

Hydroacoustics for density and biomass estimations in aquaculture ponds

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ABSTRACT

The use of hydroacoustics is currently being studied and developed as a promising non-intrusive methodology to monitor and manage fish stocks in aquaculture farms. The main objective of this study was to develop an acoustic method for the estimation of fish density and biomass in inland aquaculture farms and test the accuracy and precision of the estimates with real data provided by the company. The study was conducted in sea bass (*Dicentrarchus labrax*) production ponds located in Seville (Southern Spain). A Simrad EK60 echosounder with two split-beam circular transducers operating simultaneously at 200 kHz was used for hydroacoustic surveys. Two different hydroacoustic designs were considered: central trajectories and zigzag trajectories. The accuracy and precision of the estimates were examined in order to select the best sampling design. Due to a non-homogeneous fish distribution in the pond caused by the avoidance behaviour, as a response to the sampling disturbance presented by fish, acoustic density and biomass were corrected by applying sampling theory according to the probability of fish detection. When density and biomass were corrected, the estimates became highly accurate and precise with respect to real data, which confirms that the proposed method is adequate. Similarly, acoustic estimates of fish weight were highly in agreement with real data, due to the use of specific equations developed “in situ” for the study. Although no significant differences were recorded in the density and biomass estimates with regard to the trajectory used (central vs. zigzag), it was observed that the most accurate agreement and precision were always obtained in central trajectories. Therefore, central design is proposed as the most appropriate design for hydroacoustic measurements in inland ponds. The results obtained in this study provide estimates of density and biomass that accurately match the real data, supporting the use of hydroacoustics as a potentially valid tool to manage inland aquaculture farms.

1. Introduction

Aquaculture is a growing sector in Spain and other countries. In southern countries, sea bass (*Dicentrarchus labrax*), gilt-head sea bream (*Sparus aurata*), and turbot (*Psetta maxima*) are the most produced species, amounting to over 80% of the total market (Apromar, 2018). Most of these come from offshore facilities. Only a small percentage of them comes from inland aquaculture farms, although this sector is expanding (Espinosa et al., 2015; FAO, 2016; Magalhães et al., 2017; Rodríguez-Sánchez et al., 2018). In order to be properly managed, aquaculture companies require accurate data of the abundance, biomass and average weight of the fish farmed in their facilities. The more accurate these data are, the better the decision-making for the fish farm will be in terms of feed requirements, growth rate and food conversion calculations, medication administration, early detection of fish losses due to deaths,

robberies or escapes, splitting of farming units, economic forecasting, etc. (McCallum, 2005; Soliveres et al., 2014; Pérez et al., 2015; Hofmeester et al., 2016; Fore et al., 2018).

Production management techniques currently used are highly intrusive and do not offer the accuracy required to avoid inefficient operations in fish farms. These techniques involve an excessive handling of fish resulting in negative effects upon them, such as stress, deterioration of their immune system, decreased appetite and growth rate, diseases, etc. (Hatzithanasiou et al., 2002; HSUS, 2008; Di Marco et al., 2017; Li et al., 2020). Furthermore, these processes are highly laborious and increase production costs. Thus, one of the main goals in the aquaculture sector is to develop a more effective and profitable method that is easy to implement and maintain in order to monitor fish growth and evaluate their biomass. Different alternatives are currently being studied and developed where non-intrusive methodologies are used to

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monitor fish stock in aquaculture facilities. One of these non-intrusive methodologies consists of using hydroacoustic techniques to estimate fish abundance and biomass in production units. Hydroacoustics has become a technique which provides accurate and robust estimates of population density with an adequate balance between costs and results (Mehner and Schulz, 2002; Mackinson et al., 2004; Boswell et al., 2007; Koslow, 2009; Trenkel et al., 2011; Cushing, 2013; Zenone et al., 2017; Egerton et al., 2018; Føre et al., 2018). One of the most interesting advantages of applying this technique to aquaculture is that it is a non-invasive technique that allows for the estimation of fish abundance without any manipulation. In order to achieve this, this technique applies echosounders, which transmit a sound pulse of known characteristics within a water column and record the characteristics of the sound or echo returned by the transducer. The intensity of the returned echoes can be translated into estimates of fish density in the ensonified volume by applying the appropriate acoustic conversion equations (Simmonds and MacLennan, 2005; Knudsen, 2009; Winfeld et al., 2009; Cox et al., 2011).

At present, hydroacoustic exploration of inland farm ponds poses an unprecedented challenge as a method to obtain reliable estimates of fish abundance and biomass. In previous studies, hydroacoustics has been used to estimate fish density in open sea cages with vertical hydroacoustics (placing the transducer with the main beam perpendicular to the water surface) (Espinosa et al., 2002; Espinosa et al., 2006; Espinosa et al., 2015; Knudsen et al., 2004; De La Gándara and Espinosa, 2012; Soliveres et al., 2010; Soliveres et al., 2014; Soliveres, 2015). However, inland aquaculture ponds are shallow and as such, vertical hydroacoustics cannot be applied. Thus, horizontal hydroacoustics (placing the transducer with the main beam parallel to the water surface) must be used in these environments. Horizontal hydroacoustics works well in shallow systems, but its requirements are different from those of vertical hydroacoustics (Kubečka et al., 2000; Yule, 2000; Balk, 2001; Boswell et al., 2007; Draštik et al., 2009; György et al., 2012; Zenone et al., 2017; Johnson et al., 2019). Foremost among these, fish size estimates change depending on the fish aspect i.e. the swimming angle of the fish with respect to the axis of the transducer (Baran et al., 2017). Therefore, to estimate fish size, equations where these variations are considered must be used or developed (Lilja et al., 2000; Frouzová et al., 2005; Boswell et al., 2009; Furusawa and Amasaku, 2010; Rodríguez-Sánchez et al., 2015; Rodríguez-Sánchez et al., 2016a; Rodríguez-Sánchez et al., 2016b; Balk et al., 2017; Rodríguez-Sánchez et al., 2018).

