Feet, heat and scallops: what is the cost of anthropogenic disturbance in bivalve aquaculture?

Anthony A. Robson, Lewis G. Halsey and Laurent Chauvaud

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Review timeline
Original submission: 31 July 2015
1st revised submission: 10 December 2015
2nd revised submission: 17 January 2016
Final acceptance: 10 February 2016

Review History
RSOS-150380.R0 (Original submission)

Review form: Reviewer 1 (Helga Guderley)

Is the manuscript scientifically sound in its present form? 
No

Are the interpretations and conclusions justified by the results? 
No

Is the language acceptable? 
Yes

Is it clear how to access all supporting data? 
Fairly

Do you have any ethical concerns with this paper? 
No

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

Recommendation?
Major revision is needed (please make suggestions in comments)

Comments to the Author(s)
This is an interesting study that should be of use to a range of fields, from ecological physiology to aquaculture practices. The combination of experimental and modeling approaches is strong. The study is fairly well written, although I found a number of places where the descriptions were not clear and I was not sure of exactly how the authors had proceeded. Some of the experimental assumptions were apparently further explained in previous papers and in supplementary materials. The latter were mentioned, but I did not consult them. As is apparent from the above, I am favorable to the study, but have some reservations. My reservations are linked with the potential influence of confounding factors on some of the conclusions, notably, the decrease in the cost of activity with increasing temperature. As the authors note, this conclusion is surprising, so it needs to be better supported.

This study took considerable time during which scallops were acclimated to a randomly established constant order of temperatures. We are told that some of the scallops were used for more than one temperature. The acclimations lasted 4 weeks and then measurements were made. There were 9 acclimation temperatures, so the study had to last approximately a year. Many things change in the physiology of reproductively mature scallops in a year and the physiology of exercise is one of them. Activity in scallops is not aerobic (although the authors present it as being so in the discussion, this is clearly wrong and needs to be changed). Recuperation from exercise is aerobic. Numerous studies have demonstrated that recuperation from exercise is slowed by reproductive investment. The increase in VO2 associated with activity is loosely defined (only the word “directly” is given). This increase corresponds more with payment of an oxygen debt than with aerobic support of activity per se. If recuperation is slowed, then the time needed to pay the oxygen debt is increased. The debt may be the same, but the time needed to pay it is increased. If the authors used a set time period to assess the aerobic cost of activity, the slower recuperation by reproductively mature scallops would have suggested a lower cost. Did the analysis take this into account? How long was the oxygen trace integrated when there were increases in VO2? These critical questions need to be answered so that the reader is assured that the aerobic cost of recuperation from activity is properly assessed.

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Specifics:
Lines 11 and 428 revise to make the phrase starting with “Furthermore” a sentence.
Line 74 be more specific as to what “directly” means. Activity is not supported by aerobic metabolism directly, only recuperation from swimming activity is aerobic. This recuperation takes time and the time taken will vary with temperature (shorter at higher temperatures, presumably). Applying an arbitrary and constant time limit such as suggested by the term “directly” is likely to lead to erroneous results. The direct costs of activity would end, in my mind, when metabolic rate returns to RMR.
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Line 96 overtime should be over time
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Line 221 Greater anthropogenic disturbance was defined as extra swims per day than in previous studies. Extra relative to what? Than in or as in?

Line 344 activity in scallops is not aerobic, recuperation from activity is.

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

Is it clear how to access all supporting data?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
I do not feel qualified to assess the statistics

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

Comments to the Author(s)
Title: Heat, feet and scallops: what is the cost of anthropogenic disturbance in bivalve aquaculture?
Authors: Robson et al.
Journal: R. Soc. Open Sci.

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General comments:
this paper is really interesting and the quality is pretty good.
I have only few concerns that should be addressed before publication.
I definitely recommend the publication of this paper in R. Soc. Open Sci.
Details that need to be provided:

1) please develop a bit more the sentence in the introduction:
"As temperature increased, the activity metabolic rate of the scallops decreased" even if you refer to previous published works
2) Same, development is required for the assumption about :'Temperature - probably because of lower dissolved oxygen concentrations"
3) Please provide further details about the use of chlorophyll a: source ? Purchased chemical ? pure extracted solution? Phytoplankton ?
4) Could you be more specific on sediment-lined tanks ? are you talking about sediment collected from the field ? thickness of the layer ? origin ? general characteristics - this is useful for readers who wants to replicate these experiments.
5) Be consistent with the use of litres; L is ok but use it thus for the rest of the ms. E.g. : mL instead of ml in line251.

