**Response letter to the manuscript JoVE55161 “Protocol for microplastic sampling on the sea surface and sample analysis”**

**Editorial comments:**

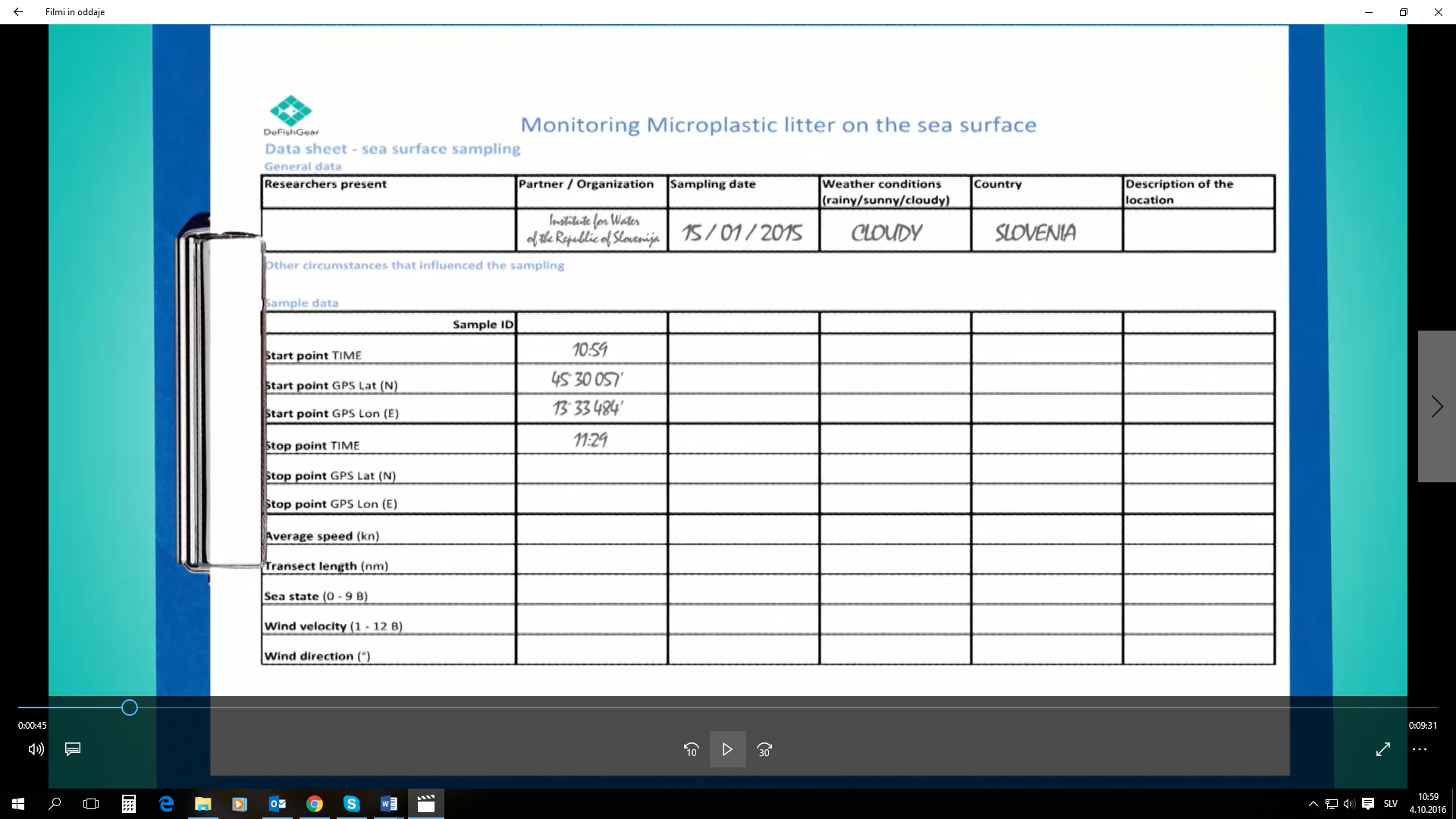
*“Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.*

*•Graphics/screen capture issues*

*• 0:33, 0:50 - When the video is scaled to our webplayer's size, the text in this graphic is very difficult to read clearly. I would recommend scaling the graphic up or making the text larger.”*

**Answer:** We do not know where exactly is the problem because on our side the resolution of these pictures is sufficient to clearly read the data (see picture bellow, Prt Scr from our computer).