Other relevant problems when studying fish populations in farming ponds are not directly derived from hydroacoustics, but they are inherent to the sampling of wild animal populations in their natural habitats. Several authors have highlighted that the sampling has a direct or indirect effect on fish detectability and, ultimately, on its abundance or biomass estimation (Brehmer et al., 2004; Ona et al., 2007; MacNeil et al., 2008; Guillard et al., 2010; Kulbicki et al., 2010; Bozec et al., 2011; Kaartvedt et al., 2012; Glennie et al., 2015; Prato et al., 2017; Pais and Cabral, 2018; Brehmer et al., 2019). Specifically, two types of bias can occur in acoustic explorations: 1) positive bias, when fish move toward the transducer which leads to an overestimation of abundance and/or biomass; 2) negative bias, when fish move in the opposite direction of the transducer (avoidance), which leads to an underestimation of density and biomass. Avoidance is the most frequent behaviour, and its intensity can change from species to species (Lucas et al., 2002; Vabø et al., 2002; Gerlotto et al., 2004; Brehmer et al., 2019).

When fish distribution in the explored systems is uniform, density and biomass adjust efficiently to those provided by the echosounder based on the volume or area ensonified by the transducer (Draštik and Kubečka, 2005). However, a non-uniform distribution can lead to biased density and biomass results, as may occur due to the shock of sampling on the fish population (Mitson and Knudsen, 2003; Jørgensen et al., 2004; Marques, 2004; Marques, 2009; Marques et al., 2013; De Robertis and Wilson, 2010; De Robertis et al., 2010; De Robertis and Handegard, 2013). In these cases, it is crucial to correct the bias in the data to obtain

correct density and biomass estimates. This is especially important in inland production ponds since they are extremely shallow and relatively small (compared to a natural ecosystem) and, therefore, fish can be found in high densities, tending to gather themselves in groups (Zhao and Ona, 2003; Draštik and Kubečka, 2005; Godlewska et al., 2009; Wheeland and Rose, 2015). Likewise, they are highly likely to present unwanted behaviour during the sampling process, such as escapes or burials, which results in a non-uniform distribution in relation to the transducer. Therefore, it is necessary to verify the existence of density gradients which occurred during the sampling to add them to the estimation method and thus avoid potential bias in the estimates (Hjellvik et al., 2008; Cox et al., 2011; Marques et al., 2013; Pais and Cabral, 2017).

In light of the above, the motivation behind this study is to contribute not only to the improvement of the aquaculture sector, but also to advance the related scientific and methodological fields. The intention of this study is to develop an estimation method to obtain the density, biomass and average weight of fish farmed in shallow aquaculture facilities using hydroacoustic techniques. The main milestones in this study are: 1) To develop an efficient sampling design for this facility type, 2) to study fish distribution patterns during the hydroacoustic exploration, verifying the existence or not existence of density gradients, 3) To prove a model that includes density gradients to correct the bias caused by a non-uniform distribution during the sampling, 4) to verify the accuracy and precision of the estimates obtained by employing hydroacoustic methods. We generally do not know about the abundance or biomass when estimates are made. Without knowing the true density or biomass of fish in a sampled area, a true accuracy cannot be determined. In our study, actual density data, biomass and average weight from sowing and fish harvesting was provided by the fish farmers for all sampled ponds. In this sense, this experiment presents a great opportunity to validate our hydroacoustic methodology.

Developing a reliable and non-intrusive method to accurately determine density and biomass in aquaculture inland farms will result in better control and management of the fishery production and greater efficiency in the aquaculture sector.

2. Material and methods

This study was conducted in the sea bass production ponds of the company Pesquerías Isla Mayor S.A. (located in Seville, Spain). The ponds were rectangular (230 m × 12 m × 2 m) with a surface area of 2700 m² and a volume of 3150 m³. A total of four production ponds were studied, one of them corresponds to a just planted pond (P-1; small fish), one corresponding to intermediate fish size (P-2) and two corresponding to big fish (P-3 and P-4) that were fished after the hydroacoustic survey. At the end of the acoustic study, the company provided us with reliable abundance data, biomass and average weight of the fish planted in the P-1 pond, those of the fish extracted from P-3 and P-4 ponds, and those estimated from the rutinary control of the P-2 pond. Pond P-1 was surveyed two times because the company supplied information on a high mortality rate occurring in this pond two months after the planting. Although the company did not provide us with new density or biomass data, we thought it would be relevant to include this pond in the study in order to verify if the decrease in the population caused by such deaths could be detected acoustically.

For hydroacoustic surveys, we used a Simrad EK60 echosounder (Simrad Kongsberg Maritime AS, Horten, Norway) with two split-beam circular transducers operating simultaneously at 200 kHz (ES200-7C). Both transducers were mounted on a stainless-steel frame fixed to the side of a boat, with the beam aligned horizontally, perpendicular to the navigation axis, and with each transducer, considered as channel 1 and channel 2, aimed in opposite directions. The positioning of the transducers enabled horizontal sampling, perpendicular to the direction of the boat movement. The sailing speed remained constant at around 6 km·h⁻¹ using a 600 W electric outboard motor. The transducer was

placed 1 m below the surface. The pulse duration was 0.128 ms, and the repetition rate was 10 pings per second. The acoustic unit was calibrated with a calibration copper sphere using the standard calibration method (Simrad, 2004).

Two different hydroacoustic designs were considered to determine the most appropriate one for this approach. These designs were named central design (C) and zigzag design (ZZ). In the central design, the transducer moved straight through the centre of the pond from one end to the other. In the zigzag design, the transducer moved from one end to the other following a zigzag trajectory. Given that the transducers were aimed in opposite directions, the entire pond could be scanned.