Review form: Reviewer 3

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

Is it clear how to access all supporting data?
Yes, but authors should add units where missing.

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
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Recommendation?
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Comments to the Author(s)

General:
This study examines the interesting topic how the impact of anthropogenically-induced disturbance on energy metabolism of king scallop varies with temperature thereby affecting growth – an important issue for commercial animal production. I only have little experience with modelling procedures but the study seemed to be well conducted, the results are interesting and the discussion is well written. My main concern is related to the puzzling findings presented in Fig.1. Please see my specific comments below.

I only have minor comments:

Line 28: “ambient temperature is likely to affect…”. Please exchange by “is known to affect…” because it is well known that metabolic rate of ectotherms is affected by temperature.
Line 42: Please specify/clarify by adding more information. i) Reference 17 observed lowered haemolymph PO2 levels in the warmth, and ii) Relationship between high temperature-induced hypoxia, rapid calcification rates and poor tissue growth.

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Figures:
In general:
Please add “O2” after mg.
“0.5” should be replaced by “0.52”, shouldn’t it?
Fig.1.
In the text it is “AMR” (line 259), in figure it is “VO2” (axe title) and in the legend it is “activity metabolic rate”. Please be more consistent.

For better clarity use consistently RMR, AMR or total MR (as defined in M&M) throughout the ms and figures.

AMR decreases with warming (e.g. at 0.2g it is ~17 mgO2/min/g at 6°C vs. ~4 mgO2/min/g at 24°C). Furthermore, when extrapolating VO2 values to 0g to estimate RMR (see e.g. Lurman et al. J Exp Biol 2007 ) RMR is higher in the cold than in the warmth - contrasting to warming-induced rise in RMR shown in Fig 2b. Please comment.

Fig.2.
Information of N should be added to legend.

Supplement file:
Please add units where missing.

Decision letter (RSOS-150380)

05-Nov-2015

Dear Dr Robson:

Manuscript ID RSOS-150380 entitled "Heat, feet and scallops: what is the cost of anthropogenic disturbance in bivalve aquaculture?" which you submitted to Royal Society Open Science, has been reviewed. The comments from reviewers are included at the bottom of this letter.

In view of the criticisms of the reviewers, the manuscript has been rejected in its current form. However, a new manuscript may be submitted which takes into consideration these comments.

Please note that resubmitting your manuscript does not guarantee eventual acceptance, and that your resubmission will be subject to peer review before a decision is made.

You will be unable to make your revisions on the originally submitted version of your manuscript. Instead, revise your manuscript and upload the files via your author centre.

Once you have revised your manuscript, go to https://mc.manuscriptcentral.com/rsos and login to your Author Center. Click on "Manuscripts with Decisions," and then click on "Create a Resubmission" located next to the manuscript number. Then, follow the steps for resubmitting your manuscript.

Your resubmitted manuscript should be submitted by 04-May-2016. If you are unable to submit by this date please contact the Editorial Office.

We look forward to receiving your resubmission.

Sincerely,
Emilie Aime
Senior Publishing Editor, Royal Society Open Science

on behalf of
Kevin Padian, Royal Society Open Science
opencience@royalsociety.org
Associate Editor Comments to Author (Dr Sean Rands):

Comments to the Author:

Three reviewers have commented on your manuscript, and their comments should be attached. Pay attention to the concerns of reviewer #1, who questions your methodology (particularly given the presumed timeframe of your experiments), and calls for some extra information to be made available. Given these concerns, I can't say at this stage whether this manuscript hits journal criteria, but would be happy to consider a revised version of the manuscript.

Reviewers' Comments to Author:

Reviewer: 1

Comments to the Author(s)

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Authors: Robson et al.
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Fig.2.
Information of N should be added to legend.