Actually, the spreadsheet is only an example of a spreadsheet, the labels in the fields are not crucial for the understanding of the protocol. Each researcher should prepare his/her own spreadsheet for the field work. The only important thing is, that data such as the date, start and stop GPS coordinates of the transect and wind speed are noted as this is mention in the text (audio and manuscript).



**•** *9:17, 9:31- If any of the text in these figures is important for the viewer to see, it should be enlarged.*

**Answer: T**he labels of the peaks are not important for the viewers, since we are not talking about specific peak wavelengths, but about general spectra that is acquired from the sample and later compared with the spectral library.



**•Audio issues**

**•** *1:17 - It sounds like the diegetic sound was left in for this shot. The sound should be removed for clarity and consistency.*

**Answer:** We think that the diegetic sound is not disturbing the viewers’ concentration since the sound in not loud and does not interfere with the narrator voice. We showed the video to several people, and no one mentioned that this part would be disturbing the clarity of the speech and the protocol**.**

**•** *If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.*

**Answer:** All the figures and tables are made by authors or video producer.

**•** *JoVE reference format requires that the DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.*

**Answer:** We checked the references again and added the DOIs where they were available**.**

**•***IMPORTANT: Please copy-edit the entire manuscript for any grammatical errors you may find. The text should be in American-English only. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol and the manuscript. Please thoroughly review the language and grammar prior to resubmission. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.*

**Answer:** Before submission the manuscript was edited by native English speaker (from USA). The language and grammar will be checked again prior to the resubmission.

**Reviewers` comments:**

**Reviewer #1:**

Manuscript Summary:

Comments on JoVE55161 by Kovač Viršek et al. (see also attached manuscript R\_1\_annotated):

*Merits of the study: This manuscript describes an instructional video for microplastic sampling. This is very helpful because there are more and more people starting to sample microplastics and it is very important that they all work in the same way. This video can help here. However, there are a number of things that can be improved.*

*1) Title: ok*

*2) Abstract: ok*

*3) Introductory part: ok, motivates the study well.*

*4) Protocol: ok, but there is confusion with the numbers. Also, there are some steps that are not clearly emphasized neither in the written nor spoken text, but that obviously do happen - for example, no mentioning is made of the second label that is placed in the jar, but in one video-sequence, this label can clearly be seen.*

*Also, some parts that are important for the methodology, e.g. using a flowmeter, are not shown in the protocol or video, even though they are very important! This description is first mentioned in the discussion section, but should be part of the protocol.*

*Also, the lab control should be mentioned here.*

*5) Representative results: ok, but the figures and tables need captions! Some of the tables look as if they were copied straight out of excel without any editing work! These should be improved.*

*6) Discussion: the discussion is a bit confusing and in some sections not really a true discussion, but instead reads like a protocol description. Some of these sections (e.g. the lab control, or the flowmeter section), if they are maintained here in the discussion section, will need references, which are out there and the authors should search for them.*

*7) Literature cited: I have not checked whether all papers are cited in the text and viceversa.*

*In summary, this video and the related text can become very useful documents in the future, but they require careful revision before they should be published in the journal.*

*There are a number of additional comments in the annotated manuscript. In case there are any questions about our comments, we invite the authors to contact us directly.*

**Answer:** We thank the Reviewer #1 for the thorough review of the manuscript and the video, and all the valuable comments. We took all the given comments into consideration and replied on the reviewer’s comments bellow in the response letter and made correction in the manuscript. Therefore we explained the confusion with numbers which emerged after editing by the journal editorial office of the manuscript. We proposed that in order to provide logical sequence and content of the steps in the protocol, the original enumeration and division of paragraphs should be accepted.

We added into the protocol a suggestion about the second label of the samples.

Our protocol is describing the sampling without flowmeter, therefore we mentioned the use of flowmeter first in the discussion as an additional option. The recommendation of using controls during laboratory analyses was already included into the submitted manuscript (lines 357-360).

