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Article

# A Package of Script Codes, POSIBIOM for Vegetation Acoustics; *POSIdonia* BIOMass

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**Abstract:** Macrophytes and seagrasses play a crucial role with a variety of the functions in the marine ecosystem and respond synchronized-promptly to the changing climate, followed by ecological status. Monitoring of the seagrasses was one of the paramount topics at the marine environment. One of the fast monitoring techniques is the vegetation acoustics with the advantages compared to the other remote sensing techniques. The acoustic method alone is ambitious to sea-truth identities of the backscatterers at the sea. Therefore, a package of computer programs was developed to identify, estimate leaf biometrics (leaf length and biomass) of one of the common seagrasses, *Posidonia oceanica*. Some troubleshooting in the acoustical data were fixed and then solved to reach the estimates regarding to the matters encountered for the vegetation as well as fisheries and plankton acoustics. One of the trouble was the "Lost" bottom which occurred during the data collection and post-processing due to occurrence of the acoustical noises, reverberations, interferences and intense scatterers suchlike fish schools. Another problem to remove was the occurrence as studying the near-bottom echoes belonging to submerged vegetations such as seagrasses, followed by spurious echoes. Last one was recognition of the seagrass to estimate leaf length and biomass calibrating the sheaths/vertical rhizome of the seagrass and establishing relationships between acoustical units and biometrics, respectively. As a consequence, an autonomous package of the code written in language MatLab was developed to perform all processes, and the name of the package was abbreviated as POSIBIOM, *POSIdonia* BIOMass. This study presented the algorithms, methodology, acoustics-biometrics relation, and mapping of the biometrics for the first time, and discussed advantages and disadvantages of the package compared to the software purposed for the bottom types, habitat and vegetation acoustic. The future studies were recommended to improve the package.

**Keywords:** acoustics; seagrass; computer programs; sea-truthing; estimates; leaf; biomass

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## 1. Introduction

Aquatic macrophyt (algae, grass etc) is one of the important components playing a crucial role in various ways for the aquatic ecosystem [1–3]. Considering the volume and surface area of the marine system, seaweeds and seagrass have significant contribution primarily with their oxygen production and carbon absorbance/emission into a way of the ocean-atmosphere interaction. Besides microscopic algae and photosynthetic bacteria, most of marine macrophytes reveal status of the marine ecosystem health and ecological level. Some of them are indicators of pollution whereas some are indicators of the pristine environment [4,5]. There are a lot of further importance and contributions of them to the marine system. Unlike the algae, the seagrasses are associated with the sediments (benthic system) by their below-ground parts (rhizomes, and roots) [6] and pelagic system by their above-ground parts (sheaths, and leaves) [5].

One of the common seagrasses, *Posidonia* species, the phanerogam species which have been sustained well in the poor-nutrient marine environment and formed colonial life are coastal species, and are worldwide distributed in the subtropical and temperate waters from Indo-Pacific Ocean (Australia) to Atlantic Ocean (Mediterranean Sea) systems [7,8]. They are very sensitive to the pollution, climate change, global warming, biogeochemical cycle, anthropogenic effects and water

movements etc [9–11]. Their biometrical traits respond rapidly to such global or local events in order for the scientists to monitor health of the marine system. Like other seagrasses, *Posidonia* species are near coastal inhabitants giving many useful opportunities to the other organisms under their canopies [4,12–15]. One of the common *Posidonia* species is *Posidonia oceanica* (Linnaeus) Delile, 1813 which is an endemic species for the Mediterranean Sea extending to near coast of the eastern Atlantic Ocean [16]. Most (25–30%) of the Mediterranean coasts are inhabited by *P. oceanica* [6].

Like other seagrass (angiosperms), *Posidonia oceanica* (the Mediterranean meadow) is a prominent component in the zonal classification in the Mediterranean Sea, and classifies the border between the Infralittoral and the Circalittoral of the benthic environment [1,3,5,17,18]. Therefore, it is positioned as a coastal engineer and interior architect in the coastal environment with their following functions [19,20]. Besides the ecological indication, *Posidonia oceanica* also play crucial roles in the sediment stability and biogeochemical cycle, oxygen, carbon emission/absorbance, blue carbon, habitat/shelter and food niches for its interacted organisms, spawning and nursery grounds, competition with invasive macrophytes, protectors of prey from predator, and extension of their habitants in range [14,21–30]. Monitoring their dynamics is a useful tool to sustain the species under the prevailing environment, and to observe indicative acute or chronic changes derived from the allochthonous and autochthonous sources in the marine ecosystem. Consequently, this knowledge is used by the ecosystem modelers, ecologists, environmentalist, biologists, coastal engineers and managers, and species managers and protectors [31–33].

Compared to fish and zooplankton acoustics, vegetations acoustics have not however attracted significant attention of researchers. The destructive sampling methods (SCUBA sampling to pick up the seagrass) are not generally desired to sample the meadow under the protection [34–36]. Therefore, many studies were fronted to apply non-destructive methods, remote sensing systems to study sensitive and vulnerable seaweeds and seagrass [37]. There are a variety of remote sensing techniques developed as follows: satellites (especially Sentinel 2), video cameras and acoustics [38–44]. There are some limitations to sample the seagrass significantly for each technique. Visual techniques (satellite, and video-camera) are limited with requirements of the suitable atmospheric and sea conditions [45–47]. These techniques were mostly used for the estimates of coverage and mapping of the different habitats e.g., [48,49]. Compared to the techniques, the acoustical method is faster, more precise, and easier and does not need clear sea conditions to sample the data [2,50], and but have serious problems changing from correct bottom detection, removal of spurious scatterers to sea-truthing of the targets [51]. Recently, *in/ex situ* acoustical studies have increased to relate the biometrics of the seaweeds and seagrasses to the Elementary Distance Sampling Unit (EDSU) [52], for instance, Depew et al. [53] focused on *Cladophora* sp., Monpert et al. [54] on *P. oceanica*, and *Zostera marina*, Llorens-Escrich et al. [55] on *P. oceanica*, Shao et al. [56] on *Saccharina japonica*, and Minami et al. [57] on *Sargassum horneri*. There were some studies to calibrate the acoustical data versus the biometrics of two seagrasses, *P. oceanica* and *Cymodocea nodosa* [58–61] and to identify the seagrass, *P. oceanica* and *C. nodosa* [62].

Like that in the other remote sensing techniques, acoustic data alone are inherently ambiguous with regard needed to the identities of the target species. The mapping of macrophytes can be accomplished with the software developed for the submerged vegetation (EcoSAV:Echo Submerged Aquatic Vegetation, Visual Habitat and Visual Aquatic) by BioSonics Company. Further methods of vegetation acoustics were developed based on algorithms to classify bottom types [63–66]. They are OTCView, Echoplus, BioSonics VBT (Visual Bottom Typer), and EchoView [67–70]. While the acoustic reflectivity of aquatic vegetation having an acoustical impedance of 1.026 to seawater [71] is known and used for the detection of submerged aquatic vegetation (SAV) [72], the basis of this phenomenon has not yet been figured out to solve the problems. The other detection factor is the acoustical frequency used during the vegetation acoustical studies. Although the most effective frequency for these techniques is 420 kHz, it has been concluded that 200 kHz is also effective for vegetation acoustics [73].

The present study is aimed to solve the problems, detect and discriminate the Mediterranean meadow, and estimate the leaf biomass for the future works and studies needing the biomass purposed by the researches for sustaining the meadow. The presence of life on the sea bed causes

significant problems during acoustic post-processing, including spurious targets, background noise, artificially generated-noise, reverberations, dead zones, interference, 'lost' bottoms (misestimated bottom, too deep, out of range of sound penetration by the frequency limitation depending on the source level). In particular, the problem of 'lost' bottoms in stock assessments of the species creates challenges when attempting to recover bottom echoes. In some cases, commercial software and computer scripts require interventions to manually recover 'lost' bottoms, which could be time-consuming. This study is the first attempt to regard the matters of the acoustical data post-processing aimed to estimate biometrics of *P. oceanica*, and presents an auto-self (no often intervention) and easy used-package of the scripts codes to solve the problems during the estimation with advantages and disadvantages of the codes in comparison to the other software.

## 2. Material and Methods

Overall, acoustical raw data (\*.dt4) used for the present study were collected with a Biosonics DT-X digital scientific echosounder which had a split beam transducer in a shape of circle beaming a width ( $\Theta$ ) of 6.8°, operating 206 kHz using a software package of "Visual Acquisition" (v. 6.3.1.10980, BioSonics inc.). The pulse width (pulse length, pulse duration,  $\tau$ ) was set to 0.1 ms (highly recommended for the seagrass studies), a ship speed to approximately no more than 5 knots, and no deeper bottom than 50-60 m. The transducer was mounted at a draft depth of 2.5 m down-looking on the starboard of the R/V *Akdeniz Su* [74,75]. During the surveys of different sampling locations and months, different bottom types covered by the seagrass were encountered namely, rock, sand, mud and matte [5,18].

Three main script codes which run autonomously after all settings completed by the users were written in MatLab (vers. 2021a, Matworks inc.) to correct the bottom depth, to remove spurious weak and strong scatterers else than *Posidonica oceanica* and to sort out the species regarding to its vertical rhizome and sheath. To recover removal of the bugs in the package, around 4000 acoustical files (300-9000 pings, 4500 pings per file on average) derived from two different projects [74,75] were subjected to the codes. Three main codes subsequently run as follows: "AbdezdeR1", "AbemsiR1" and "SheathFinder1" to estimate ultimate data of the biomass (see Mutlu and Balaban [58] for the description of abbreviations of the algorithm names). There are several auxiliary scripts and acoustical data associated with the three main codes. All three codes run under a script as a main menu and manager to reach them. It is called in an acronym of 'POSIBIOM' as abbreviation of *POSIdonia* BIOMass. The present study was inspired from an algorithm, 'SheathFinder' [58]. However, the algorithm was not a package, easy-used, and was debugged to estimate real bottom echo without infinitive permutation until all 'lost' bottoms completed disregarding seasonal biomass estimates of the seagrass. Therefore, all algorithms were highly revised and modified completely for the present study. In the present, the acoustical data collected only with only the BioSonics inc. echosounders can be used for the POSIBIOM since I am currently a user of BioSonics echosounder. To run the scripts, the data (\*.dt4) was converted to a spread sheet of Comma-Separated Value (CSV) using a post-processing commercial program, "Visual Analyzers" (v. 4.1.2.42, Biosonics inc.). During the post-processing, the settings were configured to get output data in a vertical resolution of count-to-count (equivalent of ~one-eighth of the pulse width in cm depending on seasonal sound speed) and in a horizontal resolution of ping-to-ping. The processes were repeated to get the layered data including the seagrass from the uneven (not flat) bottom up to a stratum number of 249 (this is the last number allowed by the Visual Analyzer). This could be done once for the flat bottom to get count-to-count data of the vertical resolution. This is important to determine precise and accuracy of the vertical resolution of leaf length, hence biomass. This process is but time-consuming. It would be helpful and faster for processing the data if the scripts (POSIBIOM) read the collected data file of the BioSonics directly. In order for POSIBIOM to be professional software, the package would be coded to a programming language of the 'C' from the MatLab by its relevant tool.

The POSIBIOM is functioned as a manager to load input files (acoustical and coast line files, Marine Region [76]), to start each of three analyses, and to map the results of the biomass and leaf length of the seagrass (Appendix 1).

### 2.1. Algorithm of 'lost' bottom (AbdezdeR1)

The algorithm was scripted in a file, namely, AbdezdeR1 (see Mutlu and Balaban [58] for description of the abbreviation). Fixation of lost bottom is based on a root of adjacent bottom angles and depths. Configuration for recovery of real bottom is highly flexible during the processing. The fixed lost bottom is corrected with the following three successive methods, namely; the "chirping", "running average" and "filling gap". All methods were originated for the present study. The algorithm then estimates the dead zone using three methods. One method was created during the present study, and the other two methods have been described by EchoView [77] and Mello and Rose [78]. The algorithm has some options to alert users automatically in some cases to correct the bottom before the analyses (Appendix 2), and for last check of the bottom correction after the analyses (see Table 1, Figure 7). The algorithm is also useful for other purposes (e.g., fisheries acoustics, and partly plankton acoustics) else rather than the vegetation acoustics.

**Table 1.** Fast manual to use an algorithm of "Lost Bottom" with function, if case, recommendation and Troubleshooting/negatives for each option with number (see Figure 7 for the numbers).

