Ocean acidification affects acid–base physiology and behaviour in a model invertebrate, the California sea hare (*Aplysia californica*)

Rebecca L. Zlatkin and Rachael M. Heuer

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Original submission: 9 June 2019
Revised submission: 5 September 2019
Final acceptance: 6 September 2019

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Note: This manuscript was transferred from another Royal Society journal with peer review.

Review History

RSOS-191041.R0 (Original submission)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No
Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
This neat study shows that the model organism, Aplysia, is an acid-base regulator that accumulates HCO3- to defend pH in high CO2, and that ocean acidification relevant CO2 levels also cause behavioural changes (faster relaxation of the tail retraction reflex - an anti-predation behaviour). This is the first time that HCO3- accumulation and specific behavioral changes at elevated CO2 have been directly linked in a marine invertebrate. Moreover, because Aplysia is a model organism for studying invertebrate neurobiology, so this study shows that Aplysia is an ideal species in which to investigate how higher CO2 levels affect the behaviours of marine invertebrates, and more specifically, to test the GABA hypothesis. This is an important study that has been carefully done and the data appropriately analysed. The authors have done an excellent job responding to the original round of reviews for Proc R Soc London and I have just a few relatively minor comments and suggestions on this version of the ms, which is already in very good shape.

Minor comments
Line 41. Change “expected” to “projected”, because that’s what models do.
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Line 263. Replace “the time it took to right” with “time to right”.
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Line 340. You say: “In marine fish, the link between regulatory ability and behaviour has been more consistent, where HCO3- accumulation has been linked to impaired olfaction and lateralization [29, 30].” The problem is that HCO3- accumulation has not been tested in fish species that do NOT show behavioural changes in high CO2, so we do not know if there is actually a good correlation here or not. It’s drawing a very log bow to say it’s consistent. I suggest you delete this sentence.

Line 347. Tested in less than ten species, so not “many” species, really. Better to say some fish and invertebrates.

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
Zlatkin & Heuer have done a thorough job addressing the reviewer comments from a previous submission of the manuscript to another journal.

I have only minor comments in this review.

L41-43 – References 1 and 2 are old now. Cite IPCC reports or similar for more recent CO2 projections.
Given that “Animals were exposed for either 4 or 11 days to each CO2 level” as stated in the methods, I recommend the authors change “4-11 days” to “4 or 11 days” in the manuscript and figure legends.

Behavioral count style data – i.e. time to right and time to relax tail – would be better analyzed on raw data with a count style distribution (e.g. Poisson, etc) with a GLMM. However, looking at the graphs, it is likely that the conclusions from the current manuscript tests (using an LME with log-transformation) would be the same.

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L280 – the reference to Figure 1a; 1000 µatm CO2 is unclear or incorrect. Figure 1a has 400, 1200 and 3000 µatm as pCO2 treatment conditions. Further, global year 2100 CO2 projections are 940 ppm (RCP 8.5) or less – please change this sentence.

Figure 1 and 2 – change the pCO2 treatment conditions all to 400, 1200 and 3000 µatm. At present there is a mixture of 1000 and 1200 µatm reported for the mid-CO2 level on both these figures, however, the Supp Table 2 shows these values should all be 1200.

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Fig S1 - state the errors presented.

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Manuscript needs proof-reading.

L374 – note that the pCO2 levels at 1200 µatm in this study exceeds ‘near-future CO2 levels’, which are usually considered as those at the end of this century. I would prefer ‘near-future CO2 level’ statements to be removed from the manuscript.

Decision letter (RSOS-191041.R0)

22-Aug-2019

Dear Dr Heuer

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-191041 entitled "Ocean acidification affects acid-base physiology and behaviour in a model invertebrate, the California sea hare (Aplysia californica)" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees' comments at the end of this email.
The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

- **Ethics statement**
  If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

- **Data accessibility**
  It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

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- **Competing interests**
  Please declare any financial or non-financial competing interests, or state that you have no competing interests.

- **Authors’ contributions**
  All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

  All contributors who do not meet all of these criteria should be included in the acknowledgements.

  We suggest the following format: AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

- **Acknowledgements**
  Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

- **Funding statement**
  Please list the source of funding for each author.
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Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript before 31-Aug-2019. Please note that the revision deadline will expire at 00.00am on this date. If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create a Revision." You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referees. We strongly recommend uploading two versions of your revised manuscript:

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2) A 'clean' version of the new manuscript that incorporates the changes made, but does not highlight them.

