid of 21 transects perpendicular to the coast were obtained for the spawning area delimitation and density estimation of the daily egg production. The survey objectives also included the characterization of the oceanographic and meteorological conditions in the study area. The samples to estimate adult parameters (sex ratio, female mean weight, batch fecundity and spawning fraction) were obtained in the acoustic survey "ECOCADIZ 2020-07", carried out during the same period.

Plankton and adult samples from the BOCADEVA and ECOCADIZ surveys are being processed, and once the laboratory work is finished, eggs and adults parameters will be estimated in order to provide the SSB for anchovy through the application of the DEPM.

This working document provides a brief description of the survey and the progress status of the laboratory analysis carried out to obtain the Anchovy SSB by the application of the DEPM in the South-Atlantic Iberian Stock.

2. Methodology

Table 1 summarized a description of the methodology used to obtain egg (BOCADEVA 0720) and adult (ECOCADIZ 2020-07) samples.

Table 1. BOCADEVA 0720 & ECOCADIZ 2020-07. General sampling information.

Eggs	Anchovy DEPM survey BOCADEVA 0717
Survey area	(36º13'-36º50'N –6º07'--8º55'W)
R/V	*Ramón Margalef*
Date	9th to 17th July
Transects (Sampling grid)	21 (8x3)
Pairovet stations (150 μm)	162
Sampling maximum depth (m)	100
Hydrographic sensor	CTD SBE 911
Flowmeter	Yes
CUFES stations	152
CUFES (335μm)	3 n miles (sample unit)
Environmental data	Temperature and Salinity
Adults	**Acoustic survey ECOCADIZ 2020-07**
Survey area	(36º11'-36º47'N –6º12'--8º54'W)
R/V	*Miguel Oliver*
Date	1st to 14th August
Gears	Pelagic trawl
Trawls	25 (all of them positive for Anchovy)
Trawls time	From 07:09 to 20:00 hrs GMT (At night; hauls targeted hydrated females)
Biological sampling:	On fresh material, on board of the R/V
Sample size	At least 60 individuals randomly picked; up to 120 (adding batches of 10 randomly picked anchovies) if a minimum of 30 mature females were not found for spawning fraction estimation. A minimum of 150 hydrated females for batch fecundity estimation.
Fixation	4% Phosphate buffered Formaldehyde
Preservation	4% Phosphate buffered Formaldehyde

2.1. Surveying

2.1.1.   Plankton sampling

The strategy of egg sampling was identical to that used in previous BOCADEVA surveys. An adaptive sampling was carried out in the East - West direction using a PairoVET net in fixed stations as main sampler and a continuous recording with CUFES (*Continuous Underwater Fish Egg Sampler*) as secondary sampler (**Figure 1**).
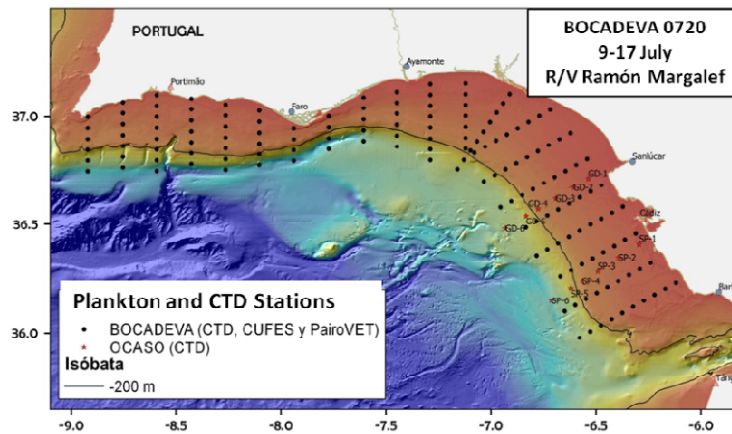
Figure 1. BOCADEVA 0720. Planned plankton and CTD stations.

