

Article

Evaluation of Anchovy Egg Density: A Study on Differences Between Vertical and Oblique Towing Samplers for Ichthyoplankton

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Abstract: The use of multiple sampling instruments during ichthyoplankton surveys could require a significant amount of time for sample collection procedures; moreover, their use is highly dependent on weather conditions. During surveys aimed at the application of the Daily Egg Production Method (DEPM), two different kinds of sampler are employed: PairoVET and Bongo 40. The possibility of using only one of these samplers may allow for a reduction in the sampling time on board and the subsequent analyses; thus, a study was carried out to test the use of the Bongo 40 sampler alone (i.e., without PairoVET sampling) in DEPM application. Samples collected during five ichthyoplankton surveys (from 2007 to 2010 and in 2014) were analyzed to compare the efficiency in anchovy egg collection. Although the compared ichthyoplankton samplers provided differences in the collected number of eggs, as well as in the filtered volume, non-significant differences between them were recorded in the egg density. An ANCOVA revealed a significant relationship ($F_{1,5} = 606.80$; $p < 0.001$) between the density estimates from the two nets, but no differences were recorded among years ($F_{1,5} = 0.99$; $p < 1$). The slopes of the linear regressions for the two datasets were close to 1, suggesting a substantial equivalence of the two samplers in estimating the egg density. Finally, since only a few sampling stations showed densities higher than 20 ind. m^{-2} , conversion equations were provided to estimate the PairoVET density from the Bongo 40 data at different density levels (i.e., 20 ind. m^{-2} or higher).

Keywords: Bongo 40; PairoVET; water volume; DEPM; *Engraulis encrasicolus*



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1. Introduction

Onboard surveys are essential tools for studying the early life stages of fish, whether the goal is to observe recruitment variability or to estimate spawning stock abundance using ichthyoplankton methods [1–4]. Conventional methods, such as towed nets at preset sampling stations, have limitations in their accuracy, precision, and sensitivity and are labor-intensive and expensive [5]. Furthermore, these sampling surveys require the dedicated use of a research vessel, often hindered by severe weather conditions, and produce numerous samples that need to be analyzed ashore. Difficulties in obtaining representative ichthyoplankton samples also arise when the study areas are very heterogeneous and less productive, such as the eastern and central-southern Mediterranean Sea [6,7].

The daily egg production method (DEPM [8]) is one of the main tools used to study the reproductive ecology of small pelagic species and to estimate the spawning biomass of

fish stocks. In the Strait of Sicily, DEPM surveys have been performed annually on anchovy stock since 2005 [7], because these surveys have provided robust data on reproductive traits, such as spawning fraction, batch fecundity, sex ratio, maturation cycle, and gonad atresia [7,9–11]. Moreover, onboard surveys are well known to produce useful information on the relationship between habitat and the reproductive biology and ecology of monitored small pelagic stocks, for both adult [10,12,13] and early life stages [14,15].

Generally, plankton sampling includes the 0–100 m layer, and the distribution of anchovy eggs and larvae is restricted to the top of the water column, where concentration peaks occur in the upper 20 m, though distinct subsurface peaks often depend on local physical conditions ([6] and references therein). The vertical CalVET or PairoVET nets are sampling tools specially developed for the purposes of DEPM application to Californian anchovy populations, and they are still employed [16,17]. However, the Bongo 40 (B40; oblique operating) or Working Party 2 (WP2; vertical operating) nets were successfully used to obtain adequate samples for DEPM applications to different species and areas [18,19], including in oligotrophic waters in the Mediterranean Sea, where anchovy egg production is lower than in the oceanic upwelling areas [6,20].

European anchovy, *Engraulis encrasicolus* (Linneus, 1758), belonging to the Engraulidae family, is one of the highly exploited resources [21] and accounts for significant biomass in the Mediterranean Sea, playing a key role in maintaining ecological processes in pelagic ecosystems [7,22,23], due to its essential intermediate trophic level in food webs [24]. This species is an indeterminate spawner that releases pelagic eggs during a protracted spawning season, which in the Strait of Sicily ranges from April to October, with a peak during summer [7]. The European anchovy is assessed within the EU data collection framework [25], to produce management plans for sustainable stock exploitation by predictive models, accounting for several biological parameters such as population structure, growth, and size at maturity [26–28]. To this aim, anchovy stocks are monitored along European coasts by both acoustic and DEPM surveys [7,10,29].