Supplement file:
Please add units where missing.

Author’s Response to Decision Letter for (RSOS-150380)
See Appendix A.
Is the manuscript scientifically sound in its present form?
No

Are the interpretations and conclusions justified by the results?
No

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes

Do you have any ethical concerns with this paper?
No

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

Recommendation?
Major revision is needed (please make suggestions in comments)

Comments to the Author(s)
The authors have made many revisions that have improved the paper, but I feel that the central problem that bothered me in the preceding version has not been adequately addressed. The authors present an unusual finding: that the energetic efficiency of activity increases with temperature. The data that support this are not shown directly, rather some regression lines supporting this idea are shown, but without the data points. Furthermore, the authors have acknowledged the fact that scallop activity is anaerobic by removing consideration of aerobic metabolic pathways from the discussion, but MANY statements remain that make it sound as though the authors feel that scallop activity is aerobic (see below). This needs to be corrected.

Specific concerns:
1) In the definition of the metabolic rate measurements (line 83 onward), AMR is defined as the rate of energy expended specifically to undertake activity and does not include the RMR component of MR during active periods. The reader that knows that scallop activity is anaerobic, needs to know what the authors considered the active period. If the period is only that when the scallops were moving, then the data do not show the aerobic cost of activity as this is only apparent as the repayment of the oxygen debt after the movement is finished. The explanation of how scallop RMR, AMR and total MR were calculated (line 160 onward) does not indicate how long measurements of AMR continued. This crucial information is only given at Line 188 – 196 and then the reader is referred to another paper and the supplementary data. While I understand that the calculations were made using R and Excel, the criteria used to decide what VO2 values were associated with activity need to be clear, since this is such a central point in this study. Line 164: were the different individuals of radically different sizes, justifying the use of mass-corrected values?

2) I would like to help the authors substantiate the validity of their central message concerning the decrease in the activity associated metabolic rate with the increase in temperature. Firstly, it would be much more convincing to have data points associated with the regression lines that are
shown in Figure 1. Providing the R2 values is a minimal gesture towards convincing readers of the validity of the data. The individual data points in Figure 2 show a wide spread in activity levels between what looks like 10C and 18C, whereas the activity for the two lower temperature (6 and 8) and the higher temperatures (21 and 23) falls in a much smaller range of values. This indicates that the range of VeDBA values in Figure 1 should be much smaller at the low and high temperatures, but this is not the case. Only the three intermediate temperatures have a greater range of VeDBA values. Providing the individual VeDBA points associated with the AMR values would be MUCH more convincing. Since the increase in energetic efficiency with an increase in temperature is a surprising finding, the authors should provide as much support as possible for it. Perhaps the data for Figures 1 and 2 were taken under different conditions, making the comparisons I undertook inappropriate, but the basic problem that the regression lines are unaccompanied by the data upon which they are based remains.

3) I am surprised that the authors state that studies concerning the relationship between temperature and metabolism do not partition resting and active metabolic rates. The seminal studies of Fry (1949, I believe) examine this question, considerations of aerobic power budgeting do as well, Wolfgang Wieser’s studies of metabolic partitioning also examine this central question.

4) Lines 366 -373, lines 381-388  The arguments in these paragraphs ignore the anaerobic nature of scallop activity. The term activity needs to be changed to “recuperation from activity”, otherwise their terminology is erroneous.

Line 413: The statement that the absolute energy costs to move are greater in larger individuals needs to be justified, either with data or with a reference.

I'm sure that the authors have guessed who I am, I can't remember whether I identified myself the last time.... Helga Guderley is my name.

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

Is it clear how to access all supporting data?
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Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
I do not feel qualified to assess the statistics

Recommendation?
Accept as is
Comments to the Author(s)
Dear authors,
well done!

Decision letter (RSOS-150679)

05-Jan-2016
Dear Dr Robson,

The Subject Editor assigned to your paper ("Feet, heat and scallops: what is the cost of anthropogenic disturbance in bivalve aquaculture?") has now received comments from reviewers. We would like you to revise your paper in accordance with the referee and Subject Editor suggestions which can be found below (not including confidential reports to the Editor). Please note this decision does not guarantee eventual acceptance.