No	Function	Case	Recommendation	Troubleshooting/negatives
1	Starting processing		First configure setting	Need to press START button after every 100 files processed
2	Input data file name for process			
3	Deletion of pings from the beginning	No bottom echo Bottom misestimated at the first ping	Enter last ping no to delete	Possible to estimate wrong bottom
4	To delete the pings		Check the box	
5	Data info of the acoustical file	Fixed during the data collection	See Appendix 4	
6	Info for processing methods to estimate running average, correct bottom	Auto-decision for cases (chirping, filling gap) Process in cycle	Allow auto-decision as long as possible Auto-switching to block solution in time	Takes time; 1. for block lost bottoms 2. Single ping lost bottom in cycle
7	Transducer depth	Deployment Below surface	Required for real depth	Enter 0 if transducer at surface
8	Calibration offset	Deviation in echosounder calibration from reference ball	Acoustic data correction If less, + difference If more, - difference	If not, uncorrected data Possible to misestimate biomass
9	Manual intervention to speed up the process	Time consuming more than usual Takes time more than 10 mins	Step-to-step slide forward until 'Lost bottom range' changes (see 28)	Speed up immediately faster resulted in misestimated bottom in some case
10	± Bottom angle	Steepness of bottom	Use default setting Too steep, adjust with bottom angle together	Suboptimum setting detects wrong bottom

11	Bottom angle	Cliff bottom No bottom echo, too deep	Use default setting Increase angle at highly rough sea Increase or decrease bottom angle to estimate the deepest depth	Strong scatterers estimated as
12	Label for dead zone estimation by 3 methods	Method of the present study embedded (see Figure 1).	Obligation	Angle estimation based on ping-to-ping, not distance
13	Echo View's dead zone	Optional, EchoView [77]	Click for future process	Works well if GPS reports every ping
14	Alternative dead zone	Optional, Mello and Rose [78]	Click for future process	Works well, regardless of GPS reporting every ping
15	Calculation of dead zone based on GPS coordinate distance	Disabled due to its troubleshooting		The GPS reports geographical coordinates once every one second, not every ping
16	White line	Optional, white area between bottom depth and dead zone	optional	
17	Last check to estimate real bottom last time	Misestimated bottom still available after the process	Optional if necessary	If bottom echo is weak, change the estimated bottom completely
18	± next bottom tracking range	Steepness of cliff bottom	Narrowing and widening the window	Jump to the strong scatterers
19	Sounding on/off the angles			Slowing the process
20	Saving the output data	Use for the next algorithm	optional	If step up to the next analysis, must be on
21	Saving the enhanced echogram by the corrected bottom	Optional for see the results later	Check button when user does not monitor the process	
22	Current ping range of lost bottom detected			
23	Showing root algorithm to detect availability of lost bottom	To see goodness of the estimation of correct bottom	optional	Slowing the process
24	Current number of lost bottom detected, Loop for auto-decision of lost-bottom correction			
25	Showing progress status in reading the data			
26	See 6			

27	See 24			
28	See 22			
29	Recovery of the real bottom (see 31, 33) from the beginning of the data to 500 pings ahead	Bottom detected too shallow or too deep	Useful for the cases	Appear after data reading complete Misrecognize the water column depth as bottom if strong scattering layer exist above the depth
30	“Chirping” method for manual intervention to recover real bottom in lost bottom range	Time consuming more than usual Takes time more than 10 mins Lost bottom range in cycle and same ping values (see 9, 22, 28)	Useful for the cases looking at function 27, 28 in cycle	Selection of the water column depth close to the bottom if strong scattering layer exist above the depth, resulted in misestimated bottom in some case (see 32)
31	Recovery of the real bottom (see 29, 33) from the last data to 500 pings backward	Bottom detected too shallow or too deep	Useful for the cases	Appear after data reading complete Selection the water column depth close to the bottom if strong scattering layer exist above the depth
32	Water column depth for cases in 30	Bottom detected too shallow or too deep	Useful for the cases	Appear after a certain number of iteration of the loop in function no 22, 27, but early view when sliding the speed (no 9) toward the faster, Selection of the water column depth close to the bottom if strong scattering layer exist above the depth (see 30)
33	Water column depth for recovery of the real bottom (see 29, 31)	Bottom detected too shallow or too deep	Useful for the cases	Appear after data reading complete Selection of the water column depth close to the bottom if strong scattering layer exist above the depth
34	“Filling Gap” method for manual intervention to recover real bottom in lost bottom range	Disabled	Not used currently	
35	Stopping the analysis	User stops the analysis	Do not stop if misestimating bottom depth in some data files, process again later when all files completed	Stop after the current file processing is finished

### 2.1.1. Real bottom recovery

Fixing misestimated bottom regards the sudden change of the bottom angle in successive pings. The angle to search the fake (second echo of the bottom, sheer of returned echo with the next pulse in the water column) bottom was followed within a certain bottom window of the successive real bottom depth. There are some flexible options to regard the depth deviations depending on steepness of the real bottom. Besides, surface, volume and bottom reverberations were considered for the process to figure out the real bottom by the algorithm. Furthermore, an additional prompt of an autonomous option appears (Appendix 2) when necessary in a case of existence of misestimated bottom at the first pings. Additionally, another subcall is functioned to track the real bottom between the last ping of the previous input file and the first ping of the next file. During the process, the script determines first the overall lost bottom in the next-to-next form of a block or single ping range. Therefore, the algorithm searches lost bottom in a narrow range for the wide block ranged-lost bottom, and then increases slowly the range to search the next lost bottom in a manner like the “spider net webbing”.

Of the three correction methods aforementioned;

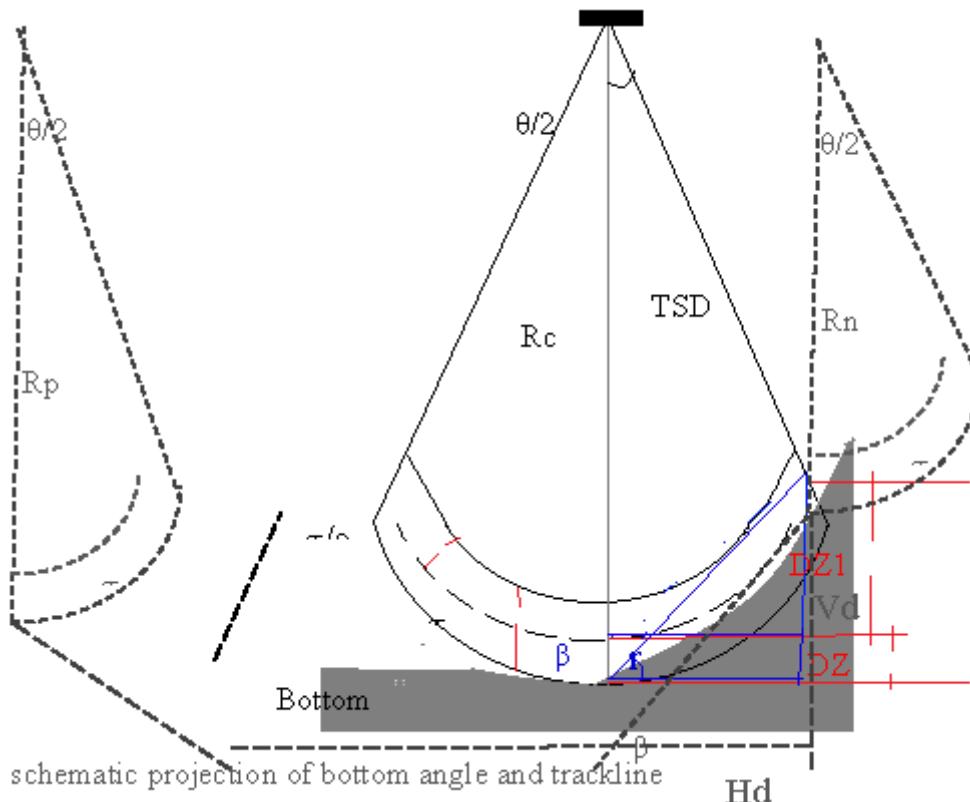
1. The chirping of the Sv of the bottom echo has a priority to the next two methods to correct the bottom. This method runs well in cases with clear bottom echo in the misestimated bottom section of the data.
2. The running average is automatically called by the processor if there is no clear (weak) bottom echo. This method is functional by correcting the bottom lost between the previous and next corrected bottom depths and angles.
3. The filling gap is then intervened to correct the bottom. In some case of occurrence of no bottom echo (a gap) due to the occurrence of surface or volume reverberation or intense fish school, the bottom echo disappears. Before filling the gap, the method checks the depths and angles.

All three methods run automatically regarding to their specific cases. In some case, for instance of bottom depth close to the near-field of the transducer, the correction could not be performed well. This action is not so rapid depending on many factors unexpected in the acoustical data by ranging pings of the lost bottoms in unicycle or multicycle. Therefore, some manual and automatic auxiliary options were added to the methods to control the range and cycle. The more the Visual Analyzer fixes the correct bottoms, the less solution of the methods takes time.

### 2.1.2. Dead zone estimates

After completing the correction for the entire data file, three different equations were used to estimate the total dead zone (TDZ) consisting of dead zones of the flat and angled bottom, regardless of the side lobe effects.

The first equation (Equations 1-2) was established with the present study, and was based on vertical range (Vd, from transducer to the bottom) and horizontal distance (Hd) estimated from the geographical successive distances or pings (Rp, Rc and Rn, choosing the shallowest distance, Rp or Rn referring to Rc) using the echosounder parameters (Figure 1). However, the GPS reports same geographical coordinates in case of echosounder pinging more than 1 per seconds. In this case, the  $\beta$  could be 90 degrees (Figure 1) so that the estimated angle could be set to zero degree. This problem was considered in another dead zone solution by equation 4 [77]. Therefore, the dead zone estimated by equations 1-2 alters to horizontal distance based on the ping instead of the geographical distance. This equation is obligatory in the algorithm to estimate the dead zone.



**Figure 1.** Schematic configuration (continuous line) and projection (dashed line colored in gray) of the successive pings (Rp: previous range, Rc: current range, and Rn: the next range) by the transducers to estimate dead zone.  $\theta$ : beam angle,  $\tau$ : pulse width,  $\beta$ : angle of bottom slope, Hd, horizontal distance to previous or next ping, Vd, vertical distance to bottom depth referring previous or next ping. DZ is dead zone height of flat bottom, and DZ1 is the height of the angled bottom referring to Rc. The shortest range is here Rn for the present study, and TSD in equation 4 for EchoView method.

Total Dead Zone, TDZ= Dead zone of flat bottom (DZ) in m + Dead zone of angled bottom (DZ1) in m

$$DZ = \frac{\tau/1000 * c}{2} \quad (1),$$

$$DZ1 = \tan(\beta) * Hd \quad (2),$$

where  $\tau$  is pulse width (msec),  $c$  is sound speed (m/s),  $\beta = \text{acot}(\frac{Hd}{Vd})$  in degree, Hd is the horizontal distance based on either geographical distance (m) or ping, Vd is the vertical distance (m) based on the range, R (not real bottom).

The second method and equation (equation 3) for estimating the dead zone was developed by Mello and Rose [78]. This equation is optional set to estimate the dead zone.

$$TDZ = \left( 2404 * \frac{d * \tan^4 \theta}{\theta^2} \right) + c * \tau/4 \dots \dots \dots (3),$$

where  $d$  is the bottom depth (m),  $\theta = \alpha/2$  in degree ( $\alpha$  is acoustical beam angle),  $\tau$  is pulse width (msec),  $c$  is sound speed (m/s),

The third equation (equation 4) was used and offered by EchoView [77]. This equation is optional to estimate the dead zone.

$$TDZ = \left( \frac{d}{\sin(90-\eta)} - d \right) + c * \tau/2 \dots \dots \dots (4),$$

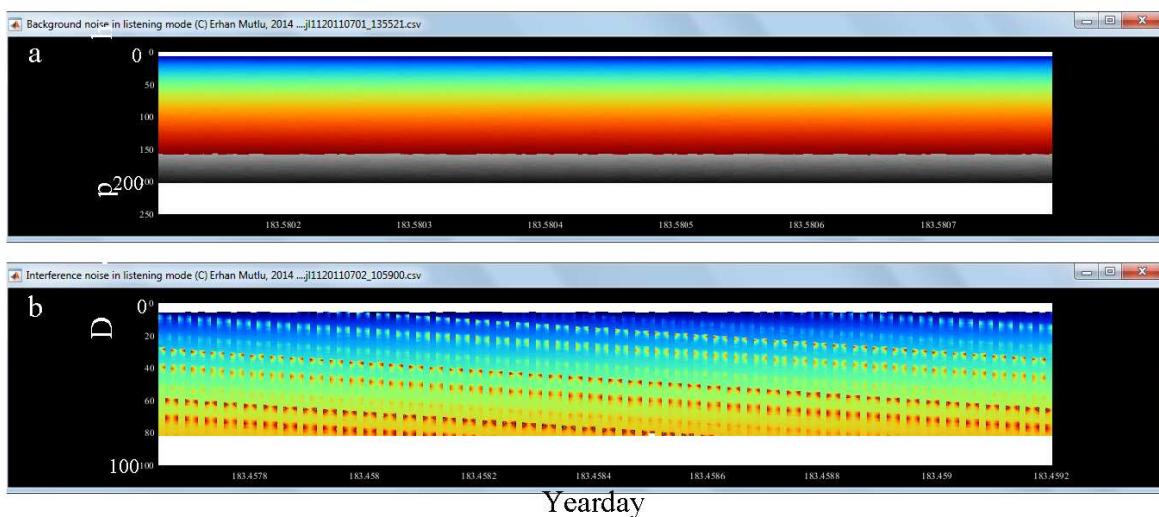
where  $d$  is the shortest range (indeed TSD) to the bottom in the acoustical beam, hereby the shortest range (Rn) between successive pings (m),  $\eta$  = the slope of bottom in degree,  $\tau$  is pulse width (s),  $c$  is sound speed (m/s),

All estimates could be similar particularly when using the equations 1, 2 and 3.

## 2.2. Algorithm of Noise and Reverberation (AbemsiR1)

Overall, determinations and removals of the noise, interference and reverberation were performed following the procedure for signal-to-noise ratio (SNR) described by De Robertis and Higginbottom [79].

The SNR estimates were based on three different algorithms in the present study modifying the procedure slightly in a script, AbemsiR1 (see Mutlu and Balaban [58] for description of the abbreviation). Two different measurements were pre-required for the present study. These measurements would be fundamental reference data in a priority to the SNR solution. The background natural noise including electrical and physical ambient noises changes in time and space. Therefore, at least one measurement is recommended for each of two noise types during an acoustical survey. During the survey with a research vessel, some other acoustical devices are used for the measurements of other purposes such as water current, bottom depth sounding, fishing net sounding etc. Such instruments can interfere with the echoes of the study echosounder, and are classified as artificial noise-generators. Furthermore, the interferences occurred owing to biological sound sources and sheering of echo returned from the bottom with echo (pulse) of the next pinging. Both noise measurements can be performed using listening mode of the echosounder and used as a hydrophone. During the present study, one noise measurement was conducted switching off the depth reader, fish finder (echosounder) or similar acoustical devices (e.g., ADCP) of the research vessel (ship) (Figure 2a) and the next measurement switching on the devices (Figure 2b).



**Figure 2.** Acoustical data measurements on enhanced echogram during the listening mode of the echosounder when the ship echosounder was off (a) and on (b).

Three methods for removal of background noise (*b*), interference (*i*) and variety (surface, volume and bottom) of reverberations (*r*) were cased conditionally with some additive functioning ranges (see Table 2, Figure 9) between measured (MSNR) [79] and expected (ESNR) data (e.g., in Figure 2) as follows:

- if  $MSNRb > ESNRb$ , removal of the data,
- if not, set  $ESNRb$  into the acoustical data for the corresponding depth, instead of -999 as suggested by De Robertis and Higginbottom [79],
- if  $MSNRir > ESNRir$ , removal of the data,
- if not, nothing done,
- if  $MSNRir/MSNRb >$  a criterion value automatically estimated between the ratios for each acoustical data file, removal of the data,
- if not, nothing changed (this option is useful tool for detection of air-bubble generated by SCUBA divers).

