

We would like to thank the reviewer for the thorough and constructive review of our manuscript “Species richness and functional attributes of fish assemblages across a large-scale salinity gradient in shallow coastal areas”.

The assessment and comments are very helpful, and we overall agree with the suggested changes and needed clarifications/elaborations to improve the manuscript.

Please find our specific responses below, with suggested new or revised text parts in italic font.

1) Manuscript structure, tables and figures

The reviewer suggests the salinity map to be shown only once in the main manuscript. We agree on the point made, and, to accomplish this while maintaining the necessary information in the main manuscript, we suggest to move Figures 4-6 into the Supplement and instead lift the previous Figures S2 and S3 into the manuscript. These show the relationships between functional groups and salinity as bi-plots (with regression lines when significant). At the same time, we would like to complete these figures, that would now be shown in the main manuscript, by including all functional groups, where we previously (i.e. in the Supplement) had only included a subset of groups. This will facilitate complete overview over the found relationships when most of the current maps should be moved to the Supplement.

By these suggested changes to the Figures, their number in the manuscript would be reduced from previously six to five. The acronyms of the sub-basins, which are given in the bar-plots of Figure 1, will be defined in the respective figure legends.

We will also follow the reviewers suggestions and place a larger-scale map for reference into the maps showing the Baltic Sea with functional patterns.

Further, we suggest to move Table 3, which gives the observed, standardized and estimated Shannon and Simpson Diversity, as well as Table 4, which gives regression information for relationships between Shannon and Simpson Diversity with salinity and temperature, from the manuscript into the Supplement. These revisions will accommodate the comment that the three estimates (std, obs, est) are, for the more common species, very similar. Figure 2, which would remain in the manuscript, will still show both the values (referring to previous Table 3) and relationships to salinity (referring to previous Table 4). By these revisions, the total number of Tables in the manuscript would be reduced from previously five to three. Further, the tables as such will become considerably simpler following the exclusion of the offshore data from the manuscript, as suggested by the reviewer.

We would suggest to keep all three estimates (i.e. std, obs and est) in Table 2 for species richness, as these differ more than for ShD and SiD (since the rare species are included). One of our central messages, i.e. that fish SR decreased about three-fold from the highest to the lowest salinity sub-basin, is based on SR_{std} . It is also relevant to compare SR_{est} with e.g. total observed SR (i.e. based on incidence data plus presence observations). For these reasons we also suggest to keep Table 2 in form of a Table, such that the precise values (and error estimates) can be directly compared. To accommodate the reviewers comment, we suggest to better explain the three different estimates in the revised Methods section, section 2.3, “Analysis of species richness data” (see below for details). In short, the purpose of the standardized estimate is to enable accurate comparisons among areas, in spite of differing sample sizes and inventory completeness. The purpose of the estimated SR is to extrapolate to a likely number of species in one area, if inventory was continued. The complementarity of

the different estimates is, for example, discussed in the last two sentences of the first discussion paragraph, and in the second paragraph of the discussion.

2) Coastal resident vs. migrating species

Since the marine migrating and visiting species are part of the coastal fish assemblage during certain parts of the year, and depend on coastal areas during certain parts of their life cycle, we included them in the statistical analyses. The mix of resident and mobile fish species indicates the dynamic nature of certain coastal areas, and their connectivity with the open sea. We agree with the reviewer that inclusion or exclusion of marine migrating and visiting fish species in the analyses does affect how the inventory completeness (IC) turns out, but find that including them results in a more complete representation of SR. To clarify this, we suggest to add a note in the respective Methods section (Sect. 2.3) which details that both resident and migrating/visiting species were included during calculation of IC, and to add a discussion around the implications of this in the discussion section, as:

“The comparatively low IC in Skagerrak (77%, Table 2) can be related to a, in this context, rather small sample size for a relatively high number of marine migrating/visiting species (which are only more rarely present; Table S5), indicating that the fish species composition in this sub-basin is more “dynamic”.”