We do not understand the comment 5, that »figures and tables need captions«, since there are captions above figures and tables in the manuscript and also in the video protocol. The tables weren't made in the Excel. The comment is very biased, since the editors didn't have any comments on the quality of our tables. Therefore we didn't consider this comment as necessary to abide.

The discussion was written by the document »Manuscript Instructions for Authors« from Jove. The Editors did some changes in splitting the paragraphs into several (there were 5 before, and after the editing there were 7). We suggested the correction of one of the paragraphs to be merged in different way than was suggested by the editors.

As already mentioned, the mention of flowmeter will stay in the discussion, and the references were added. The mention of lab control was included in the discussion and is appropriately referenced.

All the literature cited in the text is listed in the »References« section of the manuscript.

Major Concerns:

N/A

Minor Concerns:

N/A

Additional Comments to Authors:

N/A

**Manuscript comments (Reviewer 1):**

**Comments to introduction:**

**Comment A1, line 71**: »*References needed here*«

Answer: Reference has been added.

**Comment A2, line 73**: »*References needed here*«

Answer: Reference has been added.

**Comment A3, line 74**: »*The citation numbering is wrong here and throughout he whole manuscript.*«

Answer: Numbering of references have been corrected according to the JoVE manuscript instructions for authors.

**Comment A4, line 75**: »*Microplastics are not only circular.*«

Answer: The word “diameter” was deleted.

**Comment A5, line 76,77:** »*You describe below that the mesh size used during the monitoring is 300m, which therefore should be the lower limit of the microplastics analyzed with the present method. I think it is important to also standardize the lower limit of the microplastic analyzed.*«

Answer: Yes, we completely agree that also the lower limit of the microplastic has to be defined in the definition of the term microplastic. Now it is assumed that the low limit is the mesh size of the sampling net. As we did not find any new definition of microplastic where the lower limit is defined, we did not make any modification in the manuscript.

**Comment A6, line 81**: »*This sentence is not clear. Pleaser rephrase*«

Answer: Sentence has been corrected.

**Comment A7, line 83**: »*References needed here« and* ***Comment A8, line 83****: »How do sea currents break down microplastics? I think breakdown through contact, collision with other fragments or floating objects and abrasion is meant here.*«

Answer: Sentence has been corrected and reference has been added.

**Comment A9, line 91**: »*The verb here should refer to 'the small size of the microplastics', which is the subject of the sentence. However, I guess the authors wanted to write here that the microplastics are consumed*«

Answer: Sentence has been corrected to be more understandable.

**Comment A10, line 94**: “*This is mainly the hydrophobic surface of the microplastics which allows POPs to fix. Ratio surface/volume enhance that effect.*”

Answer: In the sentence also the term “hydrophobic surface” has been added.

**Comment A11, line 96**: “*But the POPs come from the environment. I think the authors here are talking about the additives part of the composition of the microplastics*.”

Answer: Sentence has been corrected to be more understandable.

**Comment A12, line 98**: “*References needed here*.”

Answer: Reference has been added.

**Comment A13, line 104**: ” *This study is useful to provide a standardize method for microplastic monitoring, not only in the EU seas but also worldwide. This argument should be better developped here to justify the importance of the present study*.«

Answer: Sentence has been corrected.

**Comment A14, line 112**: “*Where does this claim come from. References are needed here*.”

Answer: Reference has been added.

**Comment A15, line 116**: “*I do not understand the logic of the argumentation here. I recon that that part belongs to the protocol section below*.«

Answer: In this part of the introduction, the short descripton of our work is described. This paragraph has been corrected to be more understandable.

**Answers to Methodology**

**Comment A16, line 123**: *I think it could also be interesting to describe here the method in case small vessel do not have a spinnaker boom on the side. In that case, the trawl should be dragged 50 m from the stern of the boat to reduce turbulences of the sea surface generated by the boat propeller and reduce contamination from the boat.*

**Answer**: This protocol describes the sampling with a use of spinnaker or »A-frame« from a side of the vessel. For dragging a trawl behind a vessel, a separate protocol would be in place. We mentioned this option in discussion with 2 references.

For use of trawl, dragged from the stern of the boat, the distance, on which the trawl is set for sampling, should be determined individually, since the zone of turbulences caused by the vessel varies from the size of the vessel and from the speed of the boat.