When uploading your revised files please make sure that you have:

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3) Included a 100 word media summary of your paper when requested at submission. Please ensure you have entered correct contact details (email, institution and telephone) in your user account;
4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi within your manuscript. Make sure it is clear in your data accessibility statement how the data can be accessed;
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Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,
Alice Power
Editorial Coordinator
Royal Society Open Science
openscience@royalsociety.org

on behalf of Kevin Padian (Subject Editor)
openscience@royalsociety.org

Reviewer comments to Author:
Reviewer: 1

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Author’s Response to Decision Letter for (RSOS-191041.R0)

See Appendix A.

Decision letter (RSOS-191041.R1)

06-Sep-2019

Dear Dr Heuer,

I am pleased to inform you that your manuscript entitled "Ocean acidification affects acid-base
physiology and behaviour in a model invertebrate, the California sea hare (*Aplysia californica*) is now accepted for publication in Royal Society Open Science.

You can expect to receive a proof of your article in the near future. Please contact the editorial office (openscience_proofs@royalsociety.org and openscience@royalsociety.org) to let us know if you are likely to be away from e-mail contact -- if you are going to be away, please nominate a co-author (if available) to manage the proofing process, and ensure they are copied into your email to the journal.

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On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Kind regards,
Royal Society Open Science Editorial Office
Royal Society Open Science
openscience@royalsociety.org

on behalf of Mr Andrew Dunn (Associate Editor) and Kevin Padian (Subject Editor)
openscience@royalsociety.org

Associate Editor Comments to Author (Mr Andrew Dunn):
Associate Editor: 1
Comments to the Author:
(There are no comments.)

Reviewer comments to Author:

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Appendix A

22-Aug-2019

Dear Dr Heuer

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-191041 entitled "Ocean acidification affects acid-base physiology and behaviour in a model invertebrate, the California sea hare (<i>Aplysia californica</i>)" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees' comments at the end of this email.

The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

- **Ethics statement**
  If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

- **Data accessibility**
  It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad ([https://nam01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdataldryad.org%2F&amp;data=02%7C01%7Crheuer%40rsmas.miami.edu%7C7Cc1da00bbd6934bd7dca308d726e122b3%7C2a144b72f23942d48c0e6f0f17c48e33%7C0%7C0%7C637020620728940911&amp;amp;sdata=ytja9HC07spVGYae3O4s8zFnO Tx0rcwEc2ZbpmJ6UE%3D&amp;amp;reserved=0](https://nam01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdataldryad.org%2F&amp;data=02%7C01%7Crheuer%40rsmas.miami.edu%7C7Cc1da00bbd6934bd7dca308d726e122b3%7C2a144b72f23942d48c0e6f0f17c48e33%7C0%7C0%7C637020620728940911&amp;amp;sdata=ytja9HC07spVGYae3O4s8zFnO Tx0rcwEc2ZbpmJ6UE%3D&amp;amp;reserved=0)), or modify your current submission to dryad, please use the following link: ([https://nam01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdataldryad.org%2Fsubmit%3FjournalID%3DRSOS%26manu%3DRSOS-191041&amp;amp;data=02%7C01%7Crheuer%40rsmas.miami.edu%7C7Cc1da00bbd6934bd7dca308d726e122b3%7C2a144b72f23942d48c0e6f0f17c48e33%7C0%7C0%7C637020620728940911&amp;amp;sdata=8xRfp5K5h2%2BieO1PtG9KmrjaawQRpzauwEawHlXcnI0%3D&amp;amp;reserved=0](https://nam01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdataldryad.org%2Fsubmit%3FjournalID%3DRSOS%26manu%3DRSOS-191041&amp;amp;data=02%7C01%7Crheuer%40rsmas.miami.edu%7C7Cc1da00bbd6934bd7dca308d726e122b3%7C2a144b72f23942d48c0e6f0f17c48e33%7C0%7C0%7C637020620728940911&amp;amp;sdata=8xRfp5K5h2%2BieO1PtG9KmrjaawQRpzauwEawHlXcnI0%3D&amp;amp;reserved=0))

- **Competing interests**
  Please declare any financial or non-financial competing interests, or state that you have no competing interests.

- **Authors’ contributions**
  All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the...
following criteria: 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.

We suggest the following format:

AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

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Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript before 31-Aug-2019. Please note that the revision deadline will expire at 00.00am on this date. If you do not think you will be able to meet this date please let me know immediately.