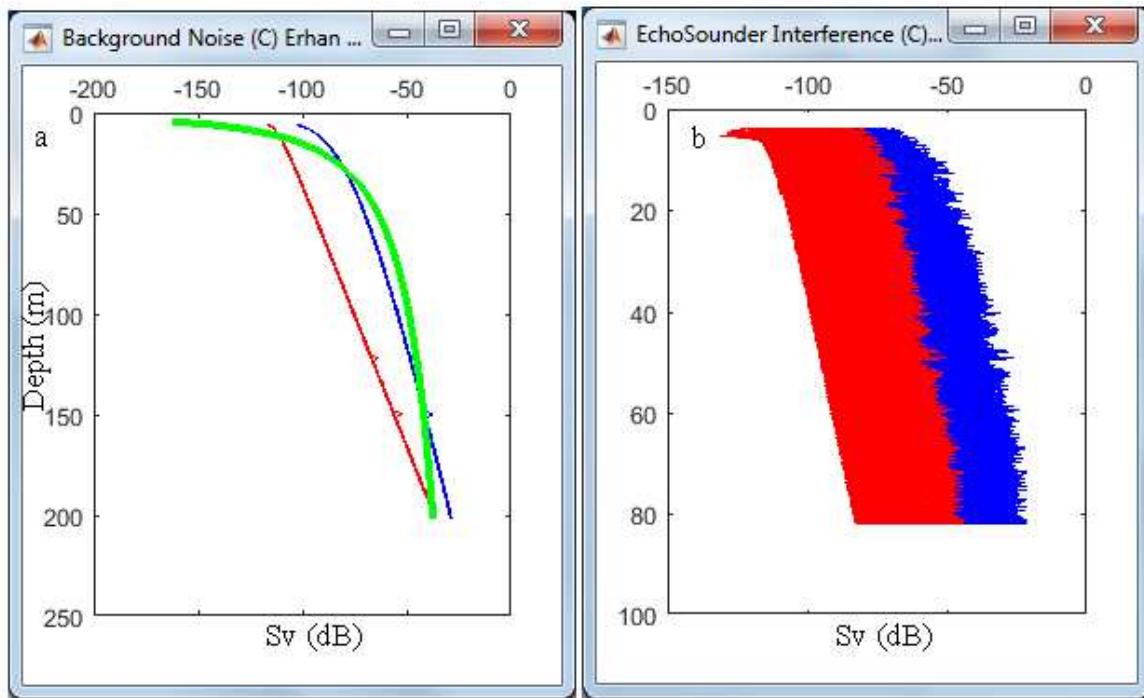
**Table 2.** Fast manual to use an algorithm of “Noise & Reverberation” with function, case, recommendation and Troubleshooting/negatives for each option with number (see Figure 9 for the numbers).

No	Function	Case	Recommendation	Troubleshooting/negatives
1	Data file name to read Label name			
2	Background noise $\pm$ label			
3	Background noise threshold	Could adjust threshold during analysis if noise not organized well for removal	Slide up and down accordingly	Depending on background noise data measured in listening mode and signal-to-noise ratio estimated (S/N) Possible spatiotemporal changes in the noise measurements
4	$\pm$ background noise threshold for removal	threshold insufficient to detect background noise	Slide up and down to increase and decrease range of the threshold	The faster adjustment the less targets (seagrass) fixed (see 3)
5	Interference & reverberation noise $\pm$	Label name		
6	Interference & reverberation noise threshold	Could adjust threshold during analysis if such noise not detected well	Slide up and down accordingly	Depending on Interference & reverberation noise data measured in listening mode Spatiotemporal changes in the noise measurements
7	$\pm$ Interference & reverberation noise threshold for removal of spurious targets	threshold insufficient to detect Interference & reverberation noise	Slide up and down to increase and decrease range of the threshold	The faster adjustment the less targets (seagrass) If no noise data file for Interference & reverberation noise, background noise data file needed
8	background noise /interference & reverberation noise ratio $\pm$	Label name		
9	background noise /interference & reverberation ratio	Alternative method for removal such noises Could adjust threshold during analysis if noise not detected well	Slide up and down accordingly looking at echogram	Depending on both background and Interference & reverberation noise data measured in listening mode Spatiotemporal changes in the noise measurements
10	$\pm$ background noise /interference & reverberation noise threshold for removal	threshold insufficient to detect Interference & reverberation noise	Slide up and down to increase and decrease range of the threshold	The faster adjustment the less targets (seagrass) If no noise data file for Interference & reverberation noise, background noise data file needed

11	Start analysis	Start button	After all setting done in data entry (see 12, 13)	Cannot change the data entry after functioning (see 12, 13)
12	Canopy height of seagrass	Change the default setting if necessary	First look at the canopy height at maxima in the echogram for a survey	Need a rough value, but not more than 1 m After starting analysis the entry disabled
13	Transducer depth at draft of R/V	Deployment depth below surface	Measurement for real bottom depth	Enter 0 if transducer at surface After starting analysis the entry disabled
14	info to start or stop	Label name		
15	Info for lower limit of background S/N ratio	No detection still if correct S/N threshold set up	Use 3 and 4 in the case	Upper limit setting not independent
16	Info for background S/N ratio	No detection still if correct S/N threshold set up	Use 3 and 4 in the case	Lower and upper limit setting not independent
17	Info for upper limit of background S/N ratio	No detection still if correct S/N threshold set up	Use 3 and 4 in the case	lower limit setting not independent
18	Info for lower limit of Interference & reverberation S/N ratio	No detection still if correct S/N threshold set up	Use 6 and 7 in the case	Upper limit setting not independent
19	Info for Interference & reverberation S/N ratio	No detection still if correct S/N threshold set up	Use 6 and 7 in the case	Lower and upper limit setting not independent
20	Info for upper limit of Interference & reverberation S/N ratio	No detection still if correct S/N threshold set up	Use 6 and 7 in the case	lower limit setting not independent
21	Info for background noise /interference & reverberation S/N ratio	No detection still if correct S/N threshold set up	Use 9 and 10 in the case	Upper limit setting not independent
22	Info for background noise /interference & reverberation S/N ratio	No detection still if correct S/N threshold set up	Use 9 and 10 in the case	Lower and upper limit setting not independent
23	Info for upper limit of background noise /interference & reverberation S/N ratio	No detection still if correct S/N threshold set up	Use 9 and 10 in the case	lower limit setting not independent

			Do not stop if misestimated noise	
24	Stopping the analysis	User wants to stop the analysis	removal in some data files, process that later again when all files completed	Stop after the current file processing, not immediately
25	Dead Zone and methods	Label name		Angle estimation based on ping-to-ping, not distance, Need to include the most precise methods of dead zone for average, At least one of three methods required for the next analysis
26	Dead zone estimated by the present study	Optional, Present study,	Click on to involve that into average Dead Zone calculation, or not	
27	Echo View's dead zone	Optional, EchoView [77]	If available in the input data, option enable	Works well if GPS reports every ping, At least one of them required for the next analysis
28	Alternative dead zone	Optional, Mello and Rose [78]	If available in the input data, option enable	Works well, regardless of GPS reporting every ping, At least one of them required for the next analysis
29	Data management to save output data, or figure, or to show S/N solution figures	Label name		
30	Saving the enhanced echogram by the corrected bottom	Optional to see the results later	Check button when user does not monitor the process	
31	Saving the processed data	Use for the next algorithm	optional	If stepping to the next analysis, must be on
32	Only S/N solution figures	To show the figures	optional	Slow down the analysis

The expected SNR (ESNR) versus the water column depth was estimated using a logarithmic regression between acoustical data (Sv) without Time-Varied-Gain (TVG, one-way transmission loss,  $TL=20\log R + 2R\alpha$ , where R is the range in m, and  $\alpha$  is absorption coefficient in dB/m) and depth for both background noise (Figure 3a) and interference (Figure 3b).



**Figure 3.** Acoustical data profile versus the depth (see Figure 2) for estimates of the expected SNRb (a) and SNRir (b). Red line denotes the  $S_v$  without TVG, blue line  $S_v$  with TVG, and green line regression curve.

### 2.3. Algorithm of "SheathFinder" & Leaf length and biomass (Sheathfinder1, and 2)

This script consisted of sections of simultaneous removal of weak and strong scatterers, and simultaneous calibration and fixation of the leaf and sheaths or vertical rhizome of *Posidonia oceanica* (Figure 4). All processes were visually performed looking at the calibration file. Visual calibration file is pre-required before switching to the next real process to estimate the biometrics. The calibration file possesses a section having the meadow (Figure 4d,e). In some temporal cases, the vertical rhizome and sheath could be too short for the detection or within the deadzone [5]. In these months, the filtration continues until leaf parts near the sheaths disappear on the enhanced echogram.

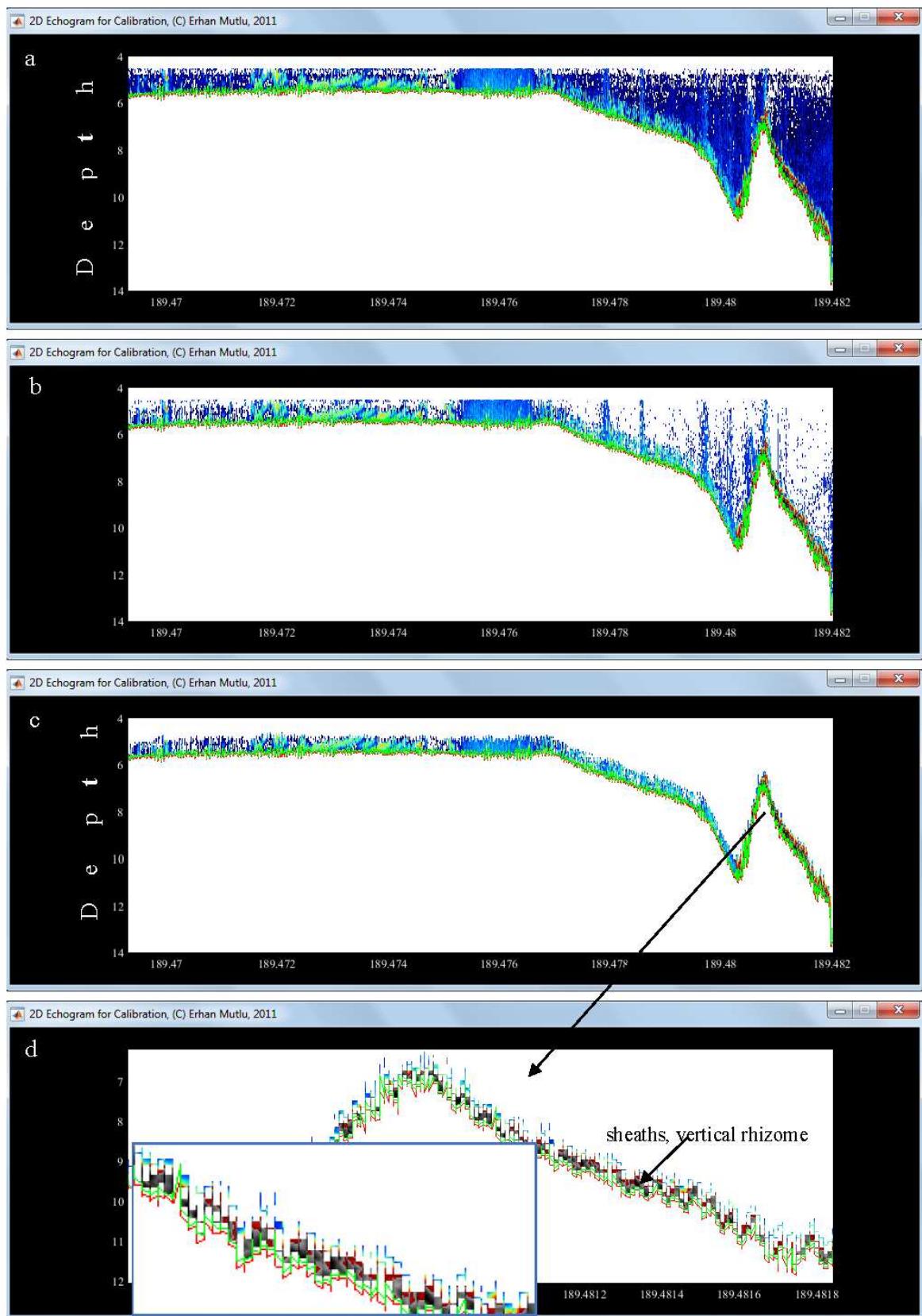
After fixation of the sheaths, the algorithm seeks the count-to-count echo upward to determine the leaves until there are a few gaps between the successive counts. The rule for the determination is that volume backscattering strength has to be in a decreasing trend from the bottom to top of the leaves.

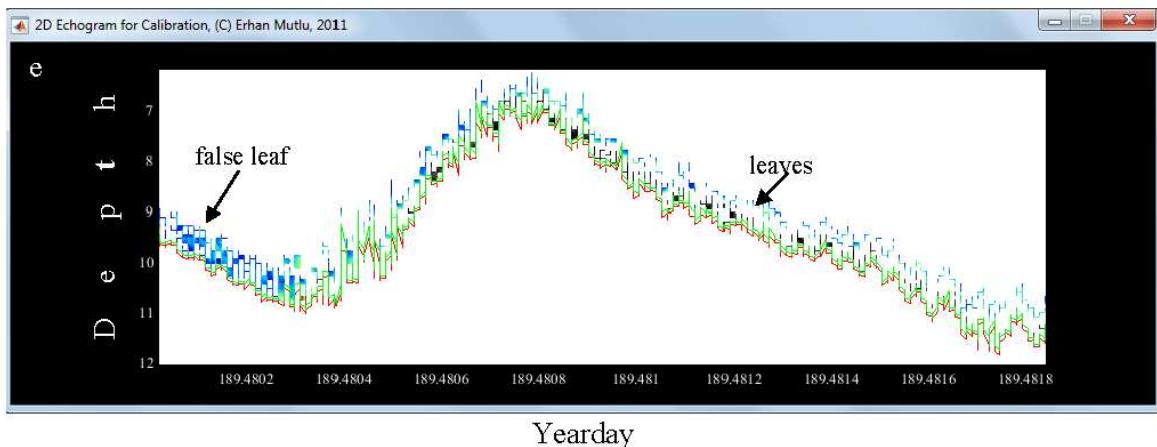
After completing the seeking above the dead zone, this height reads the leaf length in a precision of one-eighth of the pulse width depending on number of strata entered in "Visual Analyzer". Thereafter strong scatterers suchlike fish are removed from each leaf, and the EDSUs ( $S_v$ : volume backscattering strength in  $\text{dB}/\text{m}^3$  and  $S_a$ : area backscattering strength in  $\text{dB}/\text{m}^2$ ) are calculated following the methods as suggested by Simmonds and MacLennan [52]. Besides, the algorithm discards echo without sheath, and categorizes that as a false leaf (Figure 4e).