Eight to ten passes were recorded in each pond, following the same GPS navigation route for both the central and zigzag designs. Data were stored on a PC and later processed with the Sonar-5 Pro analysis software (Balk and Lindem, 2011). Raw data were converted with the 40logR function. In order to reduce the noise coming from unwanted signals, a threshold of -60 dB was selected. Moreover, a strict criterion was selected to distinguish single targets: a minimum echo duration of 0.80 ms and a maximum of 1.6 ms (rel. to the pulse length). The maximum gain compensation was -3 dB (one-way), and the maximum phase deviation was 5. Target Strength values compensated for angular location in the beam (TS, dB re 1 m²) were used for the analysis. Echo counting method was used and Single Echo Detections (SEDs) were analysed 2 and more metres away from the transducer, avoiding a possible TS deviation caused by the effect of the near field of the fish and the transducer (Tichy et al., 2003; Dawson et al., 2000; Boswell et al., 2009; Rodríguez-Sánchez et al., 2016b; Johnson et al., 2019; Koliada et al., 2019). SEDs within -55 and -25 dB were selected for the analysis since this was the size range of the fish farmed in the ponds.

All of the surveys included in this study exhibited Nv values less than 0.1, corresponding to a 1% probability of accepting multiple targets as single ones, indicating suitable conditions for fish density and biomass estimations (Warner et al., 2002; Ona and Barange, 1999; Sawada et al., 1992).

Sonar-5 Pro provides fish density (hereafter “acoustic density”) both in units of volume (fish/m³) and in units of area (fish/ha). We worked with units of area to make it possible to compare the data collected with those provided by the company. Sonar-5 Pro also allowed us to estimate fish biomass (kg/ha) based on the calculated density by incorporating TS-length and length-weight conversion equations to the program, as well as the average weight of the fish detected. For estimating fish length from acoustic size, we applied the De-convolution method for aspect correction. This method is applied to mobile horizontal survey where fish aspect cannot be obtained from the tracks, assuming random orientation. The SEDs obtained from the echogram are classified as follows, the largest size class contains echoes from the largest fish seen from the side aspect, the second largest size class contains the second largest fish plus the largest fish with some aspect, and so on (Duncan and Kubečka, 1995). The horizontal TS-length (Standard length, SL) and length (SL)-weight (W) conversion equations used in this study were those developed specifically for sea bass in the same facilities by Rodríguez-Sánchez et al. (2018):

$$TS = 27.10 \log SL - 101.23, \text{ for side aspect}$$

$$TS = 26.96 \log SL - 111.42, \text{ for Head and Tail aspects}$$

$$W = 3.50 \cdot 10^{-5} SL^{2.88}$$

with the Standard length in millimetres and the weight in grams.

Before the analysis to estimate the density and biomass of the fish present in the ponds, a study was conducted to verify fish distribution in the ponds since, in case of a non-homogeneous distribution, the estimates must be corrected accordingly.

Firstly, the behavioural response of fish in relation to the movements of the boat from one end to the other within the longitudinal axis of the

pond was checked. The pond was divided into four parts and no statistical differences in terms of fish density were detected between these divisions.

However, differences were found in the transversal axis. For that reason, the acoustic density and biomass approach proposed by Draštik and Kubečka (2005) was used, regarding the comparison of an acoustic measurement of fish density and fish biomass at different distances in relation to the position of the transducer.

The procedure was as follows: considering that a pass is an observation band, each pass was analysed by dividing it into layers with a thickness of one metre and perpendicular to the acoustic beam (Fortuna, 2001) (Fig. 1). In central design passes, a maximum of 4 layers from 2 to 6 m was established. In zigzag design passes, a maximum of 6 layers from 2 to 8 m was established. Density and biomass were estimated in each layer and the results were represented in a histogram which linked both parameters to the distance to the transducer to determine if fish were homogeneously distributed or if, on the contrary, there was a density gradient.

When the distribution is homogeneous, the acoustic density obtained in the pass is an unbiased estimator of the density in the pond ($\delta a = N/A$; where δa is the acoustic density in the pass, N is the total number of fish and A is the area). Thus, we can use the average acoustic density obtained in that pass and extrapolate it to the total of the pond, thereby obtaining its fish abundance (N). On the contrary, when the distribution is not homogeneous, N must be estimated as n/P , where P is the average probability to detect a fish in the sampled area and n is the number of fish quantified in the sampling (Nichols et al., 2000; Farnsworth et al., 2002; Bart and Earnst, 2002; McCallum, 2005). This probability is related to the detection function, g(x), which describes the probability of detecting an animal depending on the distance perpendicular to the pass (Buckland et al., 2001; Buckland et al., 2013; Buckland et al., 2015; Marques, 2009; Marques et al., 2010; Thomas et al., 2010; Martella et al., 2012; Marques et al., 2013).

In this study, one of the so-called ad hoc models was selected to correct biomass and density estimates. This model is based on the distance perpendicular to the line of the pass and uses a function based on the maximum value of the number of observed fish. This correction was applied because of its simplicity and because it is not affected by the type of probability distribution obtained (normal, binomial, etc.) (Nichols et al., 2000; Fortuna, 2001; Farnsworth et al., 2002; Cupul-Magaña, 2009).

The selected method calculates the observed visible proportion or fraction of the population (P) based on the acoustic density gradient obtained in the sampling:

$$P = \frac{\sum_{d=2}^n \delta a_d / l}{\delta max}$$

where d is the distance from the layer to the transducer, δa_d is the acoustic density obtained in the layer corresponding to that distance, l is the total number of layers in the passes, and δmax is the maximum density recorded in the pass.

Thus, the estimated density (δe) of fish in the pond would be:

$$\delta e = \delta a / P$$

Biomass estimates (β) were calculated following the same process.

The variations in the acoustic biomass and density depending on the distance to the transducer and the comparison between the estimated biomass and density in each sampling design (central vs. zigzag trajectories) were studied using an analysis of variance (ANOVA). All statistical analyses were conducted using IBM SPSS Statistics 18.0 (IBM, 2011). A significance level of 0.01 was used to contrast the null hypothesis.