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Reviewer comments to Author:
Reviewer: 1

This neat study shows that the model organism, Aplysia, is an acid-base regulator that accumulates HCO3- to defend pH in high CO2, and that ocean acidification relevant CO2 levels also cause behavioural changes (faster relaxation of the tail retraction reflex – an anti-predation behaviour). This is the first time that HCO3- accumulation and specific behavioral changes at elevated CO2 have been directly linked in a marine invertebrate. Moreover, because Aplysia is a model organism for studying invertebrate neurobiology, so this study shows that Aplysia is an ideal species in which to investigate how higher CO2 levels affect the behaviours of marine invertebrates, and more specifically, to test the GABA hypothesis. This is an important study that has been carefully done and the data appropriately analysed. The authors have done an excellent job responding to the original round of reviews for Proc R Soc London and I have just a few relatively minor comments and suggestions on this version of the ms, which is already in very good shape.

We thank the reviewer for their kind comments and thorough review of the manuscript.

Minor comments
Line 41. Change “expected” to “projected”, because that’s what models do.

We have changed this as requested. L41-43

“Average global oceanic CO2 levels are projected to increase from current levels of ~400 to ~940 µatm CO2 by the end of the century and ~1900 µatm CO2 by the year 2300 unless the rate of CO2 emissions is substantially curtailed.”

Lines 41-42. These levels are relevant to a business as usual scenario of CO2 emissions, not more conservative emissions scenarios, so I suggest you finish the sentence with “under a business-as-usual CO2 emissions scenario” or “unless the rate of CO2 emissions is substantially curtailed”

We have altered the wording and appreciate the alternative suggestions. The sentence now reads as follows L41-43:
“Average global oceanic CO₂ levels are projected to increase from current levels of ~400 to ~940 µatm CO₂ by the end of the century and ~1900 µatm CO₂ by the year 2300 unless the rate of CO₂ emissions is substantially curtailed.”

Line 57. Insert “high” or “elevated” before “CO2”. There is still CO2 in ambient conditions.

We have added the word “elevated” as suggested. L58

Line 57. Insert “animals that are” before “acid-base regulators”. Otherwise a regulator could be interpreted as some kind of tissue or organ.

Good point, we have added this to the sentence as suggested. L58-61

“Following the onset of CO₂ exposure, animals that are acid-base “regulators” counter an initial drop in blood pH through the retention and/or uptake of HCO₃⁻. This process allows acid-base regulators to correct…”

Line 59. Insert “acid-base” before “regulators”

We have added this to the sentence as suggested. L60

Line 61 Insert “is” after “

We have altered the sentence as follows: L61-62

“This compensation mechanism is generally related to how “active” an organism is, as higher metabolic rates (O₂ consumption) necessitate higher rates of CO₂ excretion.”

Para starting line 79. This should not be a new paragraph. Merge with the one above.

We have merged the paragraphs as suggested.

Para starting line 86. I think this should be part of the same single para above, too. It’s all about testing the GABA hypothesis.

We have merged the paragraphs as suggested.

Line 114. Insert “elevated” before “CO2.

We have added this to the sentence as suggested. L114

Line 137. “which” should be “that” in this instance.

We have changed this as suggested. L140-141

“Animals that experienced 11-day exposures were subjected to the same ~96-hour fasting period.”

Line 225. Insert “treatments” or “exposure levels” after “CO2”
We have added “exposure levels” as requested. L228-229.

“Linear mixed effect (LME) models were used to test for responses to CO$_2$ exposure levels for the time to complete the righting reflex and the time to complete the tail-withdrawal reflex.”

Line 228. It is not clear to me why you wanted to use post-hoc testing with multiple comparisons, which requires you to use an adjusted p-value. Aren’t you simply interested in how each of the high CO$_2$ treatments compare with the control? In that case you can simply use the contrast outputted by the LME (i.e. each treatment compared with the control) and you don’t need post-hoc tests that are then adjusted for multiple comparisons. All you need do is make sure the control is coded in a way that it is selected by the model as the contrast treatment. You only need the post-hoc tested if you specifically want to compare ALL the treatment levels to each other. None of this may matter because of the strength of your results, but thought it should be pointed out.

We decided to use the post-hoc comparisons to address a comment from a reviewer in a previous submission regarding correcting data for multiple comparisons. In addition, we were interested in the pairwise comparison between 1200 and 3000 μatm. After checking our model, we note that using the model output or the pairwise comparisons does not change the strength of the results.

Line 263. Replace “the time it took to right” with “time to right”.

We have changed this as suggested. L265-266

Line 270. Insert “high” before “CO$_2$”. This seems like a redundant analysis to me.

We have added “high” as suggested (L273). We have addressed the second portion of this comment in the Line 228 response above.

Line 281. Insert “high” before “CO$_2$”.

We have added “high” as suggested. L284

Line 296. Insert “high” before “CO$_2$”.