Please submit a copy of your revised paper within three weeks (i.e. by the 28-Jan-2016). If we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Editors, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers.

To revise your manuscript, log into http://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." Your manuscript number has been appended to denote a revision. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". Please use this to document how you have responded to each of the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

In addition to addressing all of the reviewers' and editor's comments please also ensure that your revised manuscript contains the following sections before the reference list:

• 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 (http://datadryad.org/), or modify your current submission to dryad, please use the following link:
http://datadryad.org/submit?journalID=RSOS&manu=RSOS-150679

• 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.

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.

Yours sincerely,
Matthew Allinson,
Royal Society Open Science
openscience@royalsociety.org

Associate Editor’s comments (Dr Sean Rands):
Associate Editor
Comments to the Author:
Two of the previous reviewers have commented on this much improved version of the manuscript, and one of them has a number of suggestions that could enhance the value of your manuscript further. I’d like to give you the chance to respond to these suggestions.
Reviewer: 3

Comments to the Author(s)

Dear authors,
well done!

Reviewer: 1

Comments to the Author(s)

The authors have made many revisions that have improved the paper, but I feel that the central problem that bothered me in the preceding version has not been adequately addressed. The authors present an unusual finding: that the energetic efficiency of activity increases with temperature. The data that support this are not shown directly, rather some regression lines supporting this idea are shown, but without the data points. Furthermore, the authors have acknowledged the fact that scallop activity is anaerobic by removing consideration of aerobic metabolic pathways from the discussion, but MANY statements remain that make it sound as though the authors feel that scallop activity is aerobic (see below). This needs to be corrected.

Specific concerns:

1) In the definition of the metabolic rate measurements (line 83 onward), AMR is defined as the rate of energy expended specifically to undertake activity and does not include the RMR component of MR during active periods. The reader that knows that scallop activity is anaerobic, needs to know what the authors considered the active period. If the period is only that when the scallops were moving, then the data do not show the aerobic cost of activity as this is only apparent as the repayment of the oxygen debt after the movement is finished. The explanation of how scallop RMR, AMR and total MR were calculated (line 160 onward) does not indicate how long measurements of AMR continued. This crucial information is only given at Line 188–196 and then the reader is referred to another paper and the supplementary data. While I understand that the calculations were made using R and Excel, the criteria used to decide what VO2 values were associated with activity need to be clear, since this is such a central point in this study.

Line 164: were the different individuals of radically different sizes, justifying the use of mass-corrected values?

2) I would like to help the authors substantiate the validity of their central message concerning the decrease in the activity associated metabolic rate with the increase in temperature. Firstly, it would be much more convincing to have data points associated with the regression lines that are shown in Figure 1. Providing the R2 values is a minimal gesture towards convincing readers of the validity of the data. The individual data points in Figure 2 show a wide spread in activity levels between what looks like 10C and 18C, whereas the activity for the two lower temperature (6 and 8) and the higher temperatures (21 and 23) falls in a much smaller range of values. This indicates that the range of VeDBA values in Figure 1 should be much smaller at the low and high temperatures, but this is not the case. Only the three intermediate temperatures have a greater range of VeDBA values. Providing the individual VeDBA points associated with the AMR values would be MUCH more convincing. Since the increase in energetic efficiency with an increase in temperature is a surprising finding, the authors should provide as much support as possible for it. Perhaps the data for Figures 1 and 2 were taken under different conditions, making the comparisons I undertook inappropriate, but the basic problem that the regression lines are unaccompanied by the data upon which they are based remains.
3) I am surprised that the authors state that studies concerning the relationship between temperature and metabolism do not partition resting and active metabolic rates. The seminal studies of Fry (1949, I believe) examine this question, considerations of aerobic power budgeting do as well, Wolfgang Wieser’s studies of metabolic partitioning also examine this central question.

4) Lines 366 -373, lines 381-388 The arguments in these paragraphs ignore the anaerobic nature of scallop activity. The term activity needs to be changed to “recuperation from activity”, otherwise their terminology is erroneous.
Line 413: The statement that the absolute energy costs to move are greater in larger individuals needs to be justified, either with data or with a reference.