To select the best sampling design, we examined two factors: accuracy and precision. Accuracy concerns to how close the density and

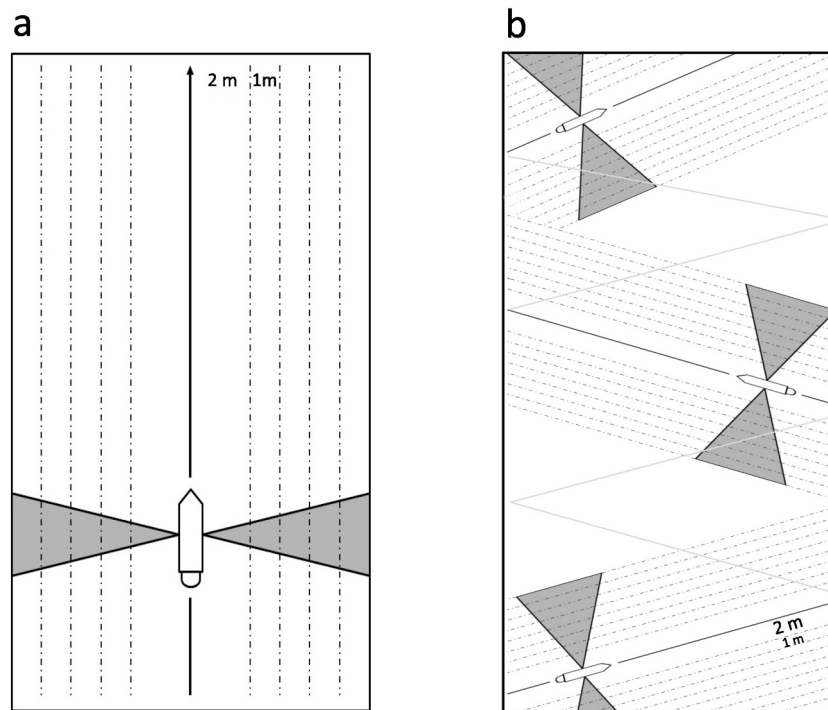


Fig. 1. Analysis layering of the acoustic survey for central (a) and zigzag (b) design.

biomass estimate are to the true population mean; precision concerns to the variability around the estimates (which may or not be accurate) (Samoilys and Carlos, 2000; Kritzer et al., 2001; Cupul-Magaña, 2009; Gallardo et al., 2010; Kowalewski et al., 2015; Pais and Cabral, 2017). To verify the accuracy of the density and biomass estimated with the method developed in the study, the results were compared with the data of fish density and biomass provided by the production company (δm and βm , respectively). An agreement index was calculated which relates the estimated density and biomass to the density and biomass provided by the farmers managers ($\delta e/\delta m$) (Gallardo et al., 2010; Johnson et al., 2019). Bias was calculated as the absolute difference from density and biomass data provided by farmers and expressed as a proportion (Pais and Cabral, 2017):

$$|\delta e - \delta m|/\delta m$$

The Relative Standard Error (RSE) of the mean, i.e., the Standard Error of the mean (SE) divided by the mean (SE/mean, expressed as a percentage) was calculated from each set of passes of each survey and it was used to determine the precision of the values obtained for the estimates of fish density and biomass (Johnson et al., 2019).

3. Results

The analysis of fish density in relation to the distance to the transducer showed that, in all ponds and all cases (passes and sampling designs), fish distribution was not homogeneous during the sampling process (Fig. 2), and the acoustic density was significantly affected by the distance to the transducer (ANOVA, $p < 0.01$). In samples with central trajectories, a gradual increase in fish density with distance was observed, which was probably caused by the fish escape behaviour to the pond edges when the vessel approached. This behaviour was observed in all passes and all ponds regardless of fish size. In samples with zigzag trajectories, the functions that link fish density to distance

were more diverse, albeit they also reflected a non-uniform distribution of density in relation to the distance to the transducer. These results were the same as those obtained in the analysis of fish biomass detected acoustically (Fig. 3).

Both results confirm that acoustic estimates of average density and biomass are not homogeneous in the pond and are biased by the effect of the sampling. To correct this deviation, we calculated the probability of detecting a fish in relation to the distance to the transducer (P) in each pass. Based on the data provided by the echosounder and the P value, we calculated the estimated values of density (δe) and biomass (βe) in each pond.

Table 1 presents the results for the density in each analysed pond and each sampling design. In addition to the mean acoustic density (δa) and mean estimated density (δe) values, the density values provided by the managers of the aquaculture facilities (δm) have also been included. There are no significant differences in the mean acoustic density obtained in the zigzag or central samples (ANOVA, $p > 0.01$). Regarding fish density, the probability of detection (P) ranged between 0.49 and 0.78, and the average of all ponds was 0.6. No significant differences in P between sampling designs were recorded in any pond (central against zigzag; ANOVA, $p > 0.01$), neither for estimated density values (δe ; ANOVA, $p > 0.01$). It can be observed that correcting acoustic density (δa) with the probability of detection P results in density values increasing significantly (δe), which indicates that acoustic density is being underestimated due to the fish avoidance behaviour caused by the disturbance of the boat during the sampling process. The mean RSE for the fish density estimate was 12.5% and ranged from 4.1% to 25.7%, being higher for zigzag designs (13.9%) with respect to central ones (11.1%).

Table 2 presents the values obtained for mean acoustic biomass (βa), the estimated biomass once the correction P has been incorporated (βe) and the biomass values provided by the managers of the aquaculture facilities (βm). No significant differences were found neither in the mean

