Dear Authors and Editor,

tanks for the possibly to review this paper again and for using dDNA I will include that in my future work.

The authors reanalyzed the data with an improved up to date bioinformatics pipeline and included most of the mentioned points. The strait forward and easy to understand methods presenting nice results. However I still have some questions regarding analyzing the final data and their interpretation, essential for the discussion. Including missing arguments and changing given statements would made this study eligible for publication. Congrats to your work.

Kind regards, Babett Günther

General short remark

There was a misunderstanding what a document with track changes are, there should be all, not only from the last corrections of the coauthors. I hardly see the changes done mentioned in the text, majority is not shown, that makes it much harder to review. You have to use using the comparing function in words. Happened to me before, so I am not judging, but please be sure you working on the correct version for the revision. I was hiding all changes and comments, so please consider this for the line numbers to review. Figures belong to the end not within the text. Lot of spaces and volatility errors, can it be that per accident the wrong version was send? Only to make sure there is no confusion with the further corrections

Major points

1. There seems to be a big misunderstanding of read abundance and their interpretation, at the end of the discussion it is stated as "read abundance (food biomass)". This is not correct; the current literature is showing across the fields and ecosystems simply an indication of a link or even trend between biomass and read abundance. However, read abundance should not be interpreted as biomass, without intense testing of the set up. Has crustacean biomass/ or volume of tissue the same amount of targeted DNA than octopus or fish; have the cells the same size, weight and density? Are they degrading differently fast via digestion based on different skeletons? We simply do not know. Relative read abundance is a useful tool to compare relatively between detection within families or even phyla, however between phyla it has to be taken with caution.

Please read Elbrecht, V., & Leese, F (see below). The PCR bias and the Primer bias are essential to understand your data. A logical point has to be, that higher ratios of Arthropods indicating the primer easier bound to arthropods than to others. It is possible that you actually have a majority of crustacean DNA in the samples; however, we simply cannot determine it with this set up. I recommend including this in the discussion to avoid any further confusion.

Elbrecht, V., & Leese, F. (2015). Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass—sequence relationships with an innovative metabarcoding protocol. PloS one, 10(7), e0130324. This based on macoinvertbrates but it

2. What Metabarcoding can clearly say id the detection of diversity. I am wondering about the deleting of OTU unique to one sample, this can have a major impact on the alpha diversity. I wonder how much this is affecting your comparison between the seasons and populations.

In addition, the "manual filtering" of OTU, of "possible not prey" without further explanation is not fulfilling scientific standards. In this logic, amphipods based on their size should be excluded as prey. Latter in the discussion it is distinguished correctly between active, passive, primary and secondary prey, but after potential prey was deleted, because it could not be prey… these logic is not fulfilling. See below in more details

3. Technical flaws of the protocol are not degrading this good study, but have to be acknowledged. The mock was for bacteria and could not be used at all, so it should be simply be deleted from the text. Secondly, the PCR products where mixed between two Primer set ups, and probably as a consequence, one fragment was sequenced insufficient. The statements made in the discussion need to be corrected urgently. See in detail below

4. Including the first three point, you could think about restructure your discussion for a better reading flow. Your comparison of literature is deep and omnibus leading to really interesting points, but they get a little lost. By avoiding your methodological limitations, it seems sometimes forced to find ecological explanations for the results. There are some singled out paragraphs for potential explanations, which than later seen as proven facts. Better is to understand what the data are able to tell and explain the indications for the ecological behavior of the birds.

Minor points

Introduction

L 89 "Selecting the correct experimental design…." is true for everything, and correct/accurate sounds a little over the top. My suggestion delete the first part of the sentence and keep "challenging based on rare direct observations". Which also true for most animals.

L 105 rewrite potential biases, as it is not clear. Define the biases, probably you want to indicate that the species identification is often not possible, and soft prey are overseen in biomass calculations.

L115 "(and specifically dDNA) "is nice, but don't belong to this sentence and in front of this publication; delete

L 144 I would delete the first part of the sentence, because based on you introduction this unnecessary, even if, this studies are old and not reflecting the current fishery impact. "The composition of….."

→At the end of your introduction, instead of using questions and statements, better define research aims I,II,III….