**Comment A17, line 128:** *The GPS coordinates only allow to calculate the area filtered. However, I realized from my own monitoring that the actual volume of water filtered during the sampling can vary greatly depending on the courant. This is why I think it is crucial to fix a flowmeter at the entrance of the mouth of the trawl to measure the volume of water filtered during the sample. I strongly recomment to add this to the method.*

**Answer:** The use of a flowmeter is a welcome addition to the method, but this protocol describes the sampling without the flowmeter and mention of the possibility of adding one is emphasized in the discussion. (See also answer to the Comment A50)

**Comment A18, line 130**: *Based on the transects we did in the bay here recently 3 knots was already too fast for the neuston net, but it may be fine for a manta net, I don't know.*

The manta net is firm and stable enough that the sampling at speed up to 3 knots, fixed from the side of the vessel, is possible without any problems. During DeFishGear project, 44 transects of sea surface were sampled two times by 6 different groups of experts, with speeds from 2 to 3 knots, and none of the expert reported any problems.

**Comment A19, line135**: *Most modern GPS devices also record the transect. If it cannot be guaranteed that the boat sampled a straight transect (for example rougher conditions) the distance could be calculated more reliably through this.*

**Answer**: We added into the protocol, that the most correct way to calculate the length of the sampled rout is from GPS coordinates.

**Comment A20, line 152**: *Should this read ''at least 70%''? (as said in the video)*

**Answer**: No, it was meant “70% ethanol”, since we prepared exactly 70% ethanol that is standard for the use in laboratory. And in the video it is not said *''at least 70%''*.

**Comment A21, line 155**: *There is a risk that the ink of the marker be washed away by the alcohol used to preserve the sample. I recommand to label the samples using masking tape and pencil.*

*and*

**Comment A22, line 155**: *And in addition you should put a pencil-written label (or previously printed with laser printer) inside the sampling jar!!! You should always have two labels, one inside the jar, and one on the outside of the jar!!!*

**Answer**: We improve the protocol with additional backup label.

**Comment A23, line 161**: *No capital letter after opening parentheses*

**Answer**: Corrected.

**Comment A24, line 162**: *So then why not reducing the sampling time to 20 min to provide more general method. I personnaly sample for 20 min due to high organic matter along the coast of Chile. Also, many studies sampled for 20 min (Gago et al. 2015, ).*

**Answer**: This protocol was developed on the basis of the document »Guidance on monitoring of marine litter in European Seas.”, provided by Marine Strategy Framework Directive Technical Subgroup on Marine Litter (Hanke, 2013), where Institute for Water of the Republic of Slovenia is also a member. And in this document the proposed sampling time is 30 min. Furthermore in our experiences after 20 minutes manta net was never clogged, so we always measured for 30 minutes to get better representative sample. We also mentioned that if you have any problems, you should adapt the sampling time.

**Comment A25, line 162**: *More to avoid contamination onboard the vessel: Make sure to not drag the net or the rope to deploy the net across the vessel. Avoid contact of net with the boat hull while deploying and capturing.*

**Answer**: We added this comment as additional notes under “NOTE: General sampling conditions”.

**Comment A26, line 167:** *Ok, but where do you put this? And will you also count this?*

*And*

**Comment A27, line 171** : *Does this refer to the objects from the previous paragraph? Why do you now further determine all objects > 25mm?? Is this identical to the video?*

**Answer**: The editors made a new paragraph in a very unfortunate place, so that now both paragraphs are confusing. We joined them back together, because we think that it is important that those two steps stays together as one, since this step is all about meso and macro litter, and there is no need to devote more space in the protocol for this as this is an optional step.

**Comment A28, line 173:** *It would be necessary to measure the objects here already. Is this desired? If so, it should be added.*

**Answer**: This is not intended and these data are not important as we talk about microplastic and not macro litter.

**Comment A29, line 173**: *This section is confusing – also in the video it is confusing, because while you are talking about ">25 mm", the object that is being manipulated is clearly <25mm!!!*

**Answer**: As mentioned in the answer on comments A26 and A27 – this step of the protocol is one step and in the video the entire step is demonstrated, in this case the particle in the video is smaller than 25 mm but larger than 5 mm, therefore meso litter that is mentioned in this step.