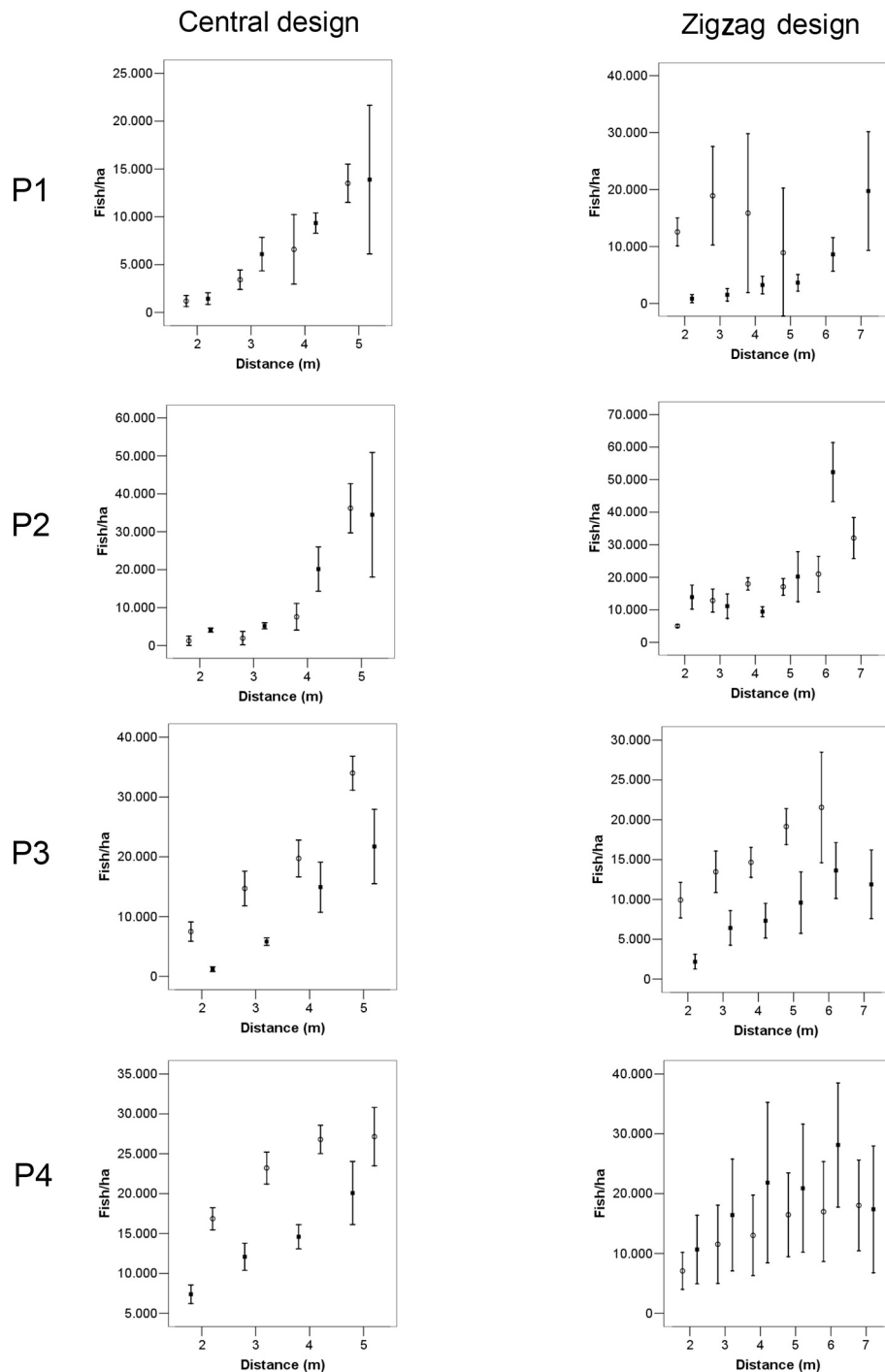


Fig. 2. Fish density in relation to the distance to the transducer. Unfilled circles for channel 1 and filled squares for channel 2.

acoustic biomass, P nor estimated biomass for any case in any of the ponds with respect to the trajectory of the sampling (zigzag vs central; ANOVA; $p > 0.01$). P ranged between 0.44 and 0.74 with an average of 0.57 for the whole group of ponds. As in the case of density, the same occurs between the biomass values calculated based on the acoustic data (βa) and those estimated incorporating the correction P (βe): the estimated values (βe) considerably increase with the correction, which shows that the acoustic value was being underestimated due to the effect of the sampling. The mean RSE for the fish biomass estimate was 15.15% and ranged from 5.9% to 22.4%, also being higher for the zigzag designs (17.5%) with respect to the central ones (12.8%). For both, the estimated density and biomass, the average precision, measured as RSE, was

greater in central designs than in zigzag.

In addition to the mean values estimated for density and biomass in each pond (δe and βe , respectively), Tables 1 and 2 show the data provided by the company for each variable (δm and βm , respectively) and their accuracy to the estimated values provided by the agreement index and the bias value. Fig. 4 shows these estimated density and biomass values and their adjustment for both types of trajectories in all studied ponds. Likewise, they also show the density and biomass values supplied by the company.

Although no significant differences were recorded in the estimates with regards to the trajectory used (ANOVA, $p > 0.01$), it was observed that the most accurate agreement between the estimated values of