Methods

Based on you answer to the mock communities, your answer was:

The inclusion of a mock community was standard practice by the sequencing company, but this mock community is designed for microbial studies. Un surpisingly, this mock community did not produce any reads, which is why we did not give any further information about it.

Than delete it out of the manuscript, because it is misleading.

190 -198? Mentioned that the primers are tagged for the two step PCR

203 not specific enough, "manufacturer's standard protocol", there are several. Please include more details about the concentrations

204 I don't think this was the best time to pool, can you give a explanation why you pooled there, if not its kay, but please include if you equalized the PCR products.

231 picking?

234 "mapped a by-sample reads to OTUs" makes no sense.

236 is the fasta including the sequence of the OTU or the asv per OTU?

238 the sentence is not really clear, please rewrite. What did you download, or did you blast by uploading the sequences to the internet? If you did blast on your computer, server, please give the exact release of the database

239-242 can't follow, you say the r script does "assign a taxonomic classification", but this is done by blast n, what do you use the r script for?

246 you don't "classify" OTU's , please change

248 "the" taxonomic assignment, discarded are OTU's……

249 be more specific with subtracted. Means all was deleted, or the number of sequences? When the OTU included 100 sequences in the negative and a sample 1500 , is the whole OTU deleted, or are 500 left at the sample.

251 singletons among samples and OTUS, makes no sense at this step anymore? You mean OTU only present in one OTU? Could it be a rare prey, only one bird catch, as you compare different population it would be interesting to see the diversity. In combination of making an presence absences FOO, you definition is a Prey has to be found by at least 2 individuals? Why? Simply contamination is not enough argumentation therefore.

252 "manual filter…" that's a clear **no no**. There is no nut picking in science which data you want to use and which not. In addition, the whole process has to be able to be repeated with leading to the same result. That's scientifitc standards. You can clearly state, that every prokaryotes and certain phyla are deleted as they are unlikely to be intentional prey. But you have to clearly set the standards here. Otherwise, you have to give a clear list of all deleted taxa, with a reasoning, at the supplementary

260 this sentence is confusing, you did a taxonomic assignment already way before, what is the meaning and intention here?

329-334 please give the number of OTUs

334 delete prey

334, is one of this 17 samples with an OTU, which was not in any other sample? If yes, you should really think about not excluding OTU only because they are unique to one poo sample

336 correct "additionnal" to additional

344 "24.02% (19 OTUs, 195,358 reads) were identified to species level, 29.11% (23 OTUs, 222,447 reads) were identified to genus level and 100% (56 OTUs, 316,587 reads)"; this calculation makes no sense. So the 19 on species level are included within the 23 at genus level? And how can 56 OTU of 79 be 100%

348 delete "Gobally,", and potential prey, that's judging but we still in the results. Combine it with the next sentence and simply state its phyla with the highest abundance. Moreover, delete the finally as well

392 make P.westlandica italic

394 delete" important "

458 again if you delete single OTU, and then compare alpha diversity, its questionable

475 "infer almost 90% of the prey species" this number based on what?

478 include more refernces and more recent ones, like Wangensteen, O. S., Palacín, C., Guardiola, M., & Turon, X. (2018). DNA metabarcoding of littoral hard-bottom communities: High diversity and database gaps revealed by two molecular markers. PeerJ, 6, e4705. doi: 10.7717/peerj.4705

479 it was definitely not "approach proved unnecessary". Having a multigene and primer approach is good practice and should be standard. However, you had PCR products, or? So the primer worked. You simply made a big mistake to pool the PCR products together incorrectly. There are ways to sequences several primers together to ensure necessary sequencing depth, you simply did not apply it. This can happen, as your other results are good, no big deal. But proven good practice, and probably good primers as a failure because of your "quick and dirty" approach did not work out, has no substance here.

481-483 delete this senctence based on the comment before

509 516 you have to clearly sate that you theory is secondary predation. You only indicate it here

517 see major point 1, Metabarcoding as biomass, is not accepted by the scientific community. Studies show that we can use relative abundance, but these systems have been tested for that, or at least to be compared to morphological data. So your logical conclsion has to be that you genetic system cannot be used to describe abundance but for detecting diversity.

560, also variation of populations could be acknowledged, or is it the same the same location as this papers?

563 no it does not confirm, you suggest/indicate/underline, but not confirm

652 , but also…. This part of the sentence is an own argument and should not be part of the conclusion.