**Comment A30, line 181:** *Please give an order of the maximum volume of each subsample that should be taken.*

**Answer**: We think that there is no need to define the size of the subsample, since the size of the sample depends on the amount of organic and inorganic (natural) and plastic material in the sample, also in the different laboratories Petri dishes of different sizes are used. In addition, the size of the subsample should also reflect the level of experience for recognizing the microplastic of the person examining the sample. Also it is up to the researcher how much of the total sample collected they will examine.

**Comment A31, line 185**: *Further: Be sure to analyze larger objects from all sides as microplastics may be stuck and therefore hidden under larger items. It may also be helpful to move already analyzed objects to one side of the petri dish. I work with a magnification between 16x and 20x to search for plastics and more to identify them (searching for plastics with 80x sounds very tedious).*

**Answer**: Comments have been added in notes in the step 2.5.

We are using magnifications from 20x to 80x all the time, 20x for general examination of the sample and larger magnifications to zoom in on specific particles to check the characteristics of the specific particle.

**A32, line 192**: *What if this is not possible? How to proceed? Possibly give a confidence rating, e.g. 1 = very sure that present item is plastic, 2 = not sure, 3 = probably no plastic? Any suggestion?*

**Answer:** This protocol describes the use of chemical characterization in the end as we believe that results are reliable only if identification of chemical structure is performed for the particles. The proposed method with the use of confidence rating would be very subjective. It is better to use polarization light on the microscope to distinguish between plastic and non-plastic material.

**A33, line 194**: *What could this be? We use a piece of a plastic mesh with a known mesh size as a scale to take pictures.*

**Answer**: Measuring equipment of a microscope means an ocular ruler calibrated by the micrometer slide for the microscope or image analysis software. The protocol was improved.

**A34, line 195**: *I think the shape and erosion of the microplastics are also important characteristics that should be described (see Table 6 in Hidalgo-Ruz et al. 2012).*

*- Shape: sharp-edged fragments may lacerate the digestive tract tissues.*

*- Erosion: degraded fragments may release additive more easily than newer particles (Andrady 2011).*

**Answer**: This protocol describes simple characterization of the microparticles for their size and color. We do not think that for general estimation of marine environment pollution with microplastic particles the shape of the particles and degree of erosion are key information.

**A35, line 200**: *Is this commonly done and feasible? What precision would the scale have to have?*

**Answer**: It is feasible in our experiences to weigh all the particles from one category on analytical scale with four decimal places of precision.

**A36, line 201**: *How do you make sure then that the sample will not get contamination from the dessicator?*

**Answer:**The protocol was corrected as “closed Petri dish”. This was our mistake.

**A37, line 201**: *Also for 24H? This is not clear from that sentence*

**Answer**: The protocol was corrected.

**A38, line 204**: *Something seems to go wrong with the enumeration here, which is jumping all over the place.*

**Answer**: Enumeration was checked and corrected where this was needed.

**A39, line 208**: *This is why I beleive geenral shape of the microplastic should be described (see comment [NO21])*

**Answer**: As already mentioned in the answer on the comment A34: We do not think that for general estimation of marine environment pollution with microplastic particles, the shape of the particles are key information, recognising the additional features of plastic particles only helps at faster recognition.

**A40, line 208**: *Alcohol helps here because it discolours the organisms and colorful plastics therefore become easier to see. Possibly add this?*

**Answer**: The protocol was improved for one additional note in the step 2.2.

**A41, line 208**: *Further signs that something is not a plastic: transparent pieces that break very easily (possibly parts of crustacea or pteropds)*

**Answer**:We had experiences with fragile and transparent pieces that were determined as plastic by chemical characterization. In our experience these are most commonly (but not exclusively) highly degraded foils. Therefore, it is “dangerous” to make a statement from which people would conclude that all microparticles that are transparent and fragile at the same time are always parts of exoskeleton of crustacean or pteropods.

It is true that researchers with little experience with work by stereomicroscope and biological samples, can confuse carapax for plastic, but if you use bigger zoom and polarization light, carapax is easily recognized in the sample.