- *Vertical sampling (PairoVET)*

The sampling grid was established on the continental shelf following a systematic sampling scheme, with transects perpendicular to the coast and spaced 8 nm. Egg samples were always taken every 3 nm in the inner shelf (ICES, 2003). The inshore limit of transects was determined by bottom depth (as close to the shore as possible), while the offshore extension was decided adaptively depending on the results of the most recent CUFES sample.

Vertical hauls were carried out with a PairoVET sampler equipped with nets of 150μm mesh size. Hauls were carried out up to a maximum depth of 100m or of 5m above the bottom in shallower depths, speed of about 1 m/s. Sampling depth were recorded using an underwater positioning systems HiPAP 500 Kongsber fitted to the net. Flowmeters were used to calculate the volume of filtered water during each haul.

Due to COVID-19 constraints, the scientific crew on board was reduced and consequently, the PairoVET samples were not processed on board as usually. Samples were preserved in a 4 % buffered formaldehyde solution.

- *Continuous sampling (CUFES)*

The CUFES sampling (Checkley *et al.*, 1997) is fixed at 5m depth. The volume of filtered water (600 l/min, approximately) was also integrated each 3 nm. The CUFES collector was arranged with a 335 μm net.

In order to decide the offshore extension of the transects, the CUFES samples collected over the 200 meters depth, were inspected on board to determine the presence/absence of the anchovy eggs. CUFES samples were all preserved in a 4 % buffered formaldehyde solution.

2.1.2. Adult fish surveying

Adult Anchovy samples for DEPM were obtained during the ECOCADIZ 2020-07 survey from pelagic trawl hauls (See Ramos et al., 2020).

Except for searching Anchovy females with hydrated gonads, fishing stations were mostly conducted during daylight hours and carried out over isobath, once echotraces supposedly belonging to Anchovy were detected by echo-sounder.

For the estimation of spawning fraction (S), a minimum of 30 mature, non-hydrated females per sample is sought, so a minimum of 60 random anchovies are sampled, adding batches of 10 random individuals to the sampling until the goal is achieved or a maximum of 120 anchovies are sampled. Sex-ratio (R), along with other parameters used in the DEPM is also obtained from this random sampling.

When hydrated females (HF) appeared, an additional sampling was done in order to obtain a minimum of 150 HF for the whole area prospected. Gonads from both hydrated and non-hydrated females were preserved in 4% buffered formaldehyde solution.

2.1.3. Hydrography

A CTD SBE 911 was used in each station to take temperature, salinity, fluorescence and oxygen of the water column. Also, a continuous sampling of sea surface temperature and salinity was carried out.

2.2. Laboratorial analyses

2.2.1. Plankton samples

At the laboratory, PairoVET samples are being sorted. Anchovy eggs and larvae from the two nets of the PairoVET samples are all identified and counted, as well as other commercial species. The anchovy eggs are classified in 11 stages of development classification according to the key proposed by Moser and Ahlstrom (1985). These samples are being processed to estimate abundances by stage.

CUFES samples have been sorted and anchovy eggs are being counted and classified in three stages: No-Embryo (I-III), Early Embryo (IV-VI) and Late Embryo (VII-XI).

Laboratory analyses for PairoVET and CUFES samples are still underway.

2.2.2. Adult fish samples

The preserved ovaries on board are then processed for histology at the laboratory; they are embedded in resin and the histological sections are stained with haematoxylin and eosin. The slides are examined and scored for their maturity stage (most advanced oocyte batch) and post ovulatory follicles (POFs) presence and age assignment to daily cohorts (Hunter and Macewicz 1985). Prior to fecundity estimation, hydrated ovaries are also processed histologically to check for POF presence and thus avoid underestimating fecundity. The individual batch fecundity is then measured, by means of the gravimetric method applied to the hydrated oocytes, on one to three whole mount sub-samples per ovary, weighing on average 50-150 mg (Hunter et al. 1985).