After finishing processing the acoustical data of a file, leaf biomass is assessed using different EDSUs in relations to the biometrics of the meadow in time [60]. Of the six different temporal relationships [60], the algorithm choices right month matched with month of the acoustical data over yearday. It shall choice the closest month if the yearday of the data is outside the six months.

Methodologically, each script needs an input file or data produced as an output file in MatLab format (\*.mat) by the previous script throughout AbdezdeR1 to Sheathfinder2. Initially, AbdezdeR1 only needs output files in CSV format, processed by "Visual Analyzer" since the package of POSIBIOM cannot directly read file.dt4 in the current status.

The package is installed according to window size based on the pixels of the monitor or display owned by the users.





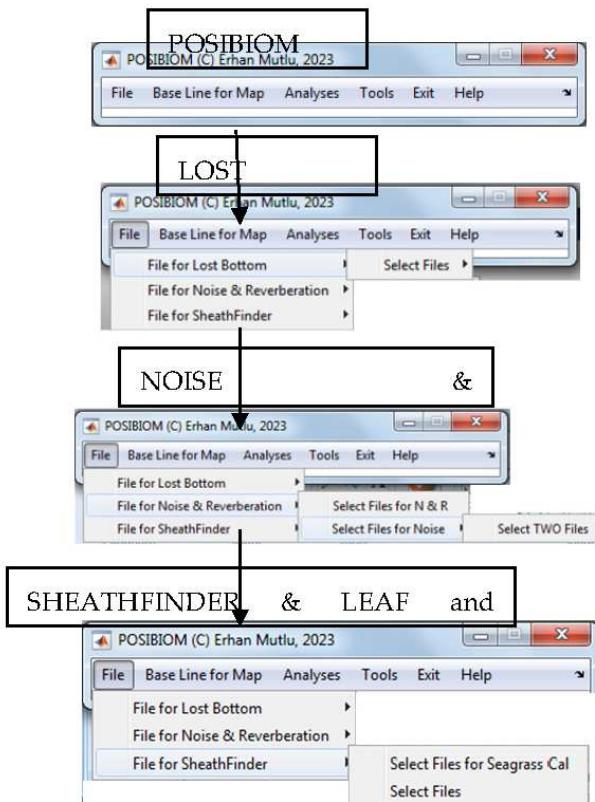
**Figure 4.** An example file or data to calibrate the meadow; unprocessed data (a), removal weak scatterers (b), removal of strong scatterers (c), removal and filter of leaf (red continuous line denotes the bottom, green the dead zone) (d) and vertical rhizome and sheaths (e) on the enhanced echogram.

### 3. Results

Besides the data (acoustical data in \*.CSV to \*.mat) and auxiliary (baseline or coast map in format of \*.bln to \*.mat, reference noises in \*.CSV, sheath calibration in \*.mat, and coast blanking in \*.bln to \*.mat) files opening/loading, a main menu for controlling all processes is configured in a window called POSIBIOM (Figure 5).

#### 3.1. Flowchart of POSIBIOM

The diagram shows the flowchart in order to finalize all analyses (Figure 5). There is also an option called “Tools” to plot results for leaf length and biomass distribution in format contour or line on the map.



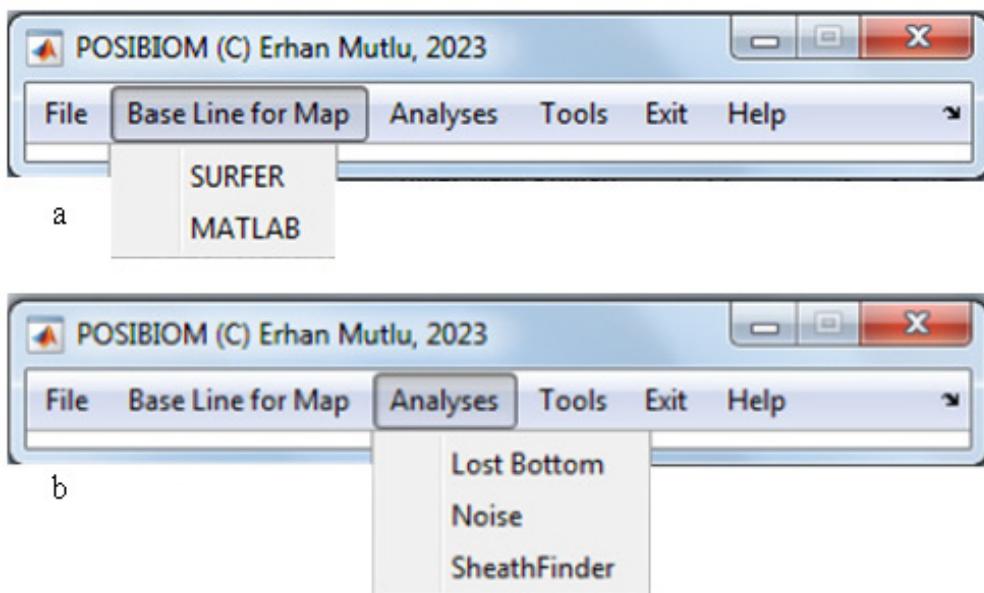
**Figure 5.** Running order to estimate leaf length and biomass of the meadow in main menu, POSIBIOM.

To load the data for acoustical or other type data, opening in “File” is associated with that of Microsoft Window, embedded in the MatLab. For each analysis, opening files is specifically called from the menu (Figure 5). Files for analysis of “Lost Bottom” are opened in format of “\*.CSV”, output of Visual Analyzer. The next analyses use format of “\*.mat” as output of the previous successive analysis.

The output files are saved in a folder “AbdezdeOut”, created by the package, under the folder where the user opens the data for “Lost Bottom” analysis. The next analyses save the outputs into a folder “AbemsiOut”, and “PosiBiom” for “Noise & Reverberation”, and “SheathFinder & Leaf and Biomass” algorithms, respectively in the way aforementioned. In other word, the previous output files become input files for the next analysis. Output file of the last analysis (Figure 5) is in format of “\*.xls\*” for the users to use the data for their different purposes.

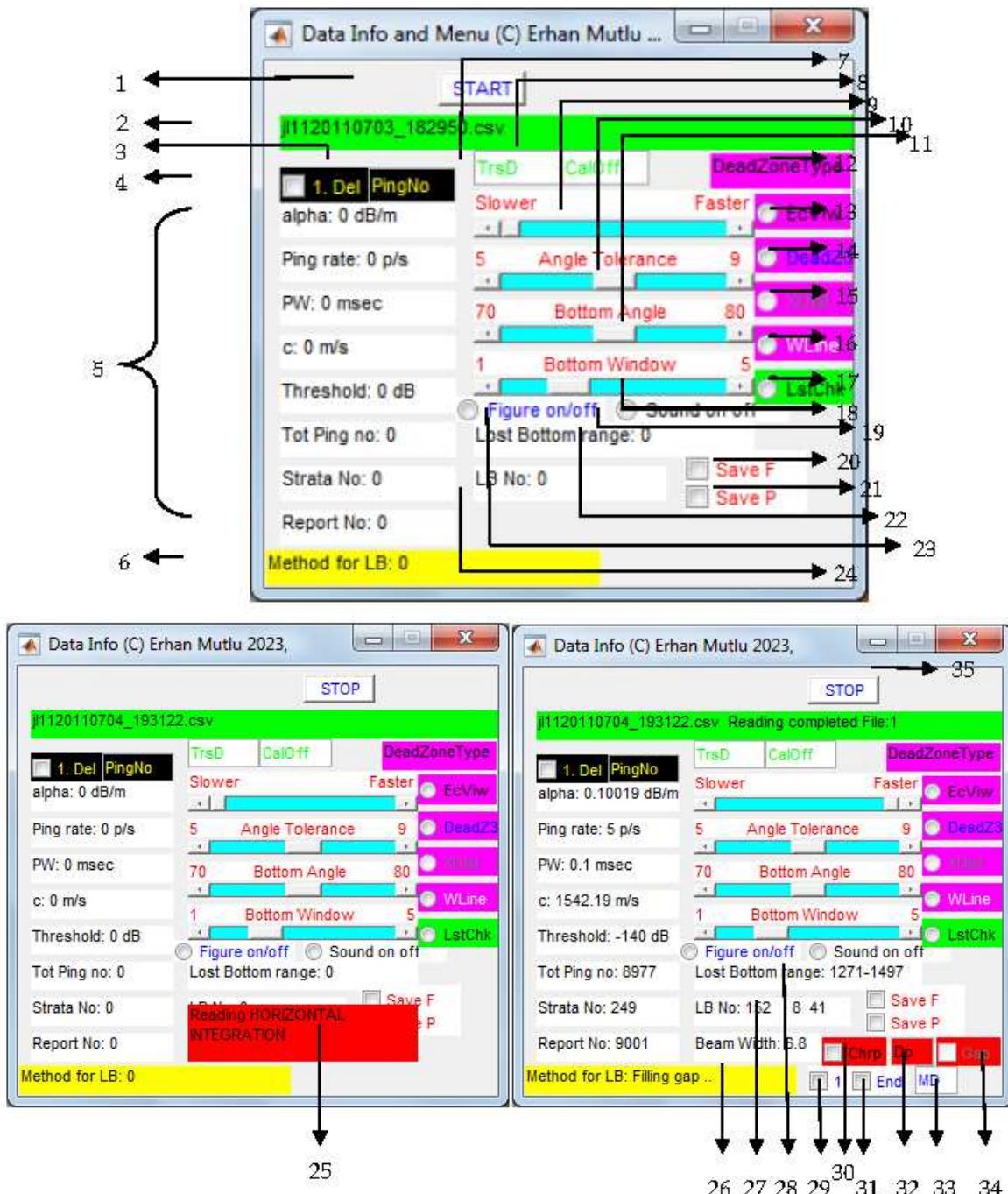
### 3.2. Lost Bottom and Dead Zone

This algorithm is purposed to correct the lost bottom, and then to estimate dead zone depth using three different equations (Equations, 1-4). After opening single or multiple input data files the users want to process (Figure 5), the algorithm needs also to open a coastline file from ‘Base Line for Map’ before starting the analysis clicking “Lost Bottom” in “Analyses” in POSIBIOM menu (Figure 6).



**Figure 6.** The procedure followed to start the “Lost Bottom” analysis. a) Loading the coast line for map, and b) starting the analysis.

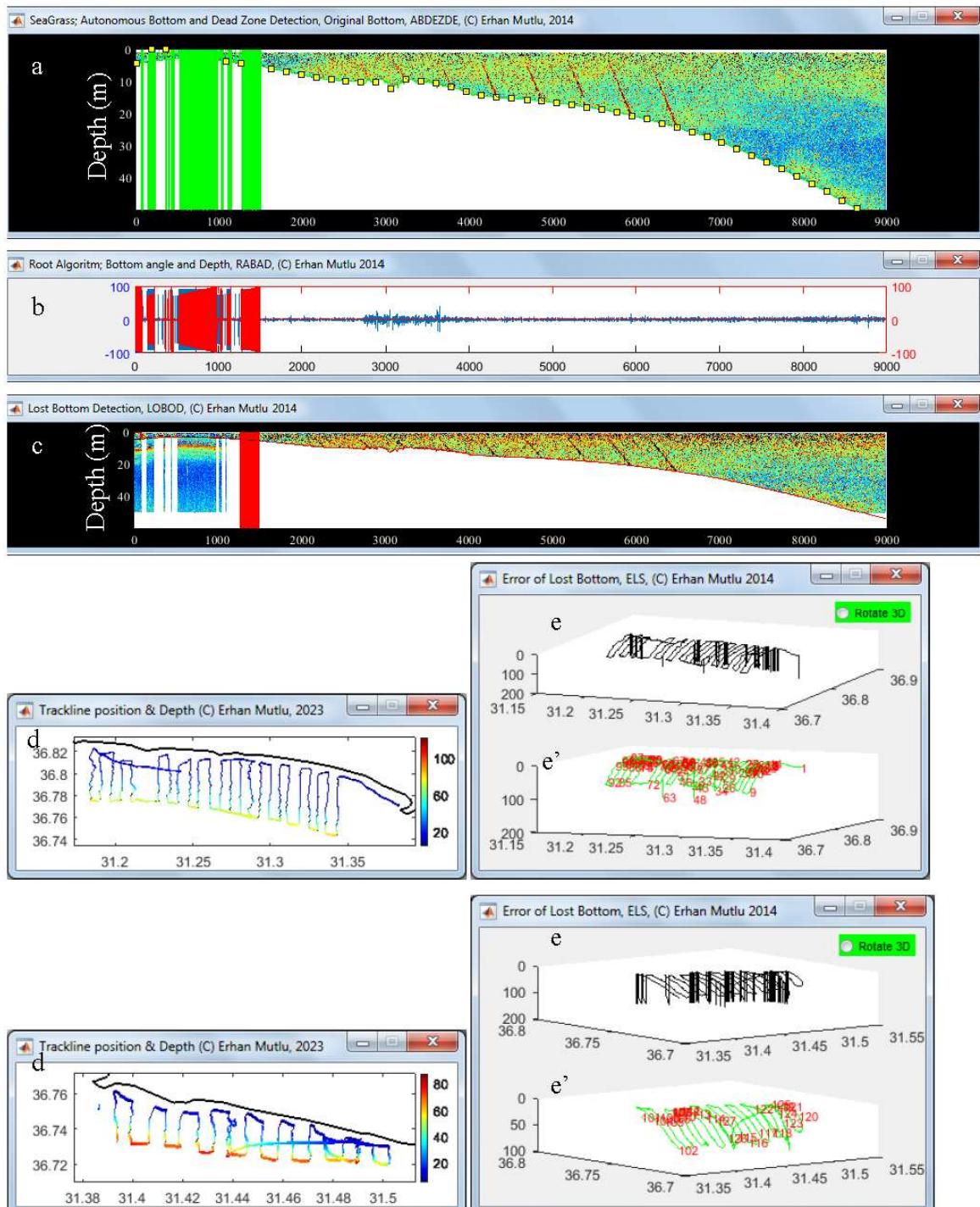
The algorithm starts to read each of the files sorted in an alphabetic order of A-to-Z after starting the analysis (Figure 7), and then a window appears to control the analysis (Appendix 3) and get information of the acoustical data and echosounder configuration during the sampling (Figure 7, Appendix 4). After all setting (transducer depth, calibration offset, types of dead zone estimates, save etc., Table 1) is performed in the window, the analysis proceeds tapping button START (Figure 7). Description of each option in Figure 7 is presented in Table 1 with their functions, case to use, recommendation and possible negatives.