We further agree with the reviewer that a lower or higher proportion of marine migrating and visiting species would influence the estimate of IC, and that changes in this proportion over time would affect the IC estimate. Adding such a temporal perspective would be interesting to follow up in a future study, but was not included in this large-scale spatial study. In the current study, monitoring data from several years were merged over time in order to obtain as accurate as possible spatial comparisons within the limits of available data. It is important to note, though, that the sample coverage (SC) in our study, i.e. the inventory completeness of the more common species, is not strongly affected by the incidence frequency of rare species, and was very similar across sub-basins (Table 2). Since SR_{std} and SR_{est} depend on SC they are rather robust against changes in the proportion of (more rarely present) migrating/visiting vs. resident species, and hence against including or excluding migrating and visiting species. We suggest to include discussion around this aspect, specifically:

“Since the rarefaction-extrapolation analyses that we used here are based on species incidence frequencies (Chao et al., 2020), the statistical results could potentially be influenced by human pressures that alter these frequencies, even if fish SR in itself may not be affected. However, given that the rarefied and extrapolated SR (i.e. SR_{std} and SR_{est}) are based on SC, where rare species are not influential, these statistics are rather robust against such effects (unless there would be severe changes in the incidence frequencies of common species).”

3) Offshore data

We agree that it will benefit the general clarity of manuscript to not include the offshore data, particularly given that only one sub-basin had enough data to conduct the statistical (rarefaction-extrapolation) analysis of fish SR. Indeed, as the reviewer assumed, data from the BITS survey carried out in the Baltic Sea were not included due to a lack of data for shallower depths (i.e. <30 m).

4) Discussion on other drivers

We agree with the reviewer, and suggest to include a new discussion paragraph on the potential influence of other drivers, such as human pressures and habitat complexity, on fish SR and on our statistical results, specifically:

“Besides salinity and temperature, which show a pronounced gradient over the large spatial scale of our study (Table 1) and were identified as likely main drivers here (Fig. 2), fish SR might also be influenced by other factors, such as human pressures. The cumulative pressure from human activities in the Baltic Sea, combining factors such as fishing, eutrophication and hazardous substances, is generally higher in the southern and south-western sub-basins, which also have both relatively higher salinity and fish SR, compared to for example the northernmost sub-basins with lower cumulative human pressure, salinity and fish SR (Table 2; Korpinen et al., 2012; HELCOM 2018). This indicates that there is no negative relationship between cumulative human pressure and fish SR on the large spatial and temporal scales studied here. It does, however, not contradict well-documented influences that human pressures can have on fish concerning other aspects, or possibly for SR on smaller spatial scales (not studied here). For example, besides direct effects of fish extraction and habitat disturbance on fish species, human-induced depletion of larger predatory fish by Baltic Sea fisheries has likely contributed to an increase in coastal mesopredatory fish abundances (Eriksson et al., 2011), and eutrophication has been connected to increasing abundances of benthic feeding fish in the Baltic Sea (Snickars et al., 2015). Since the rarefaction-extrapolation analyses that we used here are based on species incidence frequencies (Chao et al., 2020), the statistical results could potentially be influenced by human pressures that alter these frequencies, even if fish SR in itself may not be affected. However, given that the rarefied and extrapolated SR (i.e. SRstd and SRest) are based on SC, where rare species are not influential, these statistics are rather robust against such effects (unless there would be severe changes in the incidence frequencies of common species). Another potential explanatory factor to consider is habitat complexity, related to e.g. diversity of substrate or habitat-forming macrophytes, which can increase aquatic biodiversity (Soukup et al., 2021). Differences in habitat complexity may play some role in the observed large-scale patterns in fish SR given that macroalgal SR increases with increasing salinity across the Baltic Sea, with a larger share of habitat-forming and perennial species in more marine waters (Middleboe et al., 1997, Schubert et al., 2011). Hence, the greater habitat complexity with increasing salinity may enhance fish SR, further reinforcing any salinity-induced distributional pattern.”