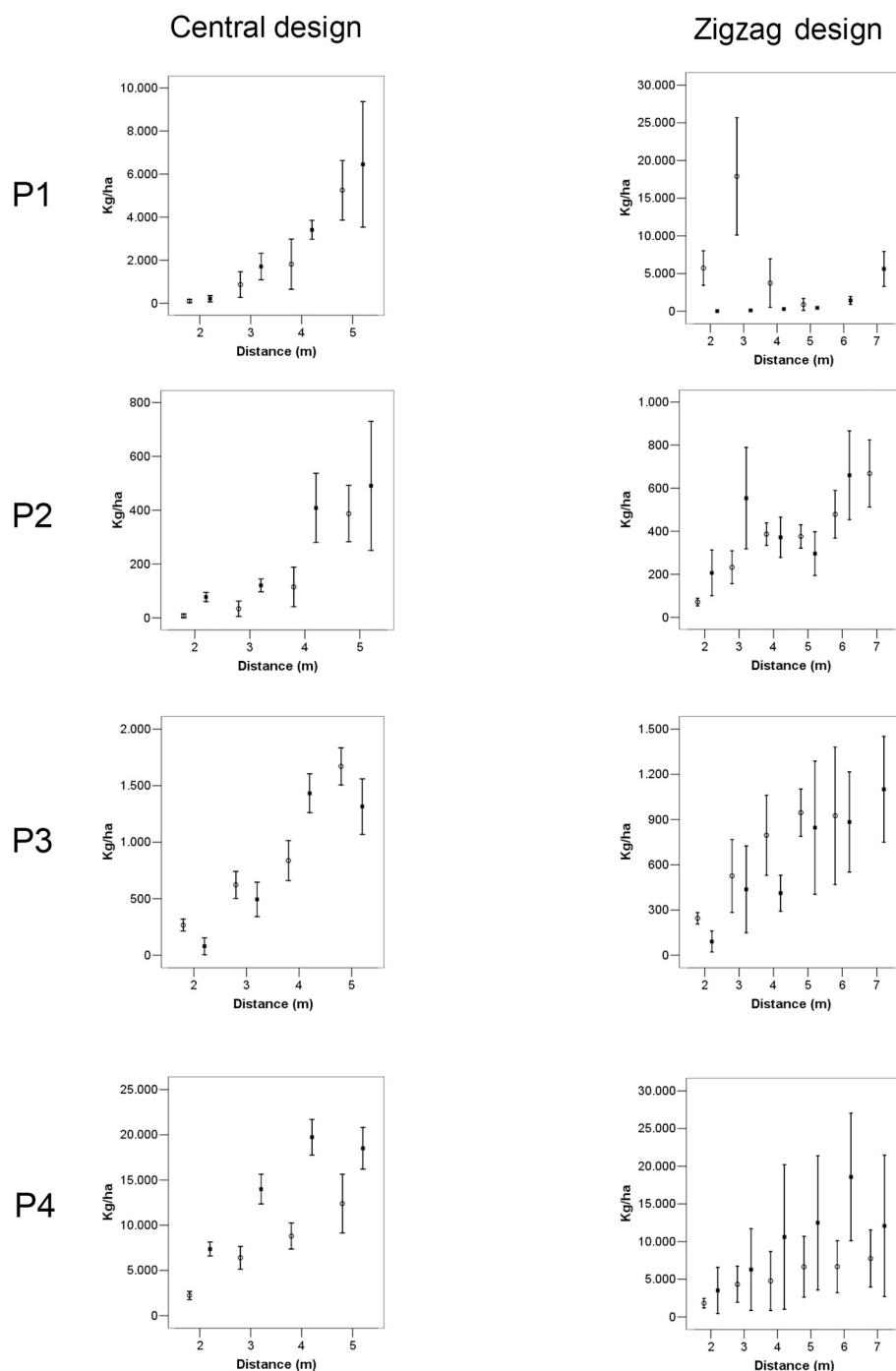


Fig. 3. Fish biomass in relation to the distance to the transducer. Unfilled circles for channel 1 and filled squares for channel 2.

density and biomass and those provided by the company was always obtained in central trajectories (especially in the case of density). Table 3 presents the average weight values estimated based on the individual detections (SEDs) of the hydroacoustic explorations conducted in all ponds and compares them with the values provided by the company. The average weight obtained with hydroacoustic methods matches the average weight provided by the company, with an agreement index close to 1 in all cases. The mean RSE was 12.1%, being similar for both sampling designs.

Regarding the fish mortality in pond P1 (small-sized sea bass), Fig. 4 clearly shows a decrease in fish stock recorded between the exploration conducted immediately after the planting and that conducted after the

deaths. This decrease is evident both in central and zigzag samples, with no significant differences in the average value of the density estimated regarding the trajectory of the sampling (ANOVA, $p > 0.01$). The difference between the estimates recorded before and after the deaths in the pond presents fish mortality values of around 40%. Specifically, the mortality rate recorded was 39.4% in central samples and 46.2% in zigzag samples.

Regarding biomass, it can be observed how it increased during two months between both explorations due to the growth of the surviving fish in the pond. However, the detected biomass growth was lower than expected, without deaths reaching mean weight and sowing density of fish. This would translate into a biomass loss of 23.3% in the estimates

Table 1

Mean acoustic density (δa), mean probability of fish detection (P), mean estimated density (δe), relative standard error (RSE), density value from the aquaculture managers (δm), agreement index ($\delta e/\delta m$) and bias for each survey design and pond.

Pond	Survey design	δa (fish/ha)	P	δe (fish/ha)	RSE (%)	δm (fish/ha)	Agreement index	Bias (%)
P1	Central	52,560.3	0.49	109,903.9	10.0	110,000	0.99	0.08
	Zigzag	46,473.5	0.63	78,298.0	20.9		0.71	28.82
P2	Central	28,638.5	0.50	62,852.1	25.7	70,000	0.89	10.21
	Zigzag	32,709.3	0.62	53,266.3	9.4		0.76	23.90
P3	Central	35,536.3	0.78	45,151.9	4.1	47,000	0.96	3.93
	Zigzag	32,612.8	0.66	48,802.7	7.7		1.04	3.83
P4	Central	25,335.7	0.52	49,336.1	4.7	49,000	1.00	0.68
	Zigzag	23,604.1	0.64	37,068.3	17.4		0.75	24.35
Mean values	Central		0.60		11.1		0.96	3.73
	Zigzag		0.60		13.9		0.82	20.23

Table 2

Mean acoustic biomass (βa), mean probability of fish detection (P), mean estimated biomass (βe), relative standard error (RSE), density value from the aquaculture managers (βm), agreement index ($\beta e/\beta m$) and bias for each survey design and ponds.