**Figure 7.** Graphical menu for control, configuration and info menu of “Lost Bottom” (see Table 1 for actions of each coded option and function with number).

During the process with the “Lost Bottom”, enhanced echogram first appears in pings versus depth for unprocessed acoustical data with marks of the estimated lost bottoms in green lineation (Figure 8a). These lineation occurred based on the algorithm of bottom angle and bottom depth in the adjacent real bottoms (Figure 8b, the figure appearance is optional because of some cases, no 23 in Table 1). As the analysis progresses, bottom depth correction starts from the beginning to the end of the pings (Figure 8c, the figure appearance is optional because of some cases, no 23 in Table 1). If there is any spurious scatterer through the water column in front of the bottom, the processing cleans them in order to reach the bottom. Nevertheless, original acoustical data are saved into output file. After finishing the analysis for the lost bottom, the algorithm estimates the dead zone depths using the equations user selects, and then final enhanced echogram comes out in the screen. If user checks option “save P”, the final echogram is saved as format of \*.jpeg (\*, input filename). If user checks

option “save F”, the final results with the variables necessary to the next analysis is saved as \*.mat (\*.input filename) to folder “AbdezdeOut”. Finally, the corrected bottom depth is plotted in line on a map after correction rate info appears on a 3D-plot to be checked by user to see uncorrected and corrected bottom depths (Figure 8e, e', respectively).



**Figure 8.** Graphical outputs of the “Lost Bottom” analysis. a) Enhanced echogram before start of the analysis (green vertical lines denote the fixed lost bottom), b) an algorithm to figure out detection of the lost bottom regarding to the bottom angle and depth (blue vertical line is bottom angle, red line bottom depth), c) correction in progress after starting analysis (red vertical lines to be corrected in progress), d) bottom depth on coastal map after correction completed, e) Uncorrected bottom depth in 3D plot (vertical lineation denotes lost the bottom, e') corrected bottom depth in 3D after finishing the analysis.

From this example in Figure 8, the result showed a significant of correction rate on comparison of plot in Figure 8e with that in Figure 8e'. Overall, goodness of the correction was estimated in a percentage of more than 95 after all files (approximately 4000 files belonging to different seasons and years) were analyzed by the “Lost Bottom”. The plots in Figure 8d, e are reset every 100 files processed, and the analysis then needs to press START to continue the next files.

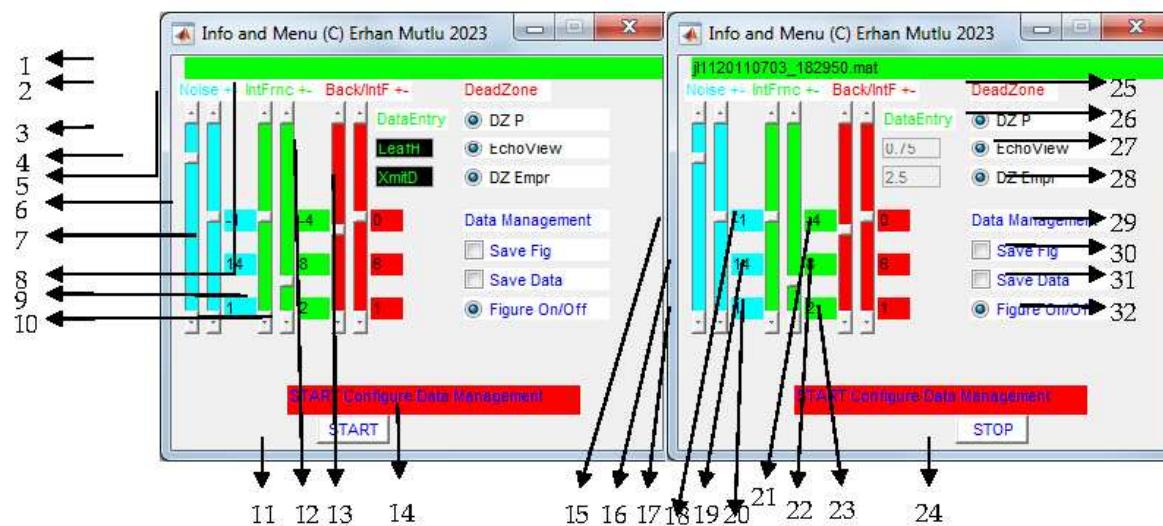
### 3.3. Noise, Reverberation and Interference

This algorithm is purposed to remove background natural noise, reverberation and interference (artificial noises) using three different conditional functions based on the SNR (Figure 9). After opening single or multiple input data files the users want to process (Figure 5), the algorithm also needs to open a coast file from ‘Base Line for Map’ in the main menu, and needs two different noise files as described in Figures 2, 3 and 5 before the analysis. Starting the analysis is performed clicking “Noise” in the “Analyses” in the POSIBIOM menu (Figure 6). This algorithm first runs a subcall in association with “Noiseback1” to estimate the expected SNR (ESNR) for background noises and interferences (Figure 3).

The algorithm “Noise & Reverberation” is achieved depending on the configuration the users perform to configure the options flexibly; particularly for the reverberation intensified at the surface and water column (volume reverberation) (Figure 10a). The surface reverberation is sourced by the wavy sea, strong winds rocking/rolling the ship, and speed of its propell at stop.

The interference had different patterns in relation to the bottom depth, and pulse width, ping rate and frequency of the ship and researcher echosounder (Appendix 5).

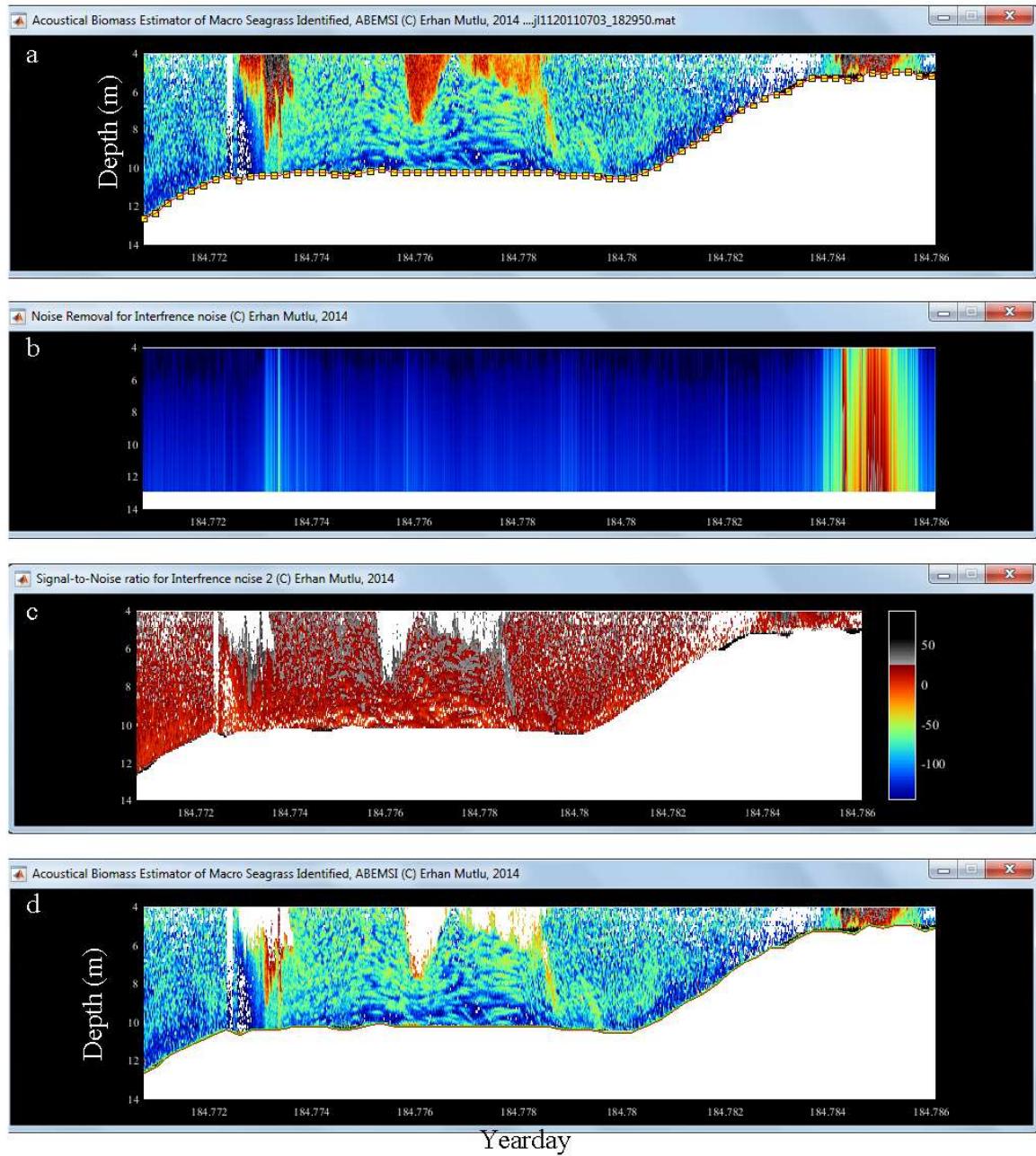
The menu needs to enter an approximate maximum canopy height of the seagrass and the transducer draft depth installed during the survey, and if no entry, the default values appear (Figure 9, Table 2).



**Figure 9.** Graphical menu for control, configuration and info menu of “Noise & Reverberation” (see Table 2 for actions of each coded option and function with number).

After finishing to run “Noiseback1”, the program calls a subprogram “Sheatfinder2” to start the process for the removal of the spurious echoes such as the noises, and reverberations. The subprogram demonstrates first output data, processed by the “Lost Bottom”, in an enhanced echogram (Figure 10a), and bottom depth and average dead zone line estimated by the methods the user checks in (option numbers of 26-28 in Figure 9). If any method is not checked by the user during the processing by “Lost Bottom”, the method option is disabled automatically in the analysis of “Noise & Reverberation” (Figure 9). The algorithm starts to fix the noises and reverberations for the removal after the trial of three conditions (Figure 10b, c) between the measured and expected SNR. Thereafter, the output file with the variables (Figure 10d) necessary to the next analysis,

“SheathFinder, Leaf & Biomass” will be saved as format of “\*.mat” in to a folder, “AbemsiOut” under folder, “AbdezdeOut” if the user wants to save the outputs (Figure 9, function no 31).



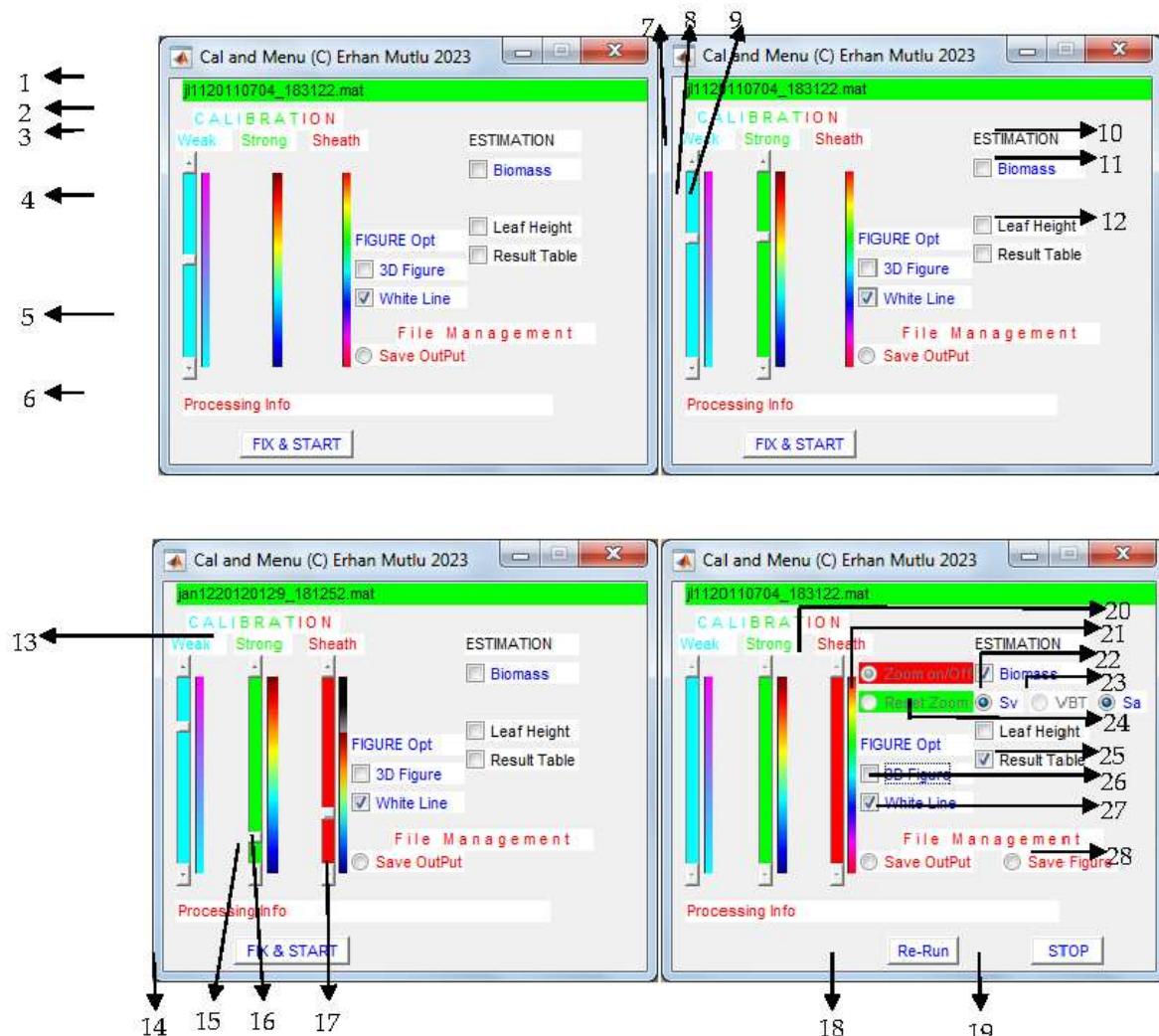
**Figure 10.** Graphical outputs of the “Noise & Reverberation” analysis. a) Enhanced echogram before the start of the analysis, b) an algorithm to figure out detection of the overall noises, spurious echoes (optional), c) the SNR for each background natural and artificial noise (optional), d) removed noises and reverberations, in this example the configuration needs optimum adjustments furthermore to remove the entire reverberations.