I'm sure that the authors have guessed who I am, I can't remember whether I identified myself the last time.... Helga Guderley is my name.

Author's Response to Decision Letter for (RSOS-150679)

See Appendix B.
Dear Dr Rands,

We are very grateful to you and the reviewers for the useful comments. The reviewers’ comments were most perceptive. We are grateful for the opportunity to resubmit our work to the journal Royal Society Open Science and have responded to all points made by the reviewers. We provide our responses (in bold) beneath the original comments (in italics). We hope you will agree that the manuscript has been substantially improved as a result.

Reviewer: 1 Comments to the Author(s)

This is an interesting study that should be of use to a range of fields, from ecological physiology to aquaculture practices. The combination of experimental and modeling approaches is strong.

Thank you for these kind words.

The study is fairly well written, although I found a number of places where the descriptions were not clear and I was not sure of exactly how the authors had proceeded. Some of the experimental assumptions were apparently further explained in previous papers and in supplementary materials. The latter were mentioned, but I did not consult them.

We have attended to this issue of general clarity and made a number of improvements throughout the manuscript (see also our specific responses, below).

As is apparent from the above, I am favorable to the study, but have some reservations. My reservations are linked with the potential influence of confounding factors on some of the conclusions, notably, the decrease in the cost of activity with increasing temperature. As the authors note, this conclusion is surprising, so it needs to be better supported.

We have now edited the text to clarify the finding – that at higher temperatures, for a given level of activity the metabolic rate of king scallops specifically to power that activity is less. Note that this is not the same as stating that at higher temperatures scallops exhibit a lower metabolic rate specifically associated with their activity – our statement is effectively about the scallops’ energy efficiency at any given level of activity, as illustrated in figure 1. Total metabolic rate is rarely split into resting metabolic rate and activity metabolic rate (Halsey et al. 2015), and we suggest in the present manuscript that this may be why our finding has not been reported before. We cite Seebacher et al.’s recent study showing that muscles are more efficient at higher temperatures as a suggested explanation for our finding.
This study took considerable time during which scallops were acclimated to a randomly established constant order of temperatures. We are told that some of the scallops were used for more than one temperature. The acclimations lasted 4 weeks and then measurements were made. There were 9 acclimation temperatures, so the study had to last approximately a year.

Many things change in the physiology of reproductively mature scallops in a year and the physiology of exercise is one of them. Activity in scallops is not aerobic (although the authors present it as being so in the discussion, this is clearly wrong and needs to be changed). Recuperation from exercise is aerobic. Numerous studies have demonstrated that recuperation from exercise is slowed by reproductive investment. The increase in VO2 associated with activity is loosely defined (only the word “directly” is given). This increase corresponds more with payment of an oxygen debt than with aerobic support of activity per se. If recuperation is slowed, then the time needed to pay the oxygen debt is increased. The debt may be the same, but the time needed to pay it is increased. If the authors used a set time period to assess the aerobic cost of activity, the slower recuperation by reproductively mature scallops would have suggested a lower cost.

We have attended to these concerns (see below for specific responses to each).

Did the analysis take this into account? Yes (see below).

How long was the oxygen trace integrated when there were increases in VO2? These critical questions need to be answered so that the reader is assured that the aerobic cost of recuperation from activity is properly assessed.

The oxygen trace was integrated until metabolic rate returned to resting levels. This is now explained in the manuscript.

Another aspect that requires better definition is the physiological condition of the animals. If they were used over such a long period of time, even though they are being fed with phytoplankton, what assurance does the reader have that their energetic condition was equivalent at the different acclimation temperatures? I feel that more information should be given about the animals used for the study. The dates of the acclimations, the status of the animals at this time, whether any spawning was observed, etc.

The Materials and Methods section now includes the following statements:

“Data were collected only from scallops with ripe gonads i.e. gonad maturity stages > 5 to 6 determined using Mason’s gonad observation index [23] and that did not spawn before, between or during experiments.”
and

“The few scallops used in multiple conditions (see 3.1 and 3.2 below) did not change their reproductive physiology (gonad maturity stage) or size (wet mass in air, volume and shell dimensions) during the period of experiments. This was due to the low temperatures these particular scallops were exposed to.”