We have added “high” as suggested. L299

Line 298. Not clear how a species can be both a regulator and non-regulator. I thought you were arguing that acid-base regulation is a species level trait. Do you mean by using different species of Aplysia? Some clarification needed here.

We agree the wording may be confusing. Theoretically, any animal could change from acid-base regulating to non-regulating if exposed to high enough CO$_2$. Rather, we are referring here to regulatory and non-regulatory responses. We have changed the sentence to reflect this detail.

L301-302: “Responses in animals showing regulatory and non-regulatory responses can be studied in the same species using Aplysia.”

Line 309. “and” in the Chilean abalone.
We have added “and” as suggested. L310

311. I don’t think you need “interestingly” here.
We have deleted this as requested.

312. Insert “elevated” before “CO2”
We have changed the sentence as suggested. L312

314 Delete “in other words”
We have made the deletion as requested.

Line 333. This is a poorly explained/constructed sentence. I think you mean there was no difference in the righting response both in crabs that are able to elevated HCO3- and sea stars that are unable to elevated HCO3-.

We agree that this sentence needs more clarity. We have revised it as follows:

L335-337: However, in other invertebrate studies, sea stars unable to elevate HCO3- (Appelhans et al. 2014; Appelhans et al. 2012) and crabs able to elevate HCO3- both showed no difference in righting (Zittier et al. 2013).

Line 340. You say: ”In marine fish, the link between regulatory ability and behaviour has been more consistent, where HCO3- accumulation has been linked to impaired olfaction and lateralization [29, 30].” The problem is that HCO3- accumulation has not been tested in fish species that do NOT show behavioural changes in high CO2, so we do not know if there is actually a good correlation here or not. It’s drawing a very log bow to say it’s consistent. I suggest you delete this sentence.

We have deleted this sentence as suggested.

Line 347. Tested in less than ten species, so not “many” species, really. Better to say some fish and invertebrates.

We have changed “many” to “some” as suggested. L347

Reviewer: 2

Comments to the Author(s)
Zlatkin & Heuer have done a thorough job addressing the reviewer comments from a previous submission of the manuscript to another journal.

We appreciate the reviewer’s summary of our revisions and for their comments.

I have only minor comments in this review.
References 1 and 2 are old now. Cite IPCC reports or similar for more recent CO2 projections.

We have removed the Meehl citation and added two more recent citations including the most recent IPCC report.
(Meinshausen et al. 2011; Portner et al. 2014)

Given that “Animals were exposed for either 4 or 11 days to each CO2 level” as stated in the methods, I recommend the authors change “4-11 days” to “4 or 11 days” in the manuscript and figure legends.

We included the 4 or 11 days to be transparent about our methods. We observed no effect of the day of exposure in any of our statistical models, so we pooled the data across days and using 4-11 days throughout the rest of the manuscript, as suggested by a previous reviewer. We have added the following sentence in the methods, where this issue is first presented to avoid confusion.

“Since day of exposure (4 versus 11) did not significantly impact any measured endpoint (see below), exposure duration is referred to as 4-11 days throughout the manuscript.”

Behavioral count style data – i.e. time to right and time to relax tail – would be better analyzed on raw data with a count style distribution (e.g. Poisson, etc) with a GLMM. However, looking at the graphs, it is likely that the conclusions from the current manuscript tests (using an LME with log-transformation) would be the same.

We agree and would prefer to stick with the current tests provided in the manuscript.

There is a typo mistake in the P value reported as “P=0.0.027”.

We have fixed this error, thank you.

The reference to Figure 1a; 1000 µatm CO2 is unclear or incorrect. Figure 1a has 400, 1200 and 3000 µatm as pCO2 treatment conditions. Further, global year 2100 CO2 projections are 940 ppm (RCP 8.5) or less – please change this sentence.

We have changed the projected level to 940, and corrected the error in both the figures and the text. The correct value is 1200 µatm CO2. This sentence now reads as follows:

“This compensatory effort led to complete pH defense at 1200 µatm CO2, an ocean acidification relevant level close to what is predicted globally by year 2100 (940 µatm CO2 under business as usual [2]) (Figure 1a; 1200 µatm CO2).”

Figure 1 and 2 – change the pCO2 treatment conditions all to 400, 1200 and 3000 µatm. At present there is a mixture of 1000 and 1200 µatm reported for the mid-CO2 level on both these figures, however, the Supp Table 2 shows these values should all be 1200.

The correct value is 1200 µatm CO2 as noted by the reviewer. We have fixed this error in the figures.