European anchovy egg occurrence and oceanographic data from summer surveys were examined to understand the mechanisms that control the spatial distribution of anchovy spawning habitat in the Strait of Sicily [30]. Ichthyoplankton distribution shows a high degree of dispersal and patchiness that may create significant challenges when trying to obtain a representative sample of a population. The sampling scheme must be designed to resolve the main physical features that govern the spatial distribution of the organisms of interest and be carried out quickly enough to avoid significant changes in distribution over the course of the survey [5]. The DEPM sampling should obtain representative samples of eggs, but also of larvae, of the target species, as well as the associated planktonic community where these eggs develop and hatch. However, often during ichthyoplankton sampling at sea, there is the need to reduce the sampling effort while safeguarding the quality and the representativeness of collected samples. The use of both samplers (Bongo 40 and PairoVET) within the sampling station grid is recommended for DEPM applications, even though the greater information obtainable from oblique plankton tows may favor the use of the Bongo net. It is well known that the B40 net is more efficient for sampling fish larvae as well, while CalVET and PairoVET (PV) nets are usually employed for fish eggs [16]. The main difference between these samplers lies in their trajectory in the water column. In the present study, vertical tows are carried out with the PV and oblique tows are carried out with the B40 at a speed of 2 knots. Finally, another difference is represented by the areas covered by the two nets. The samples provided by these nets are affected in different ways by the distribution of the planktonic organisms, with the PV catches being more sensitive to small and fine-scale patchiness. Despite previous tests on the performance of different plankton nets [31–33], very few studies have provided detailed information to compare

these two plankton samplers (e.g., [34]). Further, the great heterogeneity and habitat variability within the Mediterranean Sea strongly suggest to compare their performances in specific areas, where DEPM is routinely applied, as in the case of the Strait of Sicily [7]. To fulfill this gap, the present study analyzed a time series of anchovy egg data collected simultaneously by two different plankton samplers, the PV and the B40. Particularly, the comparability of egg density data from these two nets was assessed, in order to compare their accuracy for sampling in oligotrophic waters.

2. Material and Methods

2.1. Sampling and Analysis of Eggs

Multidisciplinary surveys took place on the continental shelf (depth < 200 m) of the northern part of the Strait of Sicily (Central Mediterranean Sea; Figure 1) during the summer period (July–August), when anchovy reproductive season reaches its peak [7]. Data on egg density and water volume were collected during five years (2007, 2008, 2009, 2010, and 2014) by two types of plankton nets (PV and B40), and each gear was equipped with two conical nets. Each mouth was instrumented with a calibrated General Oceanics mechanical flowmeter, balanced for dynamic stability and working with a high-resolution rotor for low-speed applications, as in the case of the present sampling. The sampling was carried out at the same locations, i.e., each PV sampling station represents the starting point for each B40 sample. Ichthyoplankton was collected with the B40 net towed obliquely from the surface to 100 m (where possible, according to the depth) and back to the surface, with a vessel speed of 2 knots. The ideal operational angle between the cable supporting the gear and the sea surface was 45° for optimizing the sampling collection by the B40 net. This angle was continuously monitored by expert operators working onboard, during the sampling station period, and eventually corrected if needed. This gear has two mouths with an opening diameter of 40 cm and a $200\ \mu\text{m}$ filter mesh. In each station of the sampling grid, with the vessel stationary, the PV net was brought vertically from the surface to the maximum depth (i.e., 100 m where possible or close to the bottom). The PV conical nets have a filtering mesh of $150\ \mu\text{m}$, the diameter of the two mouths is 25 cm, and the descent speed is about 1 m/s, which was constantly monitored by the research vessel crew operating with a speed-controlled winch. The biological sample was collected only during the ascent phase from maximum depth to the surface.