Pond	Survey design	βa (Kg/ha)	P	βe (Kg/ha)	RSE (%)	βm (Kg/ha)	Agreement index	Bias (%)
P1	Central	2963.6	0.45	7161.8	18.3	9708	0.73	26.22
	Zigzag	3699.9	0.74	5348.5	22.4		0.55	44.90
P2	Central	10,190.6	0.44	24,368.1	20.4	24,500	0.99	0.54
	Zigzag	12,235.7	0.63	20,245.1	18.0		0.82	17.36
P3	Central	29,981.0	0.68	44,371.9	5.9	39,500	1.12	12.33
	Zigzag	27,530.1	0.57	49,235.3	11.8		1.24	24.64
P4	Central	27,432.7	0.51	53,338.4	6.6	49,400	1.08	7.97
	Zigzag	20,775.7	0.58	36,160.9	17.8		0.73	26.80
Mean values	Central		0.50		12.8		0.98	11.77
	Zigzag		0.60		17.5		0.84	28.43

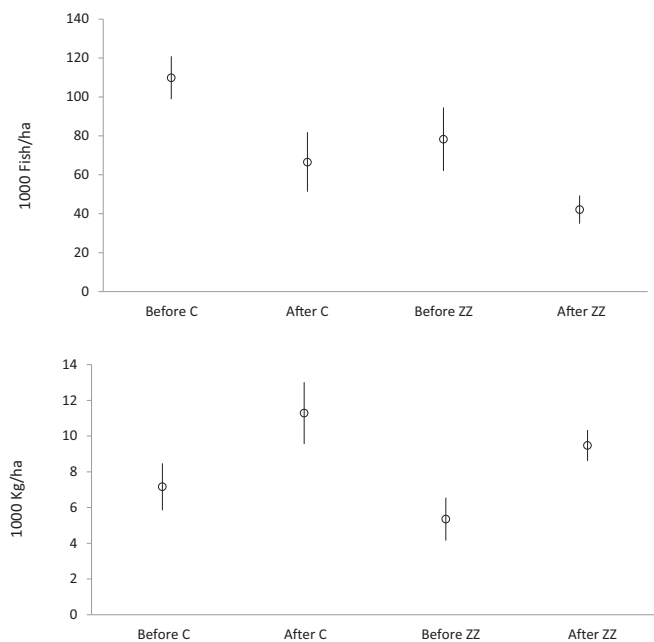


Fig. 4. Estimated density and biomass recorded between the exploration conducted immediately after the planting and that conducted after the deaths in pond P1. C for central and ZZ for zigzag design.

from the central samples, and of 35.5% in the estimates from the zigzag ones. As in the previous cases, both sampling designs (central and zigzag) delivered similar average biomass values, without any significant differences (ANOVA, $p > 0.01$).

Table 3

Acoustic average weight (ωa) estimated from single echo detections (SED), relative standard error (RSE), average weight given by the aquaculture company (ωm), agreement index and bias for each pond and survey design.

Pond	Survey design	ωa (g)	RSE (%)	ωm (g)	Agreement index	Bias (%)
P1	Central	83.1	23.3	87	0.95	4.48
	Zigzag	103.3	8.1		1.18	18.73
P2	Central	399.9	7.4	350	1.14	14.25
	Zigzag	332.5	8.8		0.95	5.00
P4	Central	881.5	9.6	1000	1.03	3.70
	Zigzag	987.1	13.6		0.98	1.29
Mean Values	Central	918.6	20.7		0.92	8.14
	Zigzag		12.4		1.03	6.60
			11.8		1.02	8.89

4. Discussion

Currently, split-beam hydroacoustic equipment allows reliable estimates of fish stock in large volumes of water to be obtained using vertical hydroacoustics (with the acoustic beam oriented perpendicular to the water surface). However, in small and shallow systems such as the one analysed in this study, this hydroacoustic technique faces greater limitations and requires further development to verify the reliability of the estimates obtained. This is why this study posed an unprecedented challenge in the use of hydroacoustic techniques in this kind of farming system, which not only presents problems derived from shallow depths, but also from fish farming in production systems with medium to high densities.

The main issue of horizontal hydroacoustics lies within the fact that there is an important variation in the relationship between the echo returned by the fish and its size depending on the aspect in which it is ensonified. However, this problem can be solved by using split-beam equipment and adding equations that incorporate the required

information about the aspect to the acoustic data conversion (Baran et al., 2017; Cox et al., 2011; Rodríguez-Sánchez et al., 2015; Rodríguez-Sánchez et al., 2016a, 2016b; Rodríguez-Sánchez et al., 2018). The excellent results obtained for the average weight of the fish detected acoustically in all explorations prove that this issue can be solved incorporating this kind of equation for the conversion of target strength. In all cases and both sampling designs, the average weight of the fish was determined to be among the variables which most accurately match the actual data provided by the company, with an agreement index close to 1, a mean bias lower than 8% and a high estimation precision (according to Johnson et al., 2019). The high quality of the results of this study is attributed to the use of acoustic and biometric equations (which, on one hand, relate dB-size and, on the other, size-weight) which were specifically developed for the studied species in the same facilities of the company. This has reduced the variability and inconsistencies which usually occur when generic equations are used or when they were developed under different environmental conditions.

High fish densities can also be an issue when using hydroacoustics as a tool to estimate density and biomass. As stated by György et al. (2012), the accuracy of acoustic estimates relies on the detectability of fish as single targets, which is a function of the distance between the single fish and the group or shoal. In this study, it was observed that fish tend to scape, avoiding the sailing of the boat. This scattering effect could suppose a particular advantage from the used method that may have facilitated the acquisition of individualised signals improving the single echoes detection (Johnson et al., 2019).

Regarding fish behaviour in relation to the sampling, the results have confirmed that fish presented avoidance behaviour as a response to the disturbance, as opposed to an attraction behaviour. Fish distribution in the ponds was not uniform in any of the cases during the sampling and there was a clear significant variation in both acoustic biomass and density depending on the distance to the transducer. Even though this result seemed to be fairly predictable, it is still relevant to demonstrate it as it drastically affects the population estimates obtained (Foote and Stefansson, 1993; Strindberg and Buckland, 2004; Hjellvik et al., 2008; Marques et al., 2010; György et al., 2012; Kaartvedt et al., 2012; Buckland et al., 2013; De Robertis and Handegard, 2013; Marques et al., 2013). Fish are very sensitive to the presence of vessels and the sound of the engines and propellers, even with small engines (Draštík and Kubečka, 2005; Janáč and Jurajda, 2005; Godlewska et al., 2009; György et al., 2012; Jacobsen et al., 2014; Magnhagen et al., 2017).