Specifics:
Lines 11 and 428 revise to make the phrase starting with “Furthermore” a sentence.
The necessary edits to these sentences have been made.

Line 74 be more specific as to what “directly” means. Activity is not supported by aerobic metabolism directly, only recuperation from swimming activity is aerobic. This recuperation takes time and the time taken will vary with temperature (shorter at higher temperatures, presumably). Applying an arbitrary and constant time limit such as suggested by the term “directly” is likely to lead to erroneous results.

This is not what we meant to imply by ‘directly’, and indeed not the way we calculated activity energy expenditure. We have deleted the word ‘directly’ and further edited this sentence to clarify that AMR is activity-specific metabolic rate, i.e. the energy expended specifically to undertake activity and thus metabolic rate during activity not including the RMR element.

The direct costs of activity would end, in my mind, when metabolic rate returns to RMR.

We agree, and our calculations of AMR, now explained in the manuscript, reflect this.

In M&M section 3.2 we have added more detail about the equation used to calculate AMR.

Lines 90-97 The description of the respirometry method is quite detailed, yet I have difficulty understanding it. In particular, I found the description of the method for establishing “stable background VO2” in the presence of the scallop unclear. Perhaps a diagram would help.

Although we prefer to give good detail about the methods we have employed, we also recognise that excess detail can be counter-productive. We have now reduced the text about accounting for background V̇O₂ levels, simply stating:

“Background V̇O₂ in the water due to aerobic organisms in the sediment was measured and accounted for in calculations of scallop MR along with the decrease in background V̇O₂ over time owing to scallop filter feeding which was determined by linear interpolation.”
Line 96 overtime should be over time
Corrected

Line 98 to determine VO2 removed from the water by of aerobic organisms in the sediment.
This sentence has been changed as part of other edits (see earlier).
The VO2 of the aerobic organisms in the sediment would be clearer.
We edited the manuscript as suggested.

Line 111 the loggers instrumented to scallops, suggest instrumented should be attached to
In the literature “instrumented” is frequently used and considered equivalent to “attached to”

Line 130 Were temperature acclimated scallops exposed to all of those temperatures? There is a statement (Line 158) “As groups of scallops at the different temperatures were mostly comprised of different individuals,…“ that leads me to several questions. If some of the scallops in the different temperature groups were the same, the total time taken to make these measurements was considerable (at least 9 months). The measurements at the beginning were under very different conditions than those at the end. How comparable can they be, both with changing size, physiological condition and endogenous rhythms. Many questions need clarification.

We hope we have clarified the referee’s concerns with the following edits and additions to the manuscript:

“Data were collected only from scallops with ripe gonads……

“The few scallops used in multiple conditions (see 3.1 and 3.2 below) did not change their reproductive physiology (gonad maturity stage) or size during the course of the experiments……

and below:

As stated in Robson et al 2012 and Robson et al in prep “Visual inspection of the data suggested no influence of tidal cycles or circadian rhythms on the movement and associated metabolic rate of scallops in the hatchery or in the wild”

In 4.1 of the current manuscript we added:

“Visual inspection of the data suggested no influence of rhythms on the movement and associated MR of the scallops.”
Line 216 two growth ranges from shell height growth from 10 to 60 mm... This formulation is not clear. It seems the word growth should be replaced with size, for the terms to make sense.

We edited the manuscript as suggested.

Line 221 Greater anthropogenic disturbance was defined as extra swims per day than in previous studies. Extra relative to what?

Now: “(c) the effect of even greater anthropogenic disturbance (defined as extra swims per day) on scallop growth than observed in the present study”

Section 3.3 Modelling growth rate against temperature in scenarios of different disturbance levels and figures 3 and 4 have been revised to make our methods and results absolutely clear.

Than in or as in?

This text has been reworded as part of the changes above.

Line 344 activity in scallops is not aerobic, recuperation from activity is.

We take this point on board. In our discussion in this section of the manuscript about why scallops may exhibit low activity levels at particularly low and high temperatures, we have removed consideration of aerobic metabolic pathways.