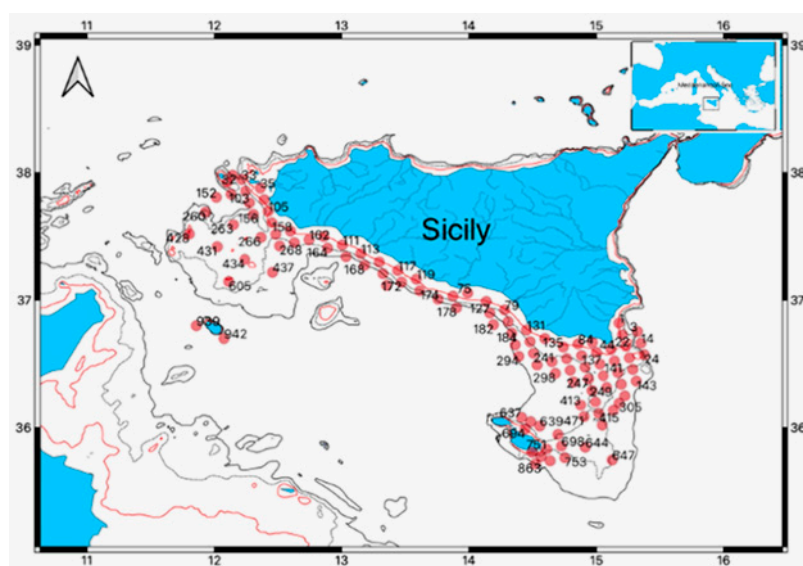


Figure 1. The study area, over the continental shelf of the Strait of Sicily. The red dots represent the sampling stations for both nets, Bongo 40 and PairoVET.

Over the study period, biological samples collected at 493 stations were analyzed; their distribution per survey is presented in Table 1. Samples from both samplers were fixed in 4% buffered formalin for subsequent laboratory analyses, where they were observed under a stereomicroscope for identifying eggs of the target species. For both PV and B40, the egg catches were standardized to m^2 by estimating the egg density in each sample according to Houde [2]:

$$\text{Egg Density} = 0.1 * N^{\circ} \text{ Eggs} * \frac{\text{Depth}}{\text{Volume}} \quad (1)$$

Table 1. Information on the samples collected by the two samplers (PairoVET, PV, and Bongo 40, B40) during the study period (2007, 2008, 2009, 2010, 2014).

	2007	2008	2009	2010	2014
N° Stations sampled	110	127	78	82	96
N° Egg PV	180	234	145	505	46
N° Egg B40	1330	1146	887	2826	305
Average density PV (#eggs m^{-2})	1.72	1.84	1.88	6.32	0.47
Average density B40 (#eggs m^{-2})	3.39	3.37	2.98	5.07	1.39
N° of PV stations with 0 eggs	77	86	53	27	83
N° of B40 stations with 0 eggs	58	83	41	8	69
N° stations with PV > 0 and B40 = 0	5	10	3	1	3
N° stations with B40 > 0 and PV = 0	24 (22%)	13 (10%)	15 (19%)	20 (25%)	17 (18%)

2.2. Statistical Analysis

In order to compare both filtered water volume and egg density between the two samplers, data were fitted using linear regression models. The negative stations (i.e., stations in which no anchovy eggs were caught by either net) were not included in these models [34].

Outliers in the data can distort predictions and compromise accuracy, especially in regression models, if they are not detected and appropriately handled. A multivariate approach was adopted for egg densities in each year to identify possible outliers. The advantage of a multivariate approach was linked to the use of Cook's distance (threshold 0.5). This method allowed for the detection of outliers, specifically, by assessing how much each observation affected the fitted values and identifying those that might alter the fitting linearity. Therefore, the regression analysis was performed on stations with an egg count greater than 0 (#egg > 0) in at least one of the samplers, after excluding the identified outliers.

Finally, to test for possible secondary effects on the linear regression model, an AN-COVA was run on the density values from the B40 and PV, including year as a factor and the interaction terms as the main effects. The regression analysis and all other computations were performed in the R statistical environment [35].

3. Results

Results for the analyzed plankton time series are summarized in Table 2, providing various descriptive statistics, including Pennington's estimators of the mean, the variance, and the variance of the mean, which are efficient descriptors for fish and plankton surveys [36].

Table 2. Basic statistics of the Bongo 40 (B40) and PairoVET (PV) samples used in the regression analysis excluding double 0 stations and outliers (256 stations). EggPV: number of eggs collected by PairoVET. EggB40: number of eggs collected by Bongo 40. VolPV: volume of water (m³) filtered by PairoVET. VolB40: volume of water (m³) filtered by Bongo 40. DepthPV: depth reached during sampling by PairoVET. DepthB40: depth reached during sampling by Bongo 40. densPV: density of eggs in PairoVET samples. densB40: density of eggs in Bongo 40 samples.