5) Discussion on functional attributes (traits)

We agree that the fish functional characteristics and their changes along the salinity gradient warrant more detailed discussion, that the statement on benthic-pelagic coupling needs to be clarified, and that it would be interesting to couple the discussion to ecosystem processes. To meet these comments, and as an overall revision in response to both reviewers' comments on this discussion section, we suggest to revise and expand the respective discussion paragraphs to:

“Our study also revealed changes in fish SR for different functional groups across the studied salinity gradient. As the different functional groups represent variability among species in e.g. use of resources or level of connectivity with other areas, this may also translate to taxonomic-driven differences in coastal ecosystem functioning across the different sub-basins (Elliott et al., 2007; Franco et al., 2008). Clearly migrating fish species are typically of marine origin (here classified as marine juvenile migrants, marine seasonal migrants or marine visitors) and cannot tolerate low salinity, explaining their predominance at higher salinities (Fig. 4), and in agreement with known patterns in European estuaries in general (Elliott & Dewailly, 1995; Franco et al., 2008). This pattern of marine fish species temporarily using coastal areas may be related to comparatively higher prey densities and to food types not encountered in marine areas, as well as to typically more turbid waters

providing better protection from predators (Franco et al., 2008). Moreover, the high migratory fish SR at higher salinity is likely relevant for the ecological connectivity between ecosystems, e.g. by transport of local “coastal” production to open sea and vice versa (Franco et al., 2008), and emphasizes the important role of higher salinity coastal areas as nursery grounds, migration routes and refuge areas for marine fish species (Elliott et al., 2007). Connectivity is also maintained in the less saline sub-basins, though the concerned functional groups are represented by only a few species (Fig. 1, S2; Berkström et al., 2021).

Benthic and demersal fish SR also decreased with decreasing salinity, corroborating previous results where demersal fish SRs decreased from the saline Kattegat to the less saline northern Baltic Proper (Pecuchet et al., 2016), and in accordance with high benthic preference of marine fish species in European estuaries (Elliott & Dewailly, 1995; Franco et al., 2008). This pattern further corresponds with that the observed SR of benthic meio- and macrofauna, which are generally the dominating prey for benthic and demersal fish, also decreased with decreasing salinity in the Baltic Sea (Broman et al., 2019; Zettler et al., 2014). Taken together, these patterns suggest that the process of benthic-pelagic coupling through fish predation likely involves a lower number of species links, or functional redundancy, towards lower salinity sub-basins. Concerning feeding habits, the general composition of feeding guilds noted in the higher-salinity sub-basins was similar to that reported on a larger European scale (Elliott & Dewailly, 1995). Also our findings of higher piscivorous fish SR in the more saline sub-basins (Fig. 6) and the pattern showing a low proportion of omnivorous fish that was unrelated to salinity levels was in agreement with findings from European estuaries (Franco et al., 2008). In summary, the found differences in functional traits of fish along the salinity gradient were largely related to the respective changes in the predominating fish origin, i.e. freshwater vs. marine species.”

Concerning the other comments:

- L32: We refer to species richness, and suggest to clarify the statement accordingly by revising to: “However, threats to coastal biodiversity from e.g. overfishing, habitat loss, pollution, eutrophication and climate change are many and profound (Duncan et al., 2015; Griffiths et al., 2017; Pan et al., 2013), and the number of species occurring in coastal habitats often remains uncertain (Appeltans et al., 2012).”
- L49: We suggest to add “on average” to accommodate the fact that certain regions experience less intense water cycling, while the general trend is an intensified hydrological cycle.
- L78: This comment does not anymore apply since offshore areas will be, upon suggestion from the reviewer, removed from the manuscript (see above).
- L116: In separated ecosystems, such as islands or lakes, SR usually increases with area (“species-area relationship”). However, such separation is not the case for our coastal sub-basins here. While the studied sub-basins are hydrographically distinct, with water exchange being separated to a certain degree by shallow sounds or sills, they are still connected. Based on this reviewer's comment we realised that giving the size of the shallow coastal areas in table and Methods may cause confusion in this regard. We therefore suggest to remove these values, which are also not used further in the study, leading to simplification of tables (as suggested would be useful by the reviewer).
- L128: We suggest to revise to “*Since each gear has a specific selectivity and efficiency this may have introduced a bias in the dataset. However, a comparison of*