Reviewer: 2 Comments to the Author(s)
Title: Heat, feet and scallops: what is the cost of anthropogenic disturbance in bivalve aquaculture?
Authors: Robson et al.
Journal: R. Soc. Open Sci.
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General comments:
this paper is really interesting and the quality is pretty good.
I have only few concerns that should be addressed before publication.
I definitely recommend the publication of this paper in R. Soc. Open Sci.

Details that need to be provided:
1) please develop a bit more the sentence in the introduction:
"As temperature increased, the activity metabolic rate of the scallops decreased" even if you refer to previous published works
We do not have space in the Abstract (where this sentence is present) to much develop the sentence, however we have adjusted it to make the finding we report clearer. The finding is this – at higher temperatures, for a given level of activity the metabolic rate of king scallops specifically to power that activity is less. Note that this is not the same as stating that at higher temperatures scallops exhibit a lower metabolic rate specifically associated with their activity – our statement is effectively about the scallops’ energy efficiency at any given level of activity, as illustrated in figure 1. Total metabolic rate is rarely split into resting metabolic rate and activity metabolic rate (Halsey et al. 2015), and we suggest in the present manuscript that this may be why our finding has not been reported before. We cite Seebacher et al.’s recent study showing that muscles are more efficient at higher temperatures as a suggested explanation for our finding. This fuller detail is now discussed when this finding is considered in the Discussion section.

Halsey LG, Matthews PGD, Rezende EL, Chauvaud L, Robson AA (2015) The interactions between temperature and activity levels in driving metabolic rate: theory, with empirical validation from contrasting ectotherms. Oecologia 177: 1117–1129.

2) Same, development is required for the assumption about: "Temperature - probably because of lower dissolved oxygen concentrations"

We take this point on board. In response to a comment by Referee 1, in our discussion in this section of the manuscript about why scallops may exhibit low activity levels at particularly low and high temperatures, we have removed consideration of aerobic metabolic pathways because scallops are mainly anaerobic when exhibiting movement.

3) Please provide further details about the use of chlorophyll a: source? Purchased chemical? pure extracted solution? Phytoplankton?

The Materials and Methods section now includes

“Before, during and between experiments scallops were provided a natural diet (unfiltered sea water) including seston, dissolved matter and benthic particulate matter.”

4) Could you be more specific on sediment-lined tanks? are you talking about sediment collected from the field? thickness of the layer? origin? general characteristics - this is useful for readers who wants to replicate these experiments.

The Materials and Methods section now includes

“In these and all other tanks and respirometry chambers used to maintain the scallops during the period of experimentation, the sediment provided was sterilised sand to a depth of 8 cm from l’anse du Dellec (48.3540°N, −4.5659°W), Plouzané.”
5) Be consistent with the use of litres; L is ok but use it thus for the rest of the ms. E.g.: mL instead of ml in line251.

Edited as suggested

Reviewer: 3 Comments to the Author(s)

General:
This study examines the interesting topic how the impact of anthropogenically-induced disturbance on energy metabolism of king scallop varies with temperature thereby affecting growth – an important issue for commercial animal production. I only have little experience with modelling procedures but the study seemed to be well conducted, the results are interesting and the discussion is well written. My main concern is related to the puzzling findings presented in Fig.1. Please see my specific comments below.

I only have minor comments:

Line 28: “ambient temperature is likely to affect...”. Please exchange by “is known to affect...” because it is well known that metabolic rate of ectotherms is affect by temperature.

Edited as suggested

Line 42: Please specify/clarify by adding more information. i) Reference 17 observed lowered haemolymph PO2 levels in the warmth, and ii) Relationship between high temperature-induced hypoxia, rapid calcification rates and poor tissue growth.

Some additional information has now been added to this statement:

“The scallops exhibit a stress response at these high temperatures [13, 15, 16] probably because these temperatures result in lower partial pressures of oxygen in their haemolymph [17], inducing rapid accumulation of calcareous shell deposits accompanied by poor tissue growth.”

Line 76: AMR is calculated as: Total MR – RMR. Why not as: Maximal MR – RMR?