	EggPV	EggB40	VolPV	VolB40	DepthPV	DepthB40	densPV	densB40
n° stations	256	256	256	256	256	256	256	256
nbr.null	89	22	0	0	0	0	89	22
pos.median	3	11	8	64	85	81	3	1
pos.mean	7	28	8	62	78	77	7	4
pos.var	123	2717	6	398	510	505	132	51
pos.std.dev	11	52	2	20	23	22	11	7
geo.mean	3	10	7	59	73	72	3	1
pen.mean	4	26	8	63	79	78	4	3
pen.var	55	5076	11	593	1008	938	58	97
pen.std.dev	7	71	3	24	32	31	8	10
pen.mean.var	0.2	12	0.04	2	4	4	0.2	0.2

nbr.null = numbers of null stations; pos.median = median of positive stations for eggs; pos.mean = mean of positive stations for eggs; pos.var = variance of positive stations for eggs; pos. std.dev = standard deviation of positive stations for eggs; geo.mean = geometric mean; pen.mean = mean of Pennington's estimators; pe.var = variance of Pennington's estimators; pen.std.dev = standard deviation of Pennington's estimators; pen.mean.var = variance of mean of Pennington's estimators.

The ichthyoplankton samplers we used showed differences in the absolute number of eggs collected, as well as in the volume filtered, while the mean depth was similar (Table 2, Figure 2). The comparison of tow depth data showed a similar depth range reached by both samplers (Table 2).

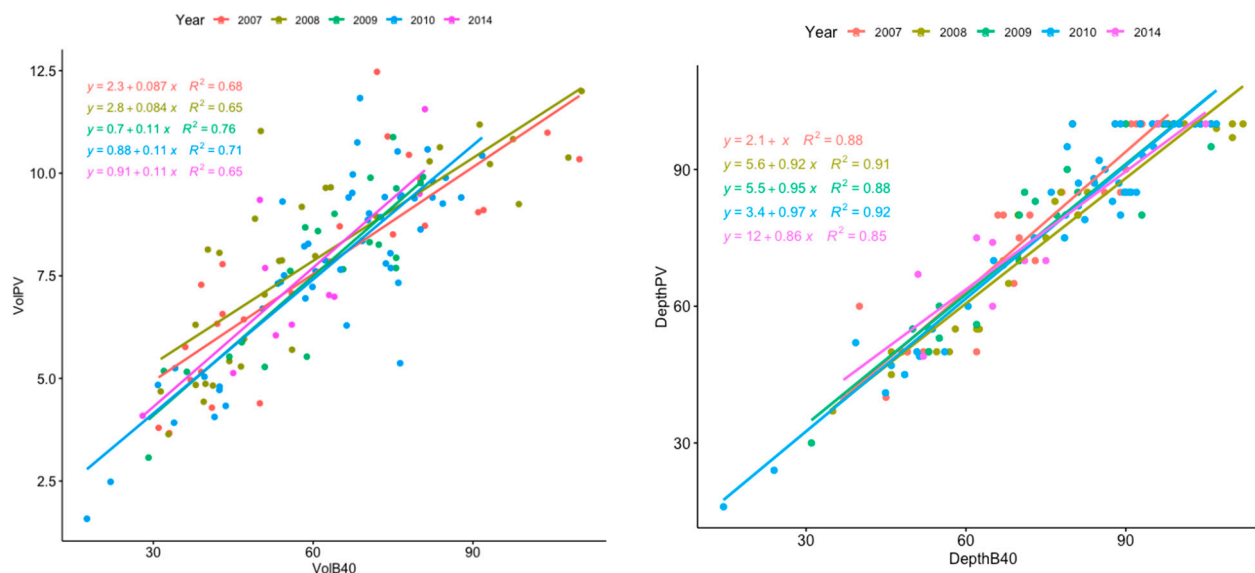


Figure 2. Relationship between the filtered volumes (left panel) and the achieved depth (right panel) of the two nets (PairoVET, PV, and Bongo 40, B40) per year. The graphs show the data (points) and linear regression for each study year, as well as the relative equation and R² value ($p < 0.001$). The legend shows the different colors according to the year of sampling.