The noise, especially interferences had different patterns extending down to the bottom in time and space (Appendix 5). This situation creates a lot of negative and spurious sources suchlike fake acoustical characteristic similar that of the sheaths and vertical rhizome. Besides, there is different reverberation rather than that generated by the ship propell; for instance, that generated by SCUBA divers as studying a purpose within beam of the transducer (Appendix 6). During the analysis both unrecovered and unfixed noises and reverberations are removed by the next analysis, “SheathFinder, leaf & Biomass”, and echoes through the canopy height are kept to the next analysis.

### 3.4. Leaf and Biomass Estimation

Recognition of different traits of *P. oceanica* in the algorithm was performed basing the descriptions on the experimental results obtained from Mutlu and Olguner [60]. After cleaning out of the spurious echoes from the acoustical data, the algorithm gives an opportunity for visual calibration to fix the seagrass (Figure 4). Therefore, this algorithm gives a favor for the users which are not much familiar with the acoustical theory. For the calibration, three sliders which guide the users with each of color scale specific to the each slider are available to remove weak, strong scatterers, and to fix the leaf and sheath or vertical rhizome, respectively (Table 3, Figs. 4, 11). For that, user needs only to load a file containing the seagrass from the main menu, POSIBIOM (Figure 5). Before starting the analysis, both acoustical and calibration data files are required to open (Figure 5). At the same time, the coast file is needed to plot results on map.

Compared to the other algorithms (Figures 7 and 9), the present algorithm is the easiest and simplest graphical demonstration during the usage (Figure 11). There are some options to save or plot the estimated results (leaf biomass and length) based on the different EDSUs. Overall, the biomass estimate based on  $Sv$  is plotted on a map on the colored trackline (Appendix 7c, d). For the contour or line plots, a further option called 'Tools' was embedded in the main menu with some more graphical properties.



**Figure 11.** Graphical menu for control, settings and info menu of "SheathFinder & Leaf and Biomass" (see Table 3 for actions of each coded option and function with number).

During the analysis, the biomass is estimated using a combination of Sv and Sa with eight equations formulated by Mutlu and Olguner [60]. When tapping “Result Table”, the results flow down ping-to-ping, but this option slows down the analysis. The algorithm saves the results comprising of three different estimate of the biomass, including other informative variables (file name, geographical coordinate, yearday informing time in format of day,hrs+min+s of a day, bottom depth, dead zone depth, canopy height (leaf height), the biomasses (based on Sv, Sa in “Cut” experiment, Sa in “Leaf” experiment [60], and month range of which the equation is used to estimate biometrics (Figure 12).

**Table 3.** Fast manual to use an algorithm of “SheathFinder & Leaf and Biomass” with function, case, recommendation and Troubleshooting/negatives for each option with number (see Figure 11 for the numbers).

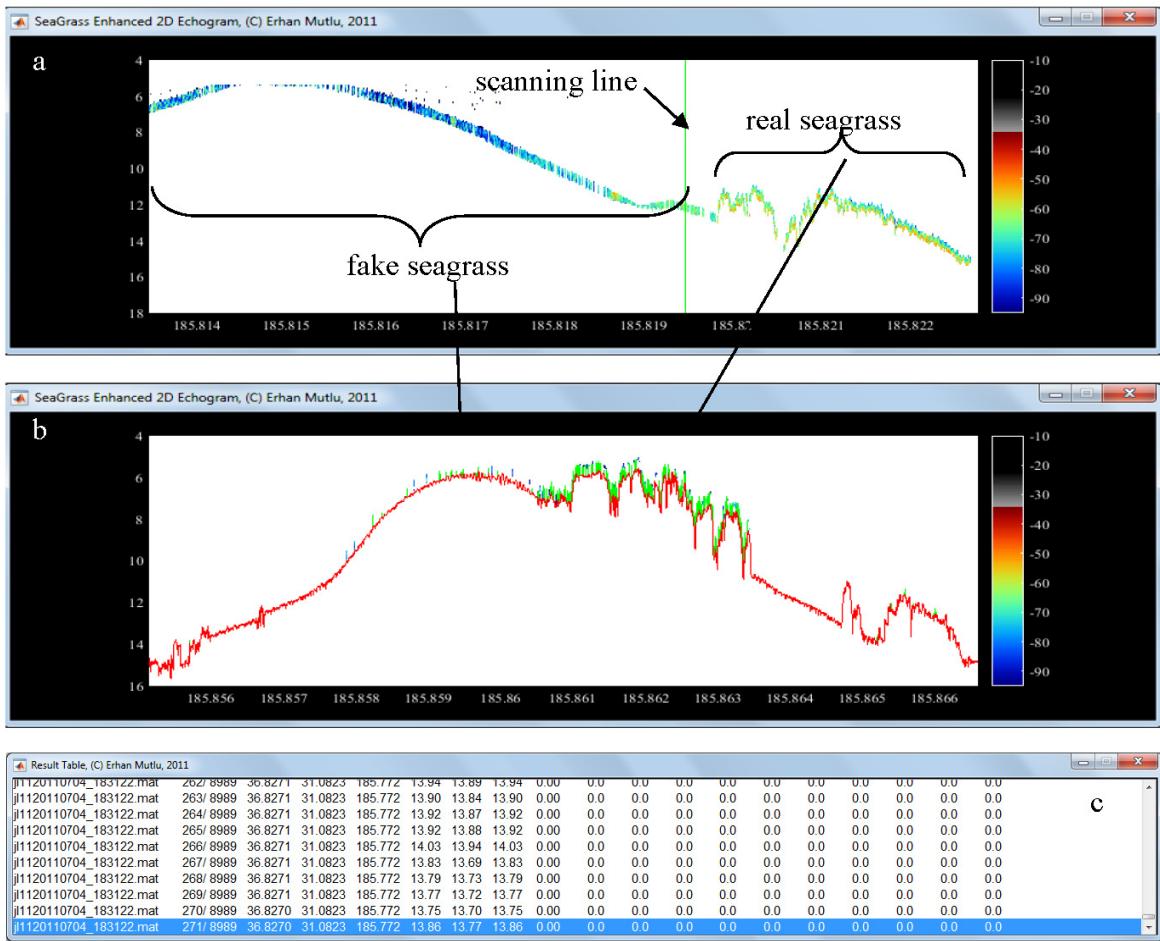
No	Function	Case	Recommendation	Troubleshooting/negatives
1	Current input file name on process	Updating by file to file		
2	Title for removal of spurious weak and strong scatterers, and sheaths	label		
3	Label of weak scattering removal	label		
4	Calibration threshold setting to remove weak scatterers	High background noise, some weak zooplankton layers	Adjust neither less nor more looking at the enhanced echogram	A good calibration data for showing clear seagrass
5	Color scale to guide user to slide up or down for weak scatterers removal	colorbar		Colors cannot fit to the acoustical data echogram in some cases, looking at the echogram
6	current info while processing the data			Strong scattering removal takes time
7	Label of strong scattering removal	label		
8	Calibration threshold setting to remove strong scatterers	Reverberation, interference, Fishes and schools, compact zooplankton layers	Adjust neither less nor more looking at the enhanced echogram	A good calibration data needed for showing clear seagrass, appears after function 4 completed
9	Color scale to guide user to slide up or down for strong scatterers removal	colorbar		Colors cannot fit to the acoustical data echogram in some cases, looking at the echogram
10	Title to show results in figure and table of the estimation	label		
11	Showing figure for estimated biomass on map with trackline	Optional,	Use “tools” after the analysis completed	Slowing the analyses, trackline width constant
12	Showing figure for estimated leaf (canopy) length on map with trackline	Optional,	Use “tools” after the analysis completed	Slowing the analyses, trackline width constant

13	Label of sheaths fixation and removal	label	
14	Fixing the settings and then start the analysis	All settings completed	Functions in 20 and 21 will be disabled after the start, Press START button after every 100 files processed
15	Calibration threshold setting to fix and remove the sheath	Calibration to fix sheath threshold	Adjust neither less nor more looking at the enhanced echogram until sheath disappear, Fix threshold ligule if sheath too short in time, use functions 20 and 21, if not well fixing settings, restart from function 4
16	Color scale to guide user to slide up or down for fixing and removing sheath	colorbar	Colors cannot fit to the acoustical data echogram in some cases, looking at the echogram
17	Saving outputs into file in *.xls format	For mapping the biomass and canopy height later	Use the "tools" or other mapping software
18	Re-start the analysis from the beginning	Not well works for the estimates	Use the option when case needed
19	Stopping the analysis	User stops the analysis	Use in case of function 18
20	Zooming on the main echogram	Fixing sheaths better for large file	Use the options
21	Zoom back to original size of the echogram	To select better sheaths on the echogram	Use the option when case needed
22	Method VBT to estimate biomass	Disabled	Need of the VBT software
23	Method to map estimate biomass based on Sa	Only estimates using regression between biomass and Sa	use if necessary

24	Method to map estimate biomass based on Sv	Only estimates using regression between biomass and Sv	Use if necessary	Slowing down the processing, appear only after checking function 11, Estimates (Sa and Sv) always available in the output file
25	Show the complex estimates with many variables in a scrolling Table	optional	optional	Slowing down the processing, Click off/on not to show the results
26	Show 3D curtain enhanced echogram	optional	optional	Slowing down the processing,
27	White line on 2D echogram	Optional, white area between bottom depth and dead zone	optional	Not in 3D echogram
28	Save figures for mapping biomass or leaf height	When function 11 or 12 checked, respectively or both	Optional, use the "tools" or other mapping software later	Saving after finishing all files processing, see function 11 and 12, appear only after checking function 11 or 12

During the analysis, the algorithm starts to scan structure suchlike the sheath or vertical rhizome of the seagrass in ping-to-ping depending on the vertical resolution analyzed with "Visual Analyzer" (Figure 12a). The green scanning vertical line proceeds slower when determining the sheaths than that during absence of sheaths in the ping. There are two theories applied to remove the fake seagrass; one is presence/absence of the sheaths, and another is structure suchlike the sheaths whether to have real leaf or not as described in Material and Methods.

After completing to scan the sheaths and leaves, strong scatterers like fish individuals among the leaves are removed according range of the expected threshold of the *Posidonia* [60]. The next step is the biomass estimates of the leaves which are regressed with seasonal equations according the current sampling month [60], followed by saving the output data in a format of ".xls" without variable names into an automatically created folder "PosiBiom". The variable names are in the order of each column as follows; geographical coordinates (latitude, longitude), yearday, bottom depth, canopy tip depth, leaf height (canopy height since orientation of leaves standing on the bottom; right, semi-flat and flat position, [62]), and three different successive biomasses (g/m<sup>2</sup>) estimated by Sv and Sa ("cut experiment"), and leaf calibration ("leaf experiment") [60]. There is an auxiliary script, namely "PosiDrwTool" in "Tools" of the main menu, POSIBIOM to draw distribution of the estimates in a format of trackline or contour (Appendices 7 and 8).



**Figure 12.** Fixation and estimates of leaves of the seagrass during the analysis of the acoustical data by the “SheathFinder& Leaf and Biomass”; a) scanning sheaths or vertical rhizome by green vertical line to determine fake or real seagrass, b) estimates of the seagrass after the analysis of current file is finished (green line denotes the canopy height), and c) Result table when checking the option (option no 25 in Figure 11).

#### 4. Discussion

The present study has discussed the advantages and disadvantages of the algorithm, POSIBIOM with the other acoustical commercial software to estimate bottom type and vegetation since the POSIBIOM is a production of the first study for *Posidonia oceanica* and its biometrics. Some scripts of the algorithm were related to solution of the general acoustical problems (e.g., lost bottom correction and noise and reverberation removal) during the post-processing of the acoustical data purposed for the other studies, such as fisheries acoustics. For such problems, there are commercial and professional software. In their some cases, a manual intervention is needed to correct the bottoms even if they have many functions for this matter. The mapping of macrophytes can be accomplished with the software developed for the submerged vegetation (Echo Submerged Aquatic Vegetation; EcoSAV, Visual Habitat and Visual Aquatic) by BioSonics Company. Further methods of vegetation acoustics were developed based on algorithms to classify bottom types [63–66]. They are OTCView, Echoplus, BioSonics VBT (Visual Bottom Typer), and EchoView [67–70]. However, previous studies on the vegetation acoustics have remained at the assessment of percent coverage and canopy height of the seagrass (e.g., van Rein *et al.* [50]; Lee and Lin [80]) depending on the limitation of echosounder parameters [81]. Unlike the POSIBIOM, there is overall a maximum bottom depth restriction with a depth value entered in the software to remove suchlike the seagrass or macrophytes at the greater depth. There were two attempts to calibrate the acoustical data to estimate the biometrics of the seagrass by developing the nonprofessional algorithm; one was based on data of VBT process [59],

and the next one was based on “SheathFinder” to fix the sheaths/vertical rhizome [58]. The present study modified and revised the nonprofessional algorithms completely and significantly to be used professionally for the seagrass studies.