data from gill and fyke net samplings at the Swedish west coast did not reveal consistent differences in biodiversity metrics, and the statistical approaches were chosen to minimize this potential bias when comparing SR among sub-basins (Bergström et al., 2013, Chao et al., 2020; see also search of additional data sources below, and Sects. 2.3 and 4)."

- L139: We suggest to revise this part to better explain the motivation for the chosen cutoff. It was essentially based on that we found that sub-basins with less than one hundred fish species incidences had too little data for statistical analysis:
"Data on observed SR, SR_{obs} , is available for all 12 sub-basins (Table 2), and was based on between 4 and 47 years of data, depending on sub-basin (Table S2). However, subsequent statistical analyses and comparisons were conducted only for the 10 sub-basins containing data from at least 30 sampling/fishing occasions, corresponding to several hundred fish incidences (i.e. Bothnian Bay, the Quark, Bothnian Sea, Åland Sea, N Baltic Proper, W Gotland Basin, Bornholm Basin, the Sound, Kattegat, and Skagerrak). This dataset is hereafter referred to as "raw data", and contained in total 160,453 entries (i.e. fish individuals caught and determined to species) from 1,638 sampling/fishing occasions at 4,571 unique locations. E Gotland Basin and Arkona Basin were not statistically analysed since we considered these sub-basins too under-sampled, with only 13 and 7 samplings, respectively, from 9 and 4 different years, and less than 100 species incidences in total (Tables 2, S2)."
While we extracted all available data from the database covering nearly five decades (1975-2021), as stated in the manuscript, only one sub-basin had annual samplings during all those years, while most sub-basins had data from 17+ years (with a few having less years, i.e. 4 and 9 years, and being excluded from statistical analysis based on that, see above). We realized this aspect would need to be clarified in the manuscript, and suggest to edit the text accordingly, and to add a new Supplementary Table detailing for which years fishing data was available, per sub-basin.
- L110-147: The incidence data is official, quality-controlled survey data for which species are correctly identified by taxonomic specialists. As an additional double-check of uncommon species we used the HELCOM list of macro-species in the Baltic Sea. Concerning the "observation databases", where e.g. citizens can report species observations and which hence are less reliable, we did a careful cross-check where unreasonable occurrences were considered falsely identified, and discarded (see L139/140). We will elaborate to clarify this in the respective text pieces.
- L167: The comment made us realise that this text part could be confusing since the rarefaction-extrapolation method used is called "Chao Richness method", and a function available in R has the same name although it includes several different calculations. Please see below for how we suggest to revise this section, to accommodate this and other comments on it.
- L170: We suggest to clarify that the observed values were standardized to the minimum SC. The purpose of SR_{std} is to allow for accurate comparisons between sub-basins, given that all standardized values give SR for the same SC, hence representing an estimate which is not biased by how completely the compared areas were sampled. During revision, we noted an unfortunate mistake in that "sample coverage" (SC) was erroneously called "inventory completeness" (IC). This is now corrected, and better explained in the methods section 2.3. We agree with the reviewer that SC was high and very similar across sub-basins, varying between 98.5-99.9%, and hence in the case of this study did not strongly influence the obtained values. However, the correction is still needed in order to allow for accurate, unbiased comparisons between sub-basins,

and we therefore find it important to keep the standardization in the manuscript rather than moving it to the Supplement. However, we understand the need of presenting the data and results more clearly, especially in the Tables. We suggest that this aim will be achieved by excluding the offshore data, as suggested by both reviewers and making text and tables considerably simpler, and by reducing the number of tables and figures into (please see above for details).