Filtered volumes by the two samplers showed a great difference in values; generally, the mean B40 values were at least seven times higher than the volume sampled by the PV (Table 2). As expected, while the average filtered volumes differed between PV and B40, the standardized square meter densities were quite similar (Table 2, Figure 2). Interannual

variability of linear regression models showed some differences in 2007 and 2008 (Figure 2), although the ANOVA revealed that such differences were not significant ($p < 0.1$; Table 3). In contrast, interannual variability with depth of the linear regression models appeared significant (Figure 2), as confirmed by the ANOVA test ($p < 0.01$; Table 3).

Table 3. Analysis of variance (ANOVA) to test for variability in (A) volumes of water filtered (Vol); (B) sampling depths achieved (Depth).

(A)						
	DF	Sum Sq	MeanSq	F value	Pr(>F)	
VolB40	1	1011.82	1011.82	612.8557	$<2.00 \times 10^{-16}$	***
Year	4	13.87	3.47	2.0998	0.08143	.
VolB40: Year	4	19.4	4.85	2.9373	0.02127	*
Residuals	246	406.15	1.65			
(B)						
DepthB40	1	118,053	118,053	2622.0383	$<2.20 \times 10^{-16}$	***
Year	4	737	184	4.0921	0.003141	**
DepthB40: Year	4	257	64	1.4263	0.225779	
Residuals	246	11,076	45			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Over the study period, the stations where at least one of the two samplers collected anchovy eggs were 256 out of 493 samples (Tables 1 and 2). As previously mentioned, those stations with 0 anchovy eggs in both samplers were excluded ($n = 111$), since they cannot be used as an element of similarity. Therefore, the dataset used for the regression analyses included 145 samples. The ANCOVA performed on egg density between the two gears, with PV as the response variable and B40 as the predictor, showed a significant relationship but no differences between years (Table 4). Since the intercept was not significantly different from 0, it was set to 0 in the final linear regression model. Moreover, since the Cook's distance index for influential observations presented values higher than the chosen threshold (i.e., 0.5), mainly for those stations with a density higher than 20 ind. m^{-2} , and considering these were very few stations (i.e., 7) (Figure 3A,C), a new subset was obtained by excluding the higher density stations (Figure 3B,D). The regression models obtained by each dataset (overall and low-density subset—i.e., <20 ind. m^{-2}) were both statistically significant, and the slopes of the estimated linear models were 1.17 ± 0.05 ($t = 23.9$, $p < 0.001$) overall and 0.97 ± 0.07 ($t = 14.1$, $p < 0.001$) for stations with densities lower than 20 ind. m^{-2} , explaining 76% and 64% of the total variance, respectively (see Table 5 and its caption for more details).

Table 4. ANCOVA on egg density (dens) between the two gears, PairoVET (PV) and Bongo 40 (B40): densPV = response variable; densB40 = predictor; Year = factor. Intercept set to 0.

	Sum of sq	Df	F Value	Pr(>F)	Significance
densB40	16,503.8	1	606.7974	2.00×10^{-16}	***
Year	135.4	5	0.9956	0.42098	
densB40:Year	501.4	4	4.6092	0.00132	**
Residuals	6690.7	246			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Table 5. (A) Regression parameter estimation of egg density estimates for all the positive (#egg > 0) Bongo 40 stations (densB40) [Residual standard error: 5.364 with 250 degrees of freedom; adjusted R-squared: 0.7505; F-statistic: 129.4; p -value: $<2.2 \times 10^{-16}$]. (B) Regression parameter estimation for the subset of stations with Bongo 40 density (densB40₂₀) values less than 20 ind. m⁻² [Residual standard error: 3.475 with 243 degrees of freedom; adjusted R-squared: 0.6253; F-statistic: 70.27; p -value: $<2.2 \times 10^{-16}$].

(A)					
Coefficients:	Estimate	Std. Error	t Value	Pr(> t)	Significance
densB40	1.17927	0.04924	23.951	$<2 \times 10^{-16}$	***
Year 2007	−0.32852	0.72656	−0.452	0.652	
Year 2008	1.07954	0.74234	1.454	0.147	
Year 2009	0.41789	0.85886	0.487	0.627	
Year 2010	1.0204	0.66644	1.531	0.127	
Year 2014	0.01232	0.98121	0.013	0.99	
<20 ind. m ⁻²					
(B)					
densB40 ₂₀	0.97452	0.06893	14.137	$<2 \times 10^{-16}$	***
Year 2007	0.23296	0.48622	0.479	0.63229	
Year 2008	1.51174	0.49425	3.059	0.00247	**
Year 2009	0.9824	0.5814	1.69	0.09237	.
Year 2010	1.09127	0.4698	2.323	0.02102	*
Year 2014	0.2691	0.64033	0.42	0.67467	
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.					