Most studies where the effect that the sampling has on fish populations is considered show that, in general, escape behaviour always occurs, especially in small and shallow systems such as the one in our study (Soria et al., 1996; Lucas et al., 2002; Mitson and Knudsen, 2003; Draštík and Kubečka, 2005; Draštík, 2008; Fewster et al., 2008; Godlewska et al., 2009; György et al., 2012; Madirolas et al., 2013; Wheeland and Rose, 2015). The reported causes for this escape behaviour during hydroacoustic explorations include visual signals, especially in clear enough waters, and, mostly, the acoustic signals coming from the engine of the vessel (Vabø et al., 2002; Simmonds and MacLennan, 2005; De Robertis and Wilson, 2010; De Robertis et al., 2010; Guillard et al., 2010; De Robertis and Handegard, 2013; Brehmer et al., 2019). Fish hearing starts at several hertz and is generally restricted up to 2 or 3 kHz (Karlsen, 1992; Knudsen et al., 1993; Knudsen et al., 1997; Mann et al., 1997; Popper, 2003). The noise frequencies of conventional engines fall within this range (Mitson and Knudsen, 2003) and, even though a small engine such as the one used in this study emits noise with a low intensity, this noise together with the movement of the propeller and of the vessel itself were the main cause of the escape behaviour found in this study. Furthermore, it must be highlighted that its effect was much greater due to the small size of the ponds. Some authors have indicated that this escape behaviour can change depending on abiotic factors, such as turbidity or temperature; or biotic factors, such as the visual capacity, experience, learning ability, physiological estate or size of the fish (Soria et al., 1996; Draštík and Kubečka, 2005). In this study,

the escape behaviour caused by the acoustic exploration was found to be similar regardless of fish size or temperature, given that the sampling was conducted in different times of the year, including both winter and summer. There was also no difference in behaviour recorded regardless of whether the sampling was the first or last one within the series conducted in the pond.

For both sampling designs (central or zigzag), the fish escape behaviour is the cause of the considerable underestimation of the fish density and biomass obtained directly from the hydroacoustic sampling (referred to as acoustic density and biomass in this study) compared with the actual density and biomass provided by the company for all ponds. Results confirm that, in these shallow and small types of ponds, the probability of detection must be included to correct the bias caused by the non-uniform distribution of fish during the sampling. When acoustic density (δ_a) and biomass (β_a) are corrected by including the probability of detection (P), the estimates (δ_e and β_e) become much more accurate and precise, even reaching agreement indices close to 1 and high accuracy, with lower variability, which reflects the high precision of the estimates (mean RSE 12.5% for density and 15.2% for biomass, for both central and zigzag designs). The accuracy of these results confirms that the method used to correct the density gradient in relation to the distance is adequate.

As stated by Fewster et al. (2008) or Marques et al. (2010), among others, a narrow percentage of the confidence interval in the estimates indicates that their precision is good. Gibbs (2000) indicates that estimates with an average variation coefficient of 14% or less can be considered reasonably good and even high, and Johnson et al. (2019) reported that a RSE of around 10% in animal population censuses could be considered high. Thus, the results obtained in this study can be considered a success in validating the use of hydroacoustics as a valid management tool in inland aquaculture systems.

There is extensive literature confirming that the use of methods which incorporate the detection function of individual fish in the sampling (P) can significantly increase the reliability of the results obtained in fish population studies (Buckland et al., 2001; Williams et al., 2002; Draštík and Kubečka, 2005; Hjellvik et al., 2008; Marques et al., 2010; Cox et al., 2011; Martella et al., 2012; Marques et al., 2013; Glennie et al., 2015; Wheeland and Rose, 2015; Pais and Cabral, 2018). The detectability of populations can vary considerably depending on the circumstances, which leads to issues in the interpretation of the raw data obtained. Thus, it is necessary to know the variability of P or, at least, manage its effects. In general, in a sampling where conditions can be considered to be equivalent, average values of P and its confidence intervals are extremely solid, as proven in this study, where the values of the function were around 0.6 (density) and 0.57 (biomass) on average, with a variation coefficient (RSE) lower than 10% in all cases.

The design with central lineal trajectories was determined to present the most accurate and precision results for density and biomass estimates in all ponds. Therefore, it can be deduced that this is the most appropriate design for hydroacoustic explorations in this kind of system. Although no significant differences in density or biomass estimates were ever statistically registered with regards to the trajectory, the results based on zigzag trajectories corresponded less accurately (agreement index more deviated than 1 and bias around 20%) with the data provided by the company. Therefore, it can be deduced that the use of this design is not recommended, at least in this kind of pond. Another result which confirms the suitability of the central design against the zigzag one is that the RSE in the estimates in the central design was lower, which results in more precise estimates.

It is worth highlighting that the least accurate values for the density from central design were obtained in pond P2 (medium-sized sea bass) with a 10% bias with respect to the data provided by the company. Due to the good results obtained in other ponds, we believe the most probable cause for this mismatch is that the data provided by the company for this pond was not sourced directly from its harvesting, but is rather an estimation by the company based on the table of probabilities of

mortality and growth together with a one-off sampling of the fish (fish planted one year before the survey). This means that, in this case, we cannot be sure whether the inaccurate value is the one estimated with the hydroacoustic methodology or the one provided by the company.

Another above average inconsistency was registered in the central samples for biomass in pond P1 (small-sized sea bass), with a bias higher than 25% (agreement index = 0.73). In this case, we believe that this inconsistency is due to the fact that the biomass data provided by the company was calculated based on the average weight during the fish planting, whereas the acoustic data confirmed a higher average weight of the fish during the sampling, which was a month after the planting, when fish had grown larger. This hypothesis could be supported by the fact that, albeit biomass values presented inaccuracies, the value obtained for the density was accurate, with an agreement index close to 1 and a bias lower than 0.1%.

Based on the results obtained in this study, it can be concluded that the developed method provides estimates of density and biomass which accurately match the actual data. They also support the use of hydroacoustics as a potentially valid tool to manage inland aquaculture farms. With this method, acoustic density and biomass can be corrected based on the gradient of fish distribution and the distance to the transducer, thereby solving the issue of fish escape behaviour during the sampling and providing realistic, accurate and precise estimates of the fish stock in this kind of farming pond.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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