**Comments to Results:**

**Comment A42, line 254**: “Unclear. Please rephrase.«

**Answer:** The natural colours have been added.

**Comment A43, line 259**: I do ot understand this sentence. Main result of what? Please clarify.

**Answer:** Sentence has been corrected to be more understandable.

**Comment A44, line 259**: “Data is the plural form: one datum, many data!!«

**Answer:** Correction was made.

**Comment A45, line 265**: “By the width of the net opening.”

**Answer:** Correction was made.

**Comment A46, line 266**: “For this it would be necessary to take pictures of the plastics. I don't think this is mentioned in the protocol.”

**Answer:** In the protocol this step (to take the picture for image analysis) is not mention, because this is the specifity of the image analyis software that researcher use it. For instance, we work by AxioVision (Zeiss) software, but we did not mention it in the protocol, while there are some specific rules from JoVE (below).

*“Style Guidelines:*

*Avoid the use of commercial language, including any TM/R symbols or the mention of company brand names before an instrument or reagent*

*TABLE OF SPECIFIC MATERIALS/EQUIPMENT:*

*This table should include information for viewers to obtain the materials used in the protocol such as company, web address and catalog number. It is appropriate to include the specific brand of reagent used in your experiments especially if this specificity influences the outcome of the experiment. However, it is not appropriate to mention specific brand names or company names throughout the manuscript text. Please avoid the use of any copyright or trademark symbols throughout the text, especially in the tables of specific reagents and equipment.”*

**Answers to the Discussion:**

**Comment A47, line 324**: *This chapter s long, repetitve, poorly written and does not have any subchapter. Please, improve the English and shorten*.

and **Comment A48, line 324**: *In most parts of the discussion, you are basically repeating what has already been written in the method. I recommend to substantially reduce the length of the text and make better use of the litterature to support your argumentation. Also, various aspect of the methodology are mentioned for the first time in the discussion (see comments below), although they should belong to the Method section.*

And **Comment A54, line 346:** *All this does not sound like a »discussion« section, but rather like a Material&Methods section.*

**Answer:** This chapter is written according to the JoVE instructions for authors (see below), where methodology have to be disscussed.

*“DISCUSSION: (3-6 paragraphs)*

*Please remember that JoVE articles are focused on the method and protocol. Thus, the discussion should be similarly focused. This should be written in full sentences and paragraph form. This section should discuss:*

*• Critical steps within the protocol*

*• Modifications and troubleshooting*

*• Limitations of the technique*

*• Significance of the technique with respect to existing/alternative methods*

*• Future applications or directions after mastering this technique”*

**Comment A49, line 329**: “*Wording is strange – please, rewrite*.”

**Answer:** Sentence has been corrected.

**Comment A50, line 337**: “*The use of a flowmeter is not described in the method. I think you need to improve the structure of the text to make it more easy to follow. That part should definitely be mentioned before.*”

And

**Comment A51, line 337**: “*It also seems very relevant to include this in the video. You probably could also include the equations that you are using in the video! Is the step of recording the station of initiation of the trawl and the end of the trawl, also in the video? If not, then maybe add?!?*”

**Answer:** The use of a flowmeter is not described in the method, because we did not use them when video was produced. There are a lot of studies, where flowmeter was not used and flow meter is just one of options that could be used if the researcher have opportunity to use them. For this reason we mention in discussion option to use the flowmeter and that the use of flowmeter could be one of the improvement of the protocol.

The step of recording the station of initiation of the trawl and the end of the trawl is already in the video.

One correction was made in the text, to make it more easily to follow.

**Comment A52, line 340**: “*Is this not very fast?!”*

**Answer:** Depends of the sea. For this reason range (2 – 3 knots) was proposed and factors on which you have to be careful were mentioned. See also the answer to the Comment A18.

**Comment A53, line 341**: “*You mentionned that wind speed should always be <2 Beaufort. This need to be clarified.*”

**Answer:** The clarification was made in the NOTE at the end of the first paragraph of the protocol (Sampling of microplastic on the sea surface).

**Comment A55, line 341**: “*References neede here*«

**Answer:** Reference has been added.

**Comment A56, line 352**: “*This also has not been mentionned in the method. Discussion should only focuss on what has been described earlier in the text*.«

**Answer:** This was not mentioned in the methods, while the separation of microplastic from the sample could also be done by the use of stereomicroscope without polarization light. This is just one option that can help researcher to find microplastic particles in the sample. And this is discussed in this chapter, according to the instructions of JoVE (see answer to the comments A47, A48 and A54).