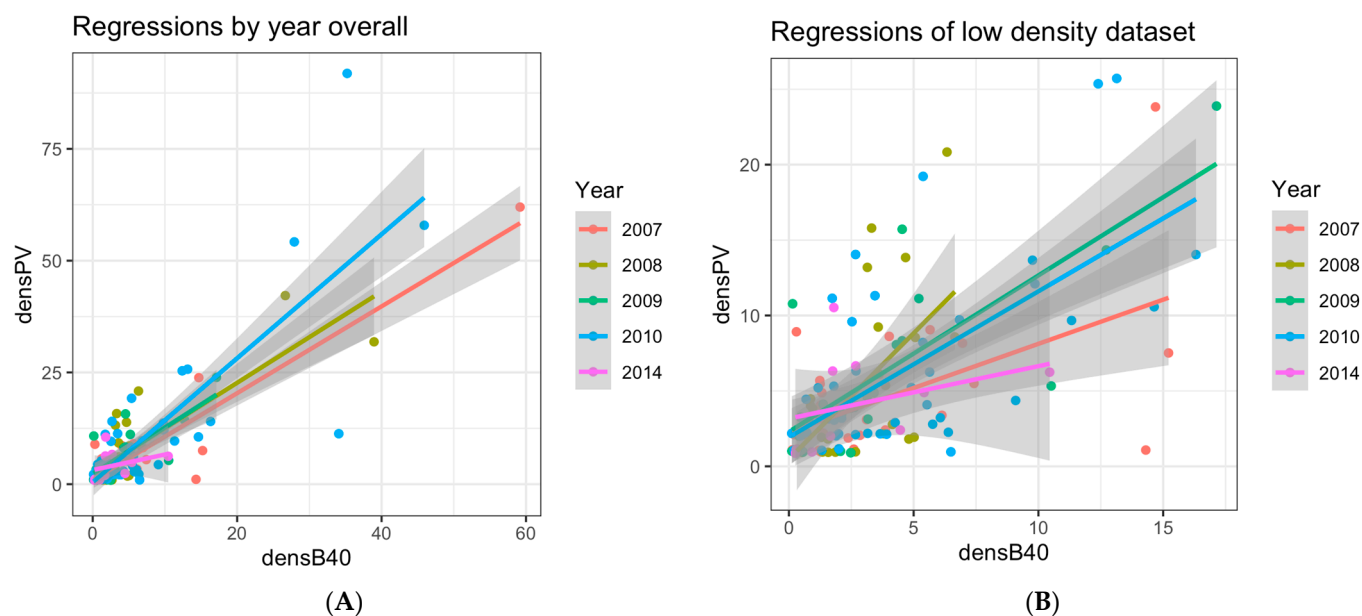


Figure 3. Cont.