Regarding to the package POSIBIOM, one issue is the avoidance from the usage of the destructive methods to study the meadows under the protection [34–36]. Besides, the previous remote sensing techniques produced only coverage and mapping of the different habitats (e.g., Fakiris *et al.* [48]; Dimas *et al.* [49]). Compared to the techniques, the acoustical method is faster, more precise, and easier to ground-truth the data [2,50], and develop the algorithms to remove spurious scatterers [51]. Another issue is detection of the seagrass by the acoustical frequency. The meadow is detected well by a frequency of 206 (200) kHz to assess biomass variables even if the higher frequency (420 kHz, Biosonics inc.) is recommended to study the seagrass and vegetation acoustics. Quintino *et al.* [82] recommended 50 kHz to study soft bottom classification, but 200 kHz is good effective for the classification of the bottom types including seagrass.

Vegetation acoustics is not however easier at practice to process its data than the fish acoustics. The fisheries acoustics had many problems overcome during the processing [52]. In many cases, the macrophytes and seagrass had unstable relationships specifically with oscillation between the EDSUs and biometrics in time and space [60,61]. These all relationships biased the estimates besides the other scatterers were available suchlike as epiphytes on the leaves and fishes among the seagrass [35,59,61]. The meadow showed inconsistent linear relationships in some seasons [60] when its biological and physiological activities were intensified [83]. Another seagrass, *C. nodosa* produced a more stable relationships in time than *P. oceanica* performed [60,61]. The POSIBIOM regarded these matters to estimate biomass by using three different equations of the relationships established in each season [60]. However, any flower (only one flower found) and fruit of the meadow was not encountered during the all surveys of the present study. The flower and fruit which occurred often in the West Mediterranean Sea [84,85] would change the acoustical traits of the meadow in other parts of the Mediterranean coasts. The main factor producing the variation in the estimates is the strength and magnification of the physiological activities of the meadow, calcification, oxygen gas releasing, beam pattern (orientation), age/type (juvenile, middle and adult leaves) and hardness of the leaves, and bottom depth of the occurrence of the meadow [60–62,86–90]. The relationships used in the present study were established by means of an *in/ex situ* study at a bottom depth of 15 for each season. The 15 m was a critical range with its inconsistent biometrics in high variation between shallow and deep *Posidonia* [5,18]. The fish removal was standardized according to the results of the acoustical ranges of the meadows, but possible significant epiphytes could be disregarded for the removal. For this, SCUBA sampling was needed for assess acoustical contribution to the EDSUs of the meadow leaves by solving the forward model solution [60]. This was not a practical way for the clear biomass estimates with frequent SCUBA samplings as a destructive method. Mutlu and Olguner [61] estimated the contribution of a calcareous alga, *Pneophyllum fragile* to the EDSUs of leaves of *C. nodosa*, and the contributions remained below the threshold of the leaves depending on abundance (ind/m<sup>2</sup>) of the alga. Another calcareous alga, *Hydrolithon boreale* occurred recently on leaves of *P. oceanica* at the present study, its abundances were found to be low as compared of that of *C. nodosa* [35,61].

The POSIBIOM could be the best offer currently for the biometric estimates regarding the matters aforementioned, and some improvements are needed during the next studies to decrease possible disadvantages. There are some biases in estimating the biomass and bottom coverage compared to the estimates by SCUBA (Appendices 8 and 9). This could be due to misestimates of bottom recovery for the coverage and the equations established at 15 m for the biomass [60]. A bottom depth of 15 m was found to be critical depth for occurrence of the high variations of the biometrics [5,18]. The negatives/troubleshooting were discussed for each algorithm in Tables 1, 2, 3.

As a conclusion from the present to future studies featured to improve the algorithms;

The algorithm would open acoustical data of the different echosounders manufactured by other companies rather than that by BioSonics,

A way would be developed for reading the acoustical raw data directly, needless of the post-processors,

The debugs would be considered to get rid of them if the feedback about that was returned by the users after the package would be a profession version converted to a programming language of C.

The multi-frequencies would be applied if the data was collected, and then the algorithm would be figured out and configured accordingly.

The experiments would be repeated to establish the relationships between the EDSUs and biometrics at the different bottom depths, else than 15 m,

Leaf types would be also taken into account of the equations of the relationships,

Mapping the results would be associated with different types of the coastal map,

Eventually, the software of the package would be hardware connected to the echosounder, which needs too much works associated with the electronics.

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**Institutional Review Board Statement:** Not applicable.

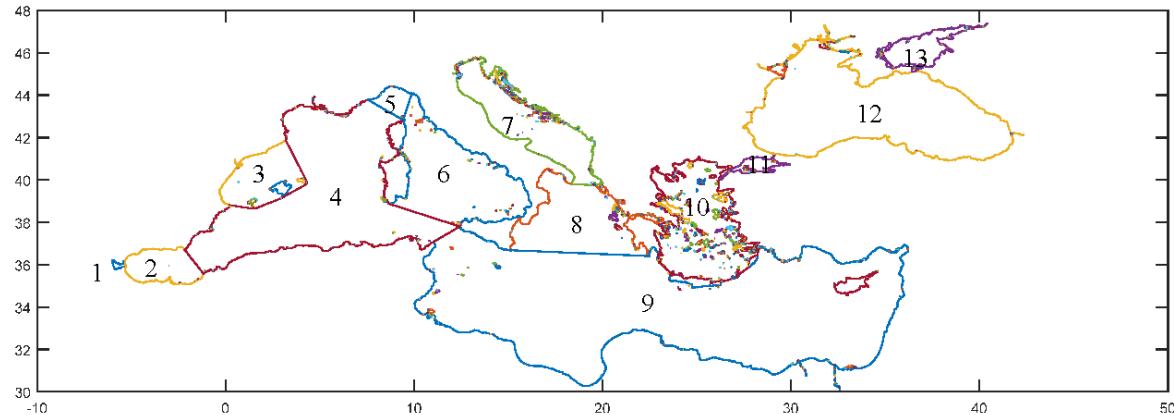
**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

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**Conflicts of Interest:** The author declare no conflict of interest.

## Appendix A



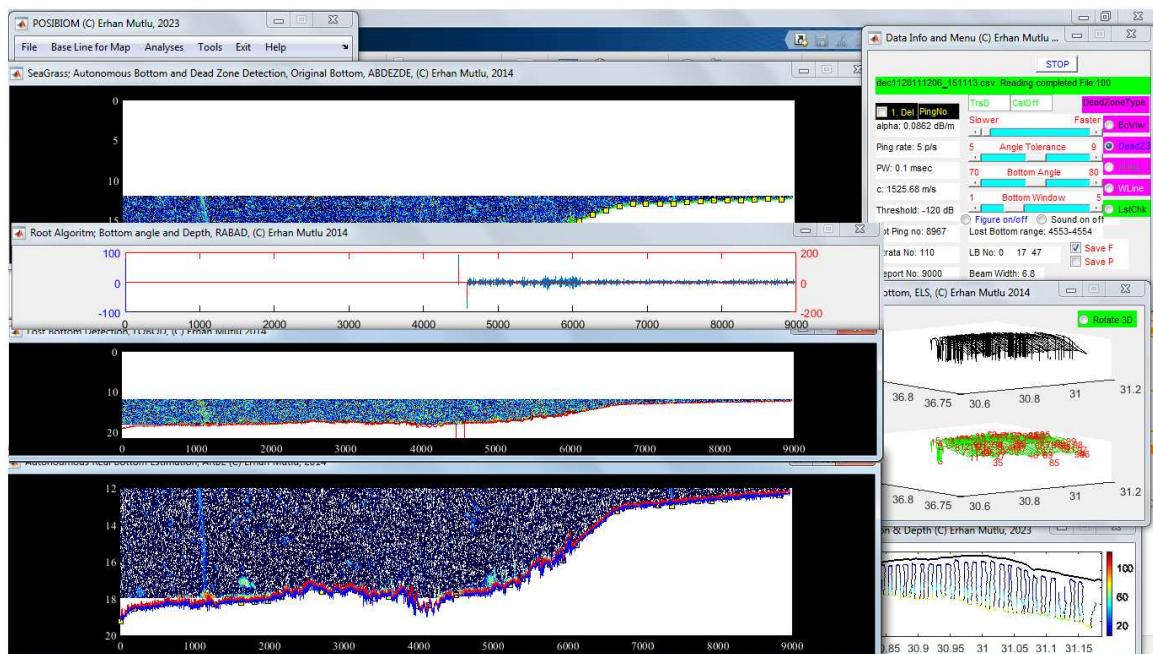
**Appendix 1.** Coast line file downloaded from Marine Region [76] and then converted to formats of SURFER (Golden Software) and MatLab (MathWorks inc.). Each colored area is a large sector in polygon (13 polygons) consisting of polygons of the islands to use for mapping and blanking data outside the Islands as formatted by Marine Region [76].

1. Gibraltar Straits
2. Alboran Sea
3. Balearic Sea
4. West Mediterranean Sea
5. Ligurian Sea
6. Tyrrhenian Sea
7. Adriatic Sea

8. Ionian Sea
9. East Mediterranean Sea
10. Aegean Sea
11. Sea of Marmara
12. Black Sea
13. Azov Sea



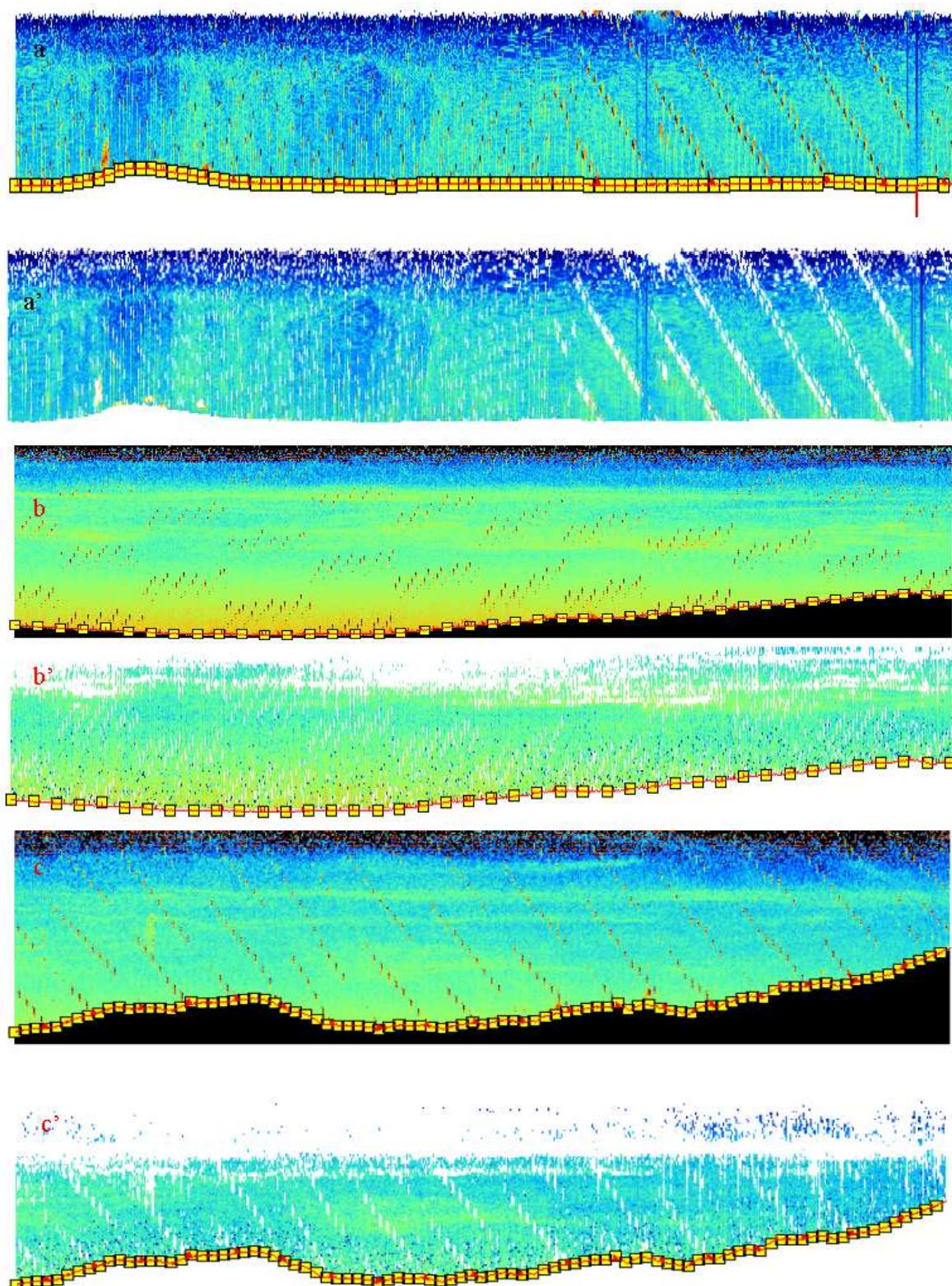
**Appendix 2.** Automatic appearance of option in a case of existence of misestimated bottom at the first pings owing to the volume or surface reverberations or the interferences.