**Comment A57, line 354**: “*Really? Particles > 0.5 mm can not be identified with FTIR spectroscopy?? It seems that this should entirely be possible*!”

**Answer:** »Particles > 0.5 mm can not be identified by FTIR spectroscopy« - this is not written in the text! In the text is written that: »particles >0.5 mm can only be identified visually, by the use of stereomicroscope«, but particles <0.5 mm have to be verified by the use of FT-IR microscope. But of course if you have option to make the chemical analysis also for the particles >0.5 mm, your results are more reliable.

**Comment A58, line 362**: “*Was this investigated somewhere or is this a hypothesis? For example, I'm not sure if the magnification (beyond ~20x) of the stereomicroscope is one of the most important criteria here.*«

**Answer:** This was written on the basis of our experiences and experiences of other scientists (e.g. Lőder and Gerdts, 2015). From our experience the quality of the microscope is one of the most important factor that influence on the results (the source of the light, the quality of lens). See also answer to the Comment A31.

**Comment A59, line 364**: “*As this appears in the discussion section, there should be some references here! There are several studies out there, where people did use these controls and you should refer to those!*”

**Answer:** Reference has been added.

**Comment A60, line 370**: “Again, this not has been mentionned before in the method. Here you could have discussed the possibility to not take in account fibers when clean air flow chambers are not available, as described in the OSPAR request on development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys.«

**Answer:** See answer to the comments A47, A48 and A54.

**Comment A61, line 370**: »Yes, but the equipment is expensive!«

**Answer:** The quality of the results can be in most cases also depended of the equipment used in the study.

**Comment A62, line 394**: “General comment about this writing of »microplastic separation« and other similar expressions – i am not a native speaker, but it is my impression that the noun »microplastic« is used as an adjective for the noun »separation« - if that is the case, then »microplastic« should always be in singular form! Check with a native speaker and if necessary, correct throughout the entire document!«

**Answer:** The phrase “microplastic separation” was replaced by the phrase “separation of microplastic”.

**Reviewer #2:**

**Manuscript Summary:**

*First and foremost, I would like to thank the authors for the insight in addressing microplastic pollution on a methodological perspective.*

*I believe this is a very significant work concerning the worldwide need for standardised protocols, and as someone working on the same research field; I use this opportunity to thank once again the authors.*

*My only comment concerns micro-FTIR techniques. I agree with everything the authors wrote, from pre-treatment of microplastic particles with 30% H2O2 when necessary, to background spectra to reduce noise, but I would also wanted to share with the authors, that sometimes there is apparent noise in the spectrum collected with is likely to be linked to polymer degradation throughout time. Fragmentation does not interfere with chemical structures, but degradation can, and that is likely to somehow affect some of the characteristic bands of some polymers. Please take this information into account. For more on this, please consult manuscripts from conservation and restoration of art pieces, from the 1970's onwards as usually these chemists have a great knowledge on polymer science and FTIR spectra analysis.*

*After everything said, I find the manuscript quite well written and suitable for publication as it is.*

**Answer:** We thank the Reviewer #2 for the thorough review of the manuscript and the video and for all the compliments.

We took the comment about the mention of degradation of the plastic as possible reason for the noise in the FT-IR spectra in consideration. We didn’t change the discussion about degradation of microplastics, since it is already mentioned (lines 359-360), that with (in-depth) analysis of the FTIR spectra, an additional information on particles, such are oxidation and level of degradation, can be acquired.

Major Concerns:

N/A

Minor Concerns:

N/A

Additional Comments to Authors:

N/A

**Authors comments to the editors:**

* We noticed the formatting changes that you made in the numbering (in the protocol) and paragraphs (in the discussion) of the manuscript. We cannot agree with these corrections. They were made in unfortunate places interfering with the understanding of the protocol. Reviewer #1 also commented that »there is confusion with the numbers« (in the protocol). We therefore suggest if we can use the original numbering of the protocol, since it was placed in order of logical steps. Step 2.2 shouldn’t be separated into two steps, since it is an optional step and we don’t want to disturb the reader with several steps he/she should over skip. In the discussion we made a new paragraph, but in a different place, where, we believe, it is more suitable.