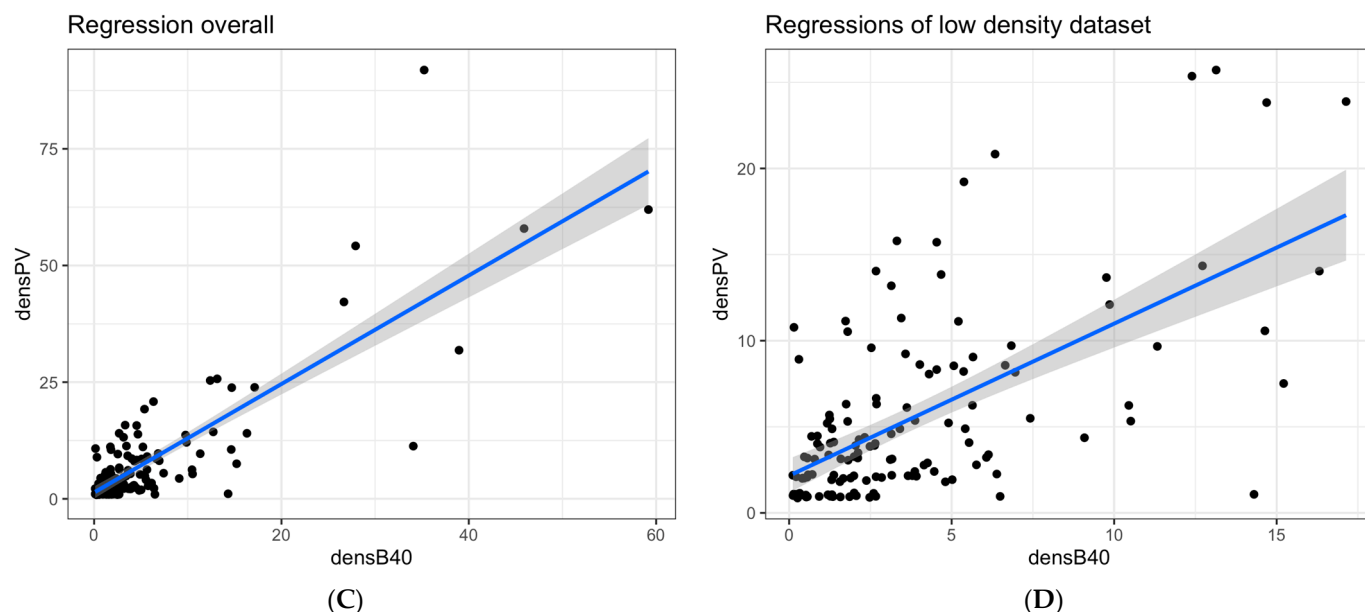


Figure 3. Scatterplot of egg density (#eggs m^{-2}) values in the PairoVET (densPV) and Bongo40 (densB40) with their confidence intervals superimposed. (A) Scatterplot of egg density from the whole dataset by sampling year (2007, 2008, 2009, 2010, 2014). (B) Scatterplot of egg density from the stations with density less than 20 ind. m^{-2} by sampling year (2007, 2008, 2009, 2010, 2014); the legend for panels A and B shows the different colors corresponding to the sampling year. (C) Scatterplot of egg density from the whole dataset for all years combined. (D) Scatterplot of egg density from the stations with density lower than 20 ind. m^{-2} for all years combined. Within all the four panels, the gray areas (i.e., the shaded areas) represent the confidence interval.

4. Discussion

Monitoring anchovy populations has been regularly carried out during scientific expeditions in the Mediterranean Sea and Atlantic Ocean to provide spatio-temporal insights into the occurrences and abundances of anchovy adults and early life stages. This monitoring may shed light on the recently recorded diminished reproductive potential [37]. The study results highlighted a general interannual variability and a negative trend in egg and larva abundance, which may be linked to both overexploitation [38] and environmental variability experienced by spawning adults and early life stages [7,39,40]. For the purposes of this study, the sampling period took place during the peak of the anchovy reproductive season [7,12] for a dual advantage. Indeed, when the DEPM is applied during the spawning period, the estimates of spawning stock biomass are considered representative of the entire stock biomass, since almost the entire population is involved in reproduction. Secondly, the reproductive peak results in a higher concentration of spawned eggs in the water column, allowing for optimal sampling by any type of ichthyoplankton sampler [1]. The comparison between PV vertical and B40 oblique tows carried out in the study area showed no significant differences in the mean density of anchovy eggs, with a small effect on this relationship related to the year of sampling. However, the interannual variance in egg production recorded during the study period did not affect the relationship between the two samplers, although some fluctuations among years could be introduced by the intrinsic variability in field sampling due to sea weather conditions, navigation requirements and similar tasks, as well as the ability of fish populations to modify their spatial distribution depending on ecosystem characteristics and density-dependent behavior ([29] and references therein).

Comparisons of several kinds of plankton samplers have been carried out for different purposes in recent decades. The sampling nets were tested for quantitative and/or qualitative efficiency regarding mesh size, species representativeness, species composition,