3.   Results

3.1.   Sampling

The surveyed area extended from Cape de Trafalgar (Spain) to Cape de S. Vicente (Portugal). This area includes the continental shelf of the Gulf of Cadiz. The survey was carried out from East to West, starting in the radial 1- station 1, located close the Strait of Gibraltar (**Figure 2**). The plankton sampling almost covered the whole 24 hours' day-time period. A total of 152 PairoVET and CTD stations were carried out and 152 CUFES samples were collected. According to the presence of anchovy eggs in the CUFES samples collected over the 200 meters depth, 13 of the 21 planned transects were extended.
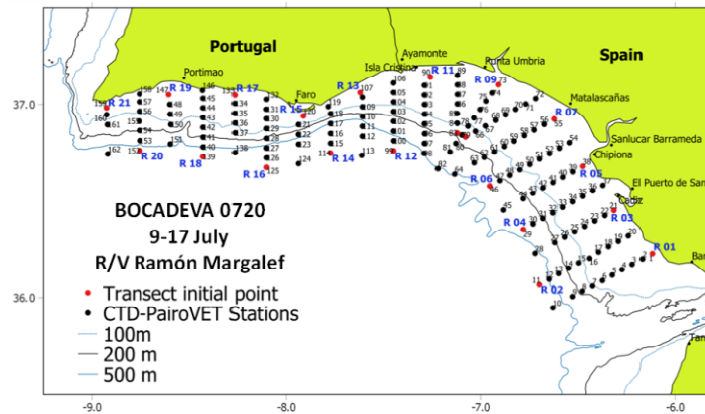
Figure 2. BOCADEVA 0720 survey. PairoVET, CUFES and CTD stations.

The samples to estimate adult parameters (sex ratio, female mean weight, batch fecundity and spawning fraction) were obtained in the acoustic survey "ECOCADIZ 2020-07". In total 21 fishing hauls (**Figure 3**) which caught anchovies were performed during the acoustic survey, complemented by 4 samples conducted by night aimed at the collection of anchovy females with hydrated egg. On the whole, almost 1691 anchovys were sampled, more than 686 ovaries were collected and *ca.* 1677 otoliths were removed for age determination. A total of 180 hydrated females were caught for batch fecundity estimation.
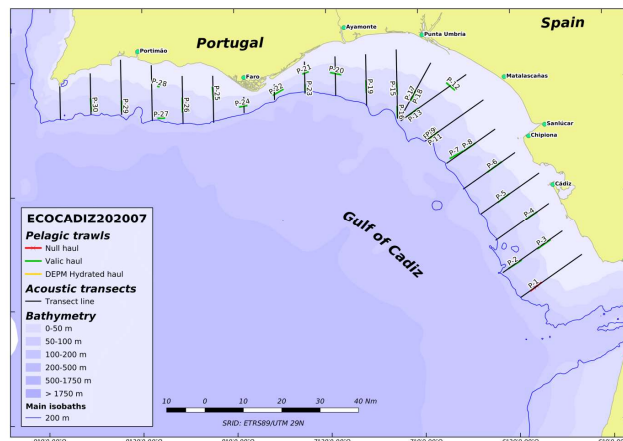
Figure 3. Spatial distribution of fishing hauls in *ECOCADIZ 2020-07* survey.

3.2.   Laboratorial analysis

At present, PairoVET samples from net 1, have already been sorted, eggs and larvae counted and the anchovy eggs staged according to the 11 stages of development classification. PairoVET samples from net 2 have been sorted, but anchovy eggs are being counted and classified in 11 developmental stages. The laboratory analyses for the CUFES samples, is still underway.

Regarding the adult samples, the histology processing for the ovary samples is not completed yet and is still in progress.

4.   Remarks

Due to COVID-19 constraints, the scientific crew on board was reduced and consequently, the plankton samples, from both CUFES and PairoVET samplers, were not processed on board as usually. At present, the plankton samples are being analysed in the laboratory and also the histology processing for the ovary samples is still in progress. At the laboratory, histology processing for ovary samples is also being affected by a decrease in technical staff due to COVID-19 health crisis. Once the laboratory work is finished, eggs and adults parameters will be estimated in order to provide the SSB for anchovy through the application of the DEPM.

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