In Supp Table 2, TA increases with pCO2. This is unusual, as TA should remain constant while pCO2 and TCO2 change in CO2 manipulation studies. Otherwise there is a confounding effect of TA and
pCO2 (i.e. TA changes at the same time as pCO2, but the authors are interpreting the results in terms of pCO2). Please can the authors detail why TA changes with pCO2 in their experiments.

We examined the data to see if there was a significant difference between the TA values for each group. We ran three one-way ANOVAs to test for treatment effects on acid-base water chemistry TA, tail-withdrawal water chemistry TA, and righting water chemistry TA. Although TA values appear to be increasing, none of these increases are statistically significant across CO2 levels. We also calculated that the percent increase from control to high CO2 (at either CO2 level), and this number was relatively low: 4.4-6.6%. This is relatively small compared to the large changes noted in pCO2 across levels.

**Fig S1 - state the errors presented.**

We have added the following to the figure caption.

“Error bars represent SEM.”

This manuscript does not test GABA, so I recommend to remove GABA mentions from key words to avoid misleading readers.

We have removed mention of gaba in the keywords.

L323-326 – this sentence needs references, since the proposal of this neural mechanism is not the authors’ own.

We have added Nilsson et al 2012 as a reference for this sentence. L326-329

**Manuscript needs proof-reading.**

We have re-read this carefully before final submission, and corrected the errors pointed out in earlier comments.

L374 – note that the pCO2 levels at 1200 µatm in this study exceeds ‘near-future CO2 levels’, which are usually considered as those at the end of this century. I would prefer ‘near-future CO2 level’ statements to be removed from the manuscript.

We have removed “near-future” and replaced it with “ocean acidification-relevant.” L373-374.

“Most importantly, the present study demonstrates that Aplysia accumulate HCO3 at an ocean acidification-relevant CO2 level.”
Abstract: Behavioural impairment following exposure to ocean acidification-relevant CO$_2$ levels has been noted in a broad array of taxa. The underlying cause of these disruptions is thought to stem from alterations of ion gradients (HCO$_3^-$/Cl$^-$) across neuronal cell membranes that occur as a consequence of maintaining pH homeostasis via the accumulation of HCO$_3^-$/Cl$^-$. While behavioural impacts are widely documented, few studies have measured acid-base parameters in species showing behavioural disruptions. In addition, current studies examining mechanisms lack resolution in targeting specific neural pathways corresponding to a given behaviour. With these considerations in mind, acid-base parameters and behaviour were measured in a model organism utilized for decades as a research model to study learning, the California sea hare ("Aplysia californica"). Aplysia exposed to CO$_2$ elevated hemolymph HCO$_3^-$/Cl$^-$, achieving full and partial pH compensation at 1200 and 3000 μatm CO$_2$, respectively. Increased CO$_2$ did not affect self-righting behaviour. In contrast, both levels of elevated CO$_2$ reduced the time of the tail-withdrawal reflex, suggesting a reduction in antipredator response. Overall, these results confirm that Aplysia are promising models to examine mechanisms underlying CO$_2$-induced behavioural disruptions since they regulate HCO$_3^-$/Cl$^-$ and have behaviours linked to neural networks amenable to electrophysiological testing.

References for response to reviewer comments:

Appelhans, Y.S., J. Thomsen, S. Opitz, C. Pansch, F. Melzner and M. Wahl. 2014. Juvenile sea stars exposed to acidification decrease feeding and growth with no acclimation potential. Marine Ecology Progress Series 509: 227-239.

Appelhans, Y.S., J. Thomsen, C. Pansch, F. Melzner and M. Wahl. 2012. Sour times: seawater acidification effects on growth, feeding behaviour and acid–base status of Asterias rubens and Carcinus maenas. Marine Ecology Progress Series 459: 85-98.

Meinshausen, M., S.J. Smith, K. Calvin, J.S. Daniel, M. Kainuma, J. Lamarque, K. Matsumoto, S. Montzka, S. Raper and K. Riahi. 2011. The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. Climatic Change 109: 213-241.

Portner, H.O., D.M. Karl, P.W. Boyd, S.E. Cheung, S.E. Lluch-Cota, Y. Nojiri, D.N. Schmidt and P.O. Zavialov. 2014. Ocean systems. pp. 411-484 in C.B. Field, V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea and L.L. White, eds. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, U.K. and New York, NY, USA.
Zittier, Z.M., T. Hirse and H.-O. Pörtner. 2013. The synergistic effects of increasing temperature and CO2 levels on activity capacity and acid–base balance in the spider crab, Hyas araneus. Marine biology 160: 2049-2062.