and larvae size [41–45]. Hydrodynamic experiments, as well as comparisons of sampler structure efficiency, were carried out to enhance stability and accuracy in ichthyoplankton surveys [46,47]. Nevertheless, few comparisons are available on ichthyoplankton sampling for DEPM, although an application on Baltic cod (*Gadus morhua*) used data from both a Bongo net and a vertical net (i.e., IKS-80 net), as no differences in catchability between these nets were previously proven [18]. The slopes of the linear regressions for both datasets (overall and lower densities) examined here were close to 1, suggesting a substantial equivalence between the two samplers in obtaining egg density values, particularly for the lower density range (0–20 ind. m⁻²). This suggests that the data could be used interchangeably to estimate egg production and the related parameters needed for DEPM application, despite the differences that occurred between the two nets in other parameters, such as filtered volume. The efficiency of the B40 in filtering a larger volume of water than the vertical ichthyoplankton nets has already been documented in the literature [6]. The oblique trajectories of the Bongo net could also explain the difference in depth reached. These aspects of variability were minimized by the standardization per unit area (m²) of the number of sampled eggs and by considering that both types of sampling are performed in the upper water layers (i.e., in the egg concentration zone of the target species) [6,41]. Moreover, the present results yielded statistically significant regression models, which explain much of the variability between the two samplers and, therefore, should be used to make spawning biomass estimates by DEPM comparable, when employing these two sampling tools (Table 5).

The CalVET net was specially designed to minimize sampling effort and post-processing time, reducing the standard error of DEPM estimates in a highly productive system, as occurs in California waters [16]. In the Bay of Biscay, for DEPM applications in European oceanic waters, the vertical sampler was changed from a CalVET (one sampling mouth) to a PairoVET (two sampling mouths) because doubling the effective sampling reduced errors, and its applications still proved good efficiency [17], also compared with a Continuous Underway Fish Egg Sample (CUFES) [48]. Similarly, it could be expected that in low-productivity regions, like several Mediterranean areas [49], the PV may produce a sampling bias in bordering the spawning ground, as well as in the estimate of the egg mortality curve, which could be reduced by the B40. In the present study, the B40 was observed to detect anchovy eggs at many more stations compared to the PV at the same location and time (Table 2).

Oblique B40 tows have been criticized in terms of their suitability for DEPM application [33] because of the vertical distribution of anchovy eggs and their high concentrations in the upper layers (sub-surface); thus, oblique tows may result in oversampling compared to vertical nets. However, a horizontal sampler proved more efficient than a vertical net in a recent study on ichthyoplankton assemblages in the Aegean Sea [50]. The distribution of anchovy early life stages in the Mediterranean Sea, and particularly eggs in the study area, appeared to be mainly concentrated in the upper 20 m depth layer [51,52], suggesting that both nets covered the layer of egg distribution, although they may reach different sampling depths. Moreover, previous DEPM applications in Mediterranean waters adopted the B40, observing that both vertical and oblique tows yielded consistent, compatible estimates of ichthyoplankton production [6,34].

In the present study, the B40 showed higher anchovy egg abundance and reduced variance in the samples (Table 1), and it allowed for better delimitation of the spawning area, since the number of stations with 0 values (i.e., no egg found) was lower than with the PV throughout the study period and overall (Tables 1 and 2). This evidence is in agreement with a previous study that used the B40 to predict the anchovy spawning habitat [14]. Moreover, the samples were affected differently by the distribution of the

planktonic organisms, with the PairoVET catches being more sensitive to small and fine-scale patchiness [6], which is the usual ichthyoplankton distribution [5]. Regarding the filtration efficiency of the PV, the cylindrical portion reduces the probability of net clogging during a single tow compared to the B40, but when equipped with an accurate flowmeter, such a problem could be avoided.

The B40 may be preferred, especially in areas with lower egg abundances compared to oceanic waters or in years with reduced egg densities. However, the years with higher egg densities (i.e., 2007 and 2010) also showed a higher proportion of positive stations with the B40, highlighting an overall performance 19% higher than the PV in catching anchovy eggs (Table 1). Although no larva data were available in the present study, it is well known that the B40 is more efficient than the PV for sampling fish larvae. Thus, the Bongo net allows for the sampling of early-hatched larvae, which could be used to obtain a more accurate mortality curve for eggs, substantially improving the precision of daily egg production estimate [6].

5. Conclusions

The results of this comparative experiment showed an agreement between the egg density data provided by the two samplers, suggesting the use of the Bongo 40 instead of PairoVET for DEPM applications, when only one plankton sampler is deployed. Especially in oligotrophic areas such as the Mediterranean Sea, where egg densities are lower than in oceanic waters, the B40 allows for an increase in the probability of capturing anchovy eggs, providing more data on the boundaries of the spawning area and the daily egg production.

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