To further accommodate this comment and the comment above we also suggest a major rewrite of the method section 2.3, to:

“The raw data was first summarized to a dataset of unique fish species caught per fishing/sampling occasion in presence/absence format, and then further aggregated to an incidence frequency format, giving the observed total incidence of each species over the number of fishing/sampling occasions. This dataset is referred to as “fish incidence database”. Each unique combination of a fishing/sampling location per date was defined as one sampling unit, and these were summed per sub-basin to obtain the sample sizes. Subsequently, incidence-based Hill diversity numbers of three “orders”, which differ in their propensity to include or exclude relatively rarer species (Hill, 1973), were calculated to quantify the species diversity of each assemblage, i.e. 1) species richness (SR), which counts all species equally irrespective their incidence frequency, 2) Shannon diversity (ShD), which considers the incidence frequency and can be interpreted as the effective number of frequent species, and 3) Simpson diversity (SiD), which can be interpreted as the effective number of highly frequent species (Chao et al., 2014; Chao et al., 2020). Calculations were performed using the R package iNEXT (Chao et al., 2020; Hsieh et al., 2016), and the values are hereafter referred to as observed SR, ShD and SiD, respectively. It should be noted that, using these methods, Shannon and Simpson diversity are expressed in terms of richness, i.e. number of species, which differs from other known formats. Specifically, ShD is the exponential of Shannon’s entropy index, and SiD is the inverse of Simpson’s concentration index (Chao et al., 2014).

SR_{obs} is highly dependent on “sample completeness” (Colwell & Coddington, 1994; Hill, 1973) and typically underestimates the “true” SR due to undetected species, an aspect referred to as under-sampling, sampling bias or sampling problem (Chao et al., 2014; Chao & Jost, 2015; Menegotto & Rangel, 2018). Similar to Hill numbers, “sample completeness” can be calculated for different “orders” (Chao et al., 2020). The zero-order sample completeness is hereafter referred to as inventory completeness (IC). It is calculated as the ratio of SR_{obs} to the estimated “true” SR (i.e. observed plus undetected SR, see “estimated SR” below), hence giving the proportion of detected species without considering the species incidence frequencies. We calculated IC for the data merged over time, and including both resident and migrating/visiting fish species. The first-order sample completeness, hereafter referred to as “sample coverage” (SC), is a measure where species are weighted by their detection probabilities, giving the proportion of incidences detected from the estimated “true” incidences (Chao et al., 2020).

To correct for the effect of differing sample completeness on SR_{obs}, and allow accurate, unbiased comparisons between sub-basins, we used a coverage-based rarefaction and extrapolation method implemented for incidence data in the R package iNEXT (Chao et al., 2014, 2020; Hsieh et al., 2016). A coverage-based method was chosen because more traditional sample size-based corrections can introduce a systematic bias, since the number of samples needed to fully characterize a community depends on its SR (Chao & Jost, 2012). For each sub-basin, we obtained 1) the rarefied SR, ShD and SiD, which were standardized to the minimum observed

SC across all included sub-basins (hereafter referred to as standardized values, i.e. SR_{std} , ShD_{std} and SiD_{std}), and 2) the fish SR extrapolated to twice the actual sample size (hereafter referred to as estimated values, i.e. SR_{est} , ShD_{est} and SiD_{est} ; Chao et al., 2014, 2020; Hsieh et al., 2016). Similar analyses were also conducted for SR of fish with different functional attributes (see Sect. 2.4). All calculations were conducted using R version 4.0.4 (R Core Team, 2021).”.