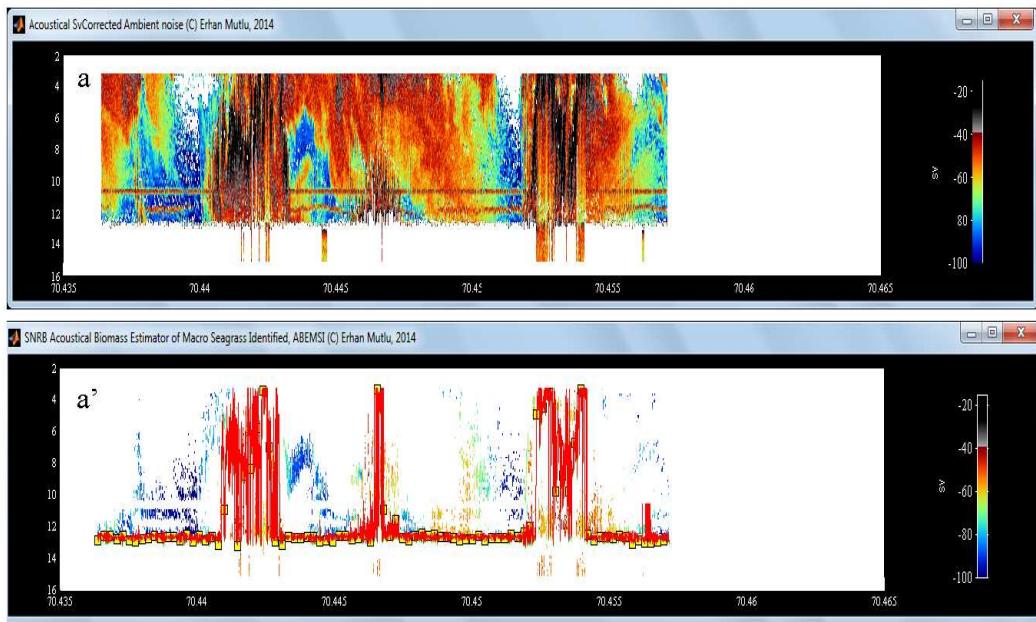
**Appendix 3.** Screen shot of analysis “Lost Bottom” during the processing. Two figures in the middle of left panel appear optionally when function “Figure on/off” is on.

**Appendix 4.** Echosounder parameters during the data collection and \*configuration parameters of Visual Analyzer during the post processing (see Figure 7).

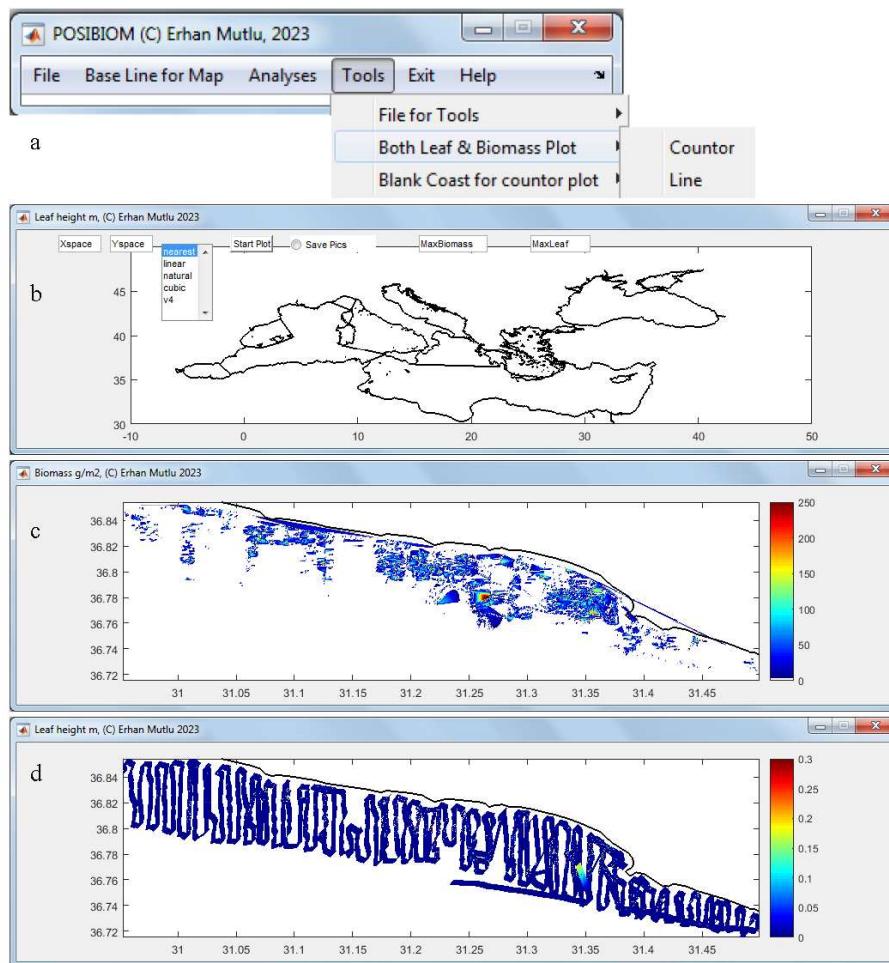
Info variables	Description
alpha	Absorption coefficient
Ping rate	Pulse rate per second
PW	Pulse width
c	Sound speed
Threshold	Minimum data collection threshold
Tot ping no*	Total number of pings
Strata*	Number of stratum (vertical resolution)
Report No*	Number of reporting data (horizontal resolution)
Beam width	Angle of main lobe of beam



**Appendix 5.** Removal of background noises; different patterns of the interferences. a, b, c) original data, and a', b', c') removal of the interferences, respectively.

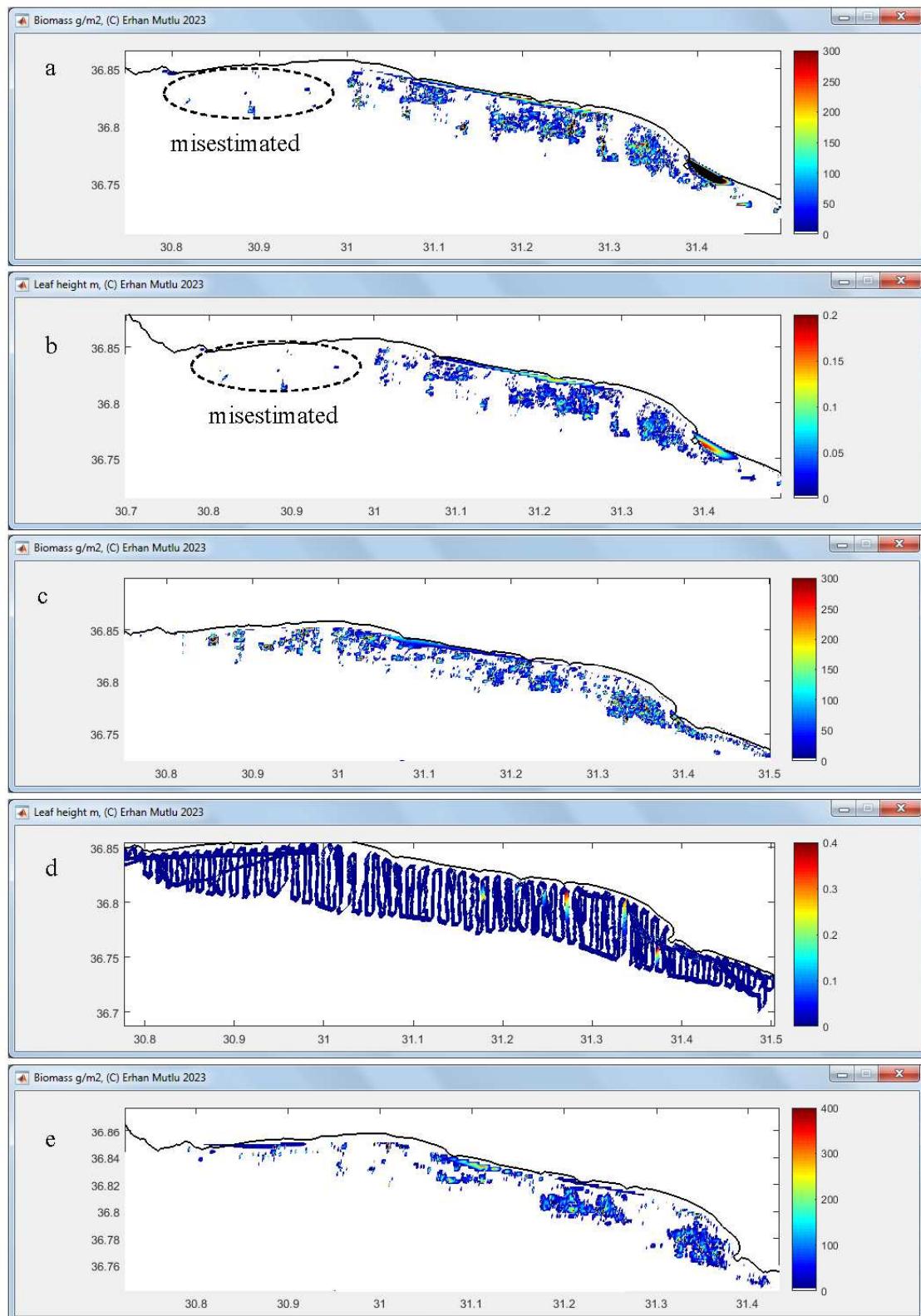


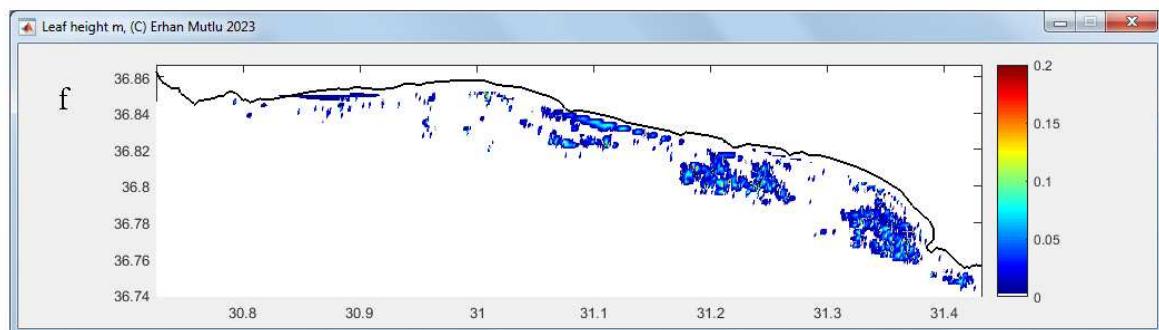
**Appendix 6.** Removal background noise and reverberation; different patterns of the surface and volume reverberations produced by SCUBA divers, a) original data, a') removal of the reverberations, respectively.



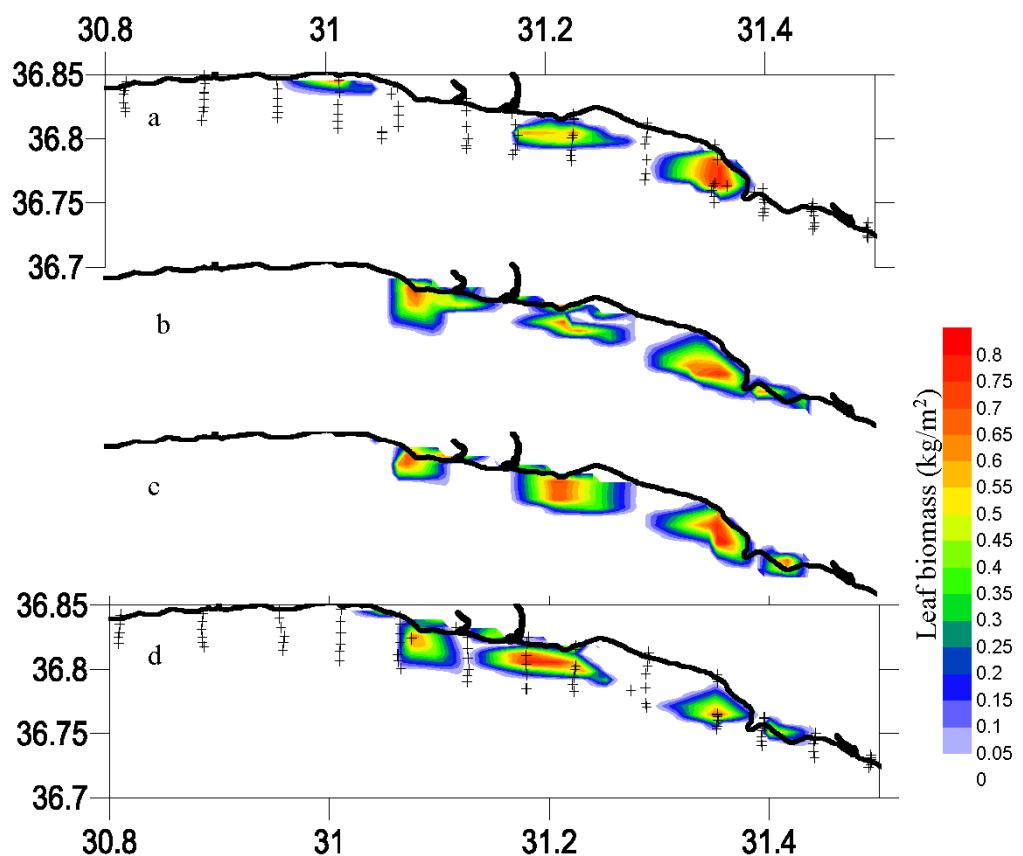
**Appendix 7.** Tools menu to load files (a), to configure the plot of the map for instance here biomass ( $\text{g}/\text{m}^2$ ) in contour (b, c) or leaf length (m) in trackline (d) depending on maximum value the user sets. When selecting contour plot, some entries appear to configure interpolation to make grid (Xspace;

longitude versus Yspace; latitude), interpolation type and save of the figure (b, c) and when selecting the line plot (b, d), an entry only for line width. The data was an example from July 2011 (c, d).





**Appendix 8.** Outputs of 'Tools' of the package program: a) leaf biomass ditribution ( $\text{g/m}^2$ ), and b) leaf length (m) in contour mode in December 2011, and c) and d) in contour mode and line mode in January 2012, and e) and f) in April 2012, respectively.



**Appendix 9.** *Posidonia oceanica*: leaf biomass ( $\text{kg/m}^2$ ) estimated from SCUBA sampling in December 2011 (a), January 2012 (b), April 2012 (c), and July 2011 (d) to compare with estimated with acoustics (see Appendices 7 and 8 for the comparison). (+: sampling stations by SCUBA).

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