- L222: This comment does not apply any more following omission of the offshore data, as suggested by both reviewers.
- L237: This part should be changed, because we realized during revision that we had by mistake interchanged the terms inventory completeness and sample coverage. Now, inventory completeness shows a larger variation, and we suggest to refer to Table 2 rather than translating the percentages into species numbers.
- L250: Extrapolation is recommended up to maximally twice the actual sample size for SR (Chao et al., 2020). We suggest to include this information in the respective methods section, and in the legend of Figure 1.
- Table 3. We suggest to move this table to the Supplement (please also see above). A graphic illustration of the trends across the salinity gradient is available in Figure 2, and, for ShD and SiD, the observed values are very similar to the standardized and estimated values, given that the missing species represent rare species. Hence, for study sites with high SC, the observed ShD and SiD are already largely unbiased estimates. To further accommodate the reviewers general comment that the manuscript may contain too many tables and figures we suggest to also move Table 4 to the Supplement. Table 4 contains the statistical information on regressions between salinity or temperature with SR, ShD and SiD. Since these regressions are already graphically shown in Figure 2 it seems justified to move the statistical details on their relationships to the Supplement.
- Table 5. We will include information about the number of observations in Table 5, and in the legend of Table 4.
- Figures 4-6. We agree it is redundant to repeat the salinity map repeatedly in these figures. We therefore suggest to move figures 4, 5 and 6 into the Supplement, and instead lift the previous figures S2 and S3 into the manuscript. These figures directly shows the relationships between functional groups (on habitat use and vertical distribution) and salinity.
- L372: Mora et al., 2008 applied rarefaction-extrapolation methods similar to the one used in our study, but used different statistical models. Appeltans et al. 2012 based their estimate of inventory completeness on a statistical model based on historical rates of species description. While the methods differ more or less from the one we used the estimated property is the same, i.e. the inventory completeness as the proportion of observed to total species, which motivates our comparison in spite of methodological variation.
- L379: We suggest to rewrite this sentence to clarify which ratio we mean, and to move it to the Results (end of Sect. 3.5), but to take it up as discussion point in this place. We also suggest a rewrite to “calculated based on data presented in Table 2”, to make clear that the ratio is not given in the Table, but that the values needed to calculate it are found in Table 2.
- L394: We understand that this statement may be confusing. We suggest a rewrite to: *“Our study reinforces that SR_{obs} is strongly dependent on sample coverage, as relatively rare species are more likely to be missed at lower sample size/sample coverage, and that comparisons of SR_{obs} of in species assemblages without accounting for this effect can lead to biased or even misleading conclusions.”.*

- L400-414: We suggest to add some more information on this aspect already in the respective Methods section, section 2.2, specifically: *“Since each gear has a specific selectivity and efficiency this may have introduced a bias in the dataset. However, a comparison of data from gill and fyke net samplings at the Swedish west coast did not reveal consistent differences in biodiversity metrics, and the statistical approaches were chosen to minimize this potential bias when comparing SR among sub-basins (Bergström et al., 2013, Chao et al., 2020; see also search of additional data sources below, and Sects. 2.3 and 4).”*
- L400: We will change accordingly.
- L417: We suggest to edit to: *“As anticipated based on earlier Baltic Sea studies on fish (e.g. Hiddink & Coleby, 2012; Ojaveer et al., 2010; Olsson et al., 2012) and other organism groups (e.g. Broman et al., 2019; Zettler et al., 2014), coastal fish SR was positively correlated with salinity (Fig. 2a, Table S), with fish SR increasing ca. threefold together with the ca. 12-fold increase in salinity (Table 2).”*
- L440: We agree and will change accordingly, i.e. remove the statement with a lag-period.
- L460: We suggest that the suggested place may not be the optimal place to add reference to the phytoplankton trend, since we here focus on prey items of benthic fish. However, we are referring to this study in the introduction.
- L463: We agree that this wording could be improved, and suggest a rewrite to: *“Taken together, these patterns suggest that the process of benthic-pelagic coupling through fish predation likely involves a lower number of species links, or functional redundancy, towards lower salinity sub-basins.”*
- L483-484: This is an interesting question, i.e. what the net effect of simultaneous warming and upper layer freshening on fish SR may be. However, given that we did not study warming, we suggest not to include further discussion on potential implications at this point.

We thank the reviewers and Editors for their efforts with this work, and are looking forward to hear from you about our manuscript.

Yours sincerely,

Birgit Koehler and co-authors