Because we have defined AMR as the element of total metabolic rate associated specifically with activity, i.e. the element of total metabolic rate that is not required for background maintenance etc. See:

Halsey LG, Matthews PGD, Rezende EL, Chauvaud L, Robson AA (2015) The interactions between temperature and activity levels in driving metabolic rate: theory, with empirical validation from contrasting ectotherms. Oecologia 177: 1117-1129.
Can you please comment on that as Total MR is “the mean MR across the entire recording period” and thus include lowest as well as highest MR.

In this study, total metabolic rate represents the total average metabolic rate of the scallops during the given recording period (i.e. any given temperature condition). It is termed ‘total’ because it includes both ‘elements’ of metabolic expenditure – routine expenditure (as defined in the manuscript) and activity expenditure. Our analysis comes from the angle of investigating how changes in resting metabolic rate and activity metabolic rate change with temperature and human disturbance and, in turn, how this affects their overall (total) metabolic rate. To achieve this we recorded a single average value of total MR and a single value of RMR (lowest 20 mins average), and from those values calculated AMR.

How do calculated values fit to literature data? Please add some information accordingly.

Previous studies focus on the MR of escape responses and valve clapping until exhaustion.

We state in our previous king scallop study: … Validity of these results is provided by comparison with previous studies. For example, in the respirometry experiments of the present study, maximum ODBA was produced by a spin involving six rapid valve adduction–abduction events at a rate of 2-3 s\(^{-1}\) although this resulted in a relatively small subsequent EPOC of less than 30 min (figure 5). This finding is similar to the results of Livingstone et al. [33], who recorded a recovery period of approximately 30 min after five rapid valve adduction, followed by abduction events at a rate of 2–3 s\(^{-1}\) in the giant scallop, Placopecten magellanicus (cf. recovery period of scallops after valve movements causing complete exhaustion [33,47,48]).

See: Robson, AA, Chauvaud, L, Wilson, RP, Halsey, LG. 2012 Small actions, big costs: the behavioural energetics of a commercially important invertebrate. J. Royal Soc. Interface. 9, 1486-1498. (10.1098/rsif.2011.0713)

In the present manuscript we compare our modelled scallop growth values to growth values in the literature under different disturbance scenarios reported for bay scallops:

“A similar pattern between total daily energy expenditure and temperature was shown for bay scallops Argopecten irradians concentricus albeit across just four temperatures [53].”

Line 79f: Information to the calibration of O\(_2\) sensors is missing. Temperature affects oxygen solubility in water why it is necessary to calibrate at each experimental temperature.

The text now includes:

“….calibrated dissolved O\(_2\) probe”
Line 94-95: Consider re-writing this sentence.

This text has been edited

Line 130: I guess the acclimation of scallops to different Ts happened outside the O2-chambers, didn’t it? Please clarify.

This is correct. The text now reads:

“Scallops in sediment-lined tanks”

Why do temperatures differ between experiments? Compare line 131 and 168.

In each case the range of temperatures is the same, but from experience we consider that calibrations using seven temperatures within that range is sufficient to generate the required prediction equations. In the aquaculture facility experiments we wanted a higher resolution within the temperature range for inclusion in the models.

Line 160: Confusing wording “dry mass”: Sometimes dry mass seemed to refer to ash-free dry tissue mass, sometimes to “real” dry mass. Please clarify by using “AFDM” and “DM”. Please check ms accordingly, including units of VO2 values.

For complete clarity we now use “ash-free dry tissue mass” and “total dry tissue mass”

Line 249-255 and line 282-284: These are no results and should better be moved to M&M part. N should be given for the mean values. Is it mean +/-SD or SE?

We consider this information most appropriately presented in the Results section (and have included similar information here in a previous Royal Society publication), however we are prepared to move them on the advice of the journal editor.

n = 111 added. From Materials and Methods: “45 scallops were used in respirometry experiments to calibrate accelerometry data with rate of energy expenditure, and 66 scallops were placed in an aquaculture facility where their activity-time budgets and energy expenditure budgets were estimated from accelerometry data.”

and

“Unless stated otherwise means are reported ± the 95% CI”

Line 272-274: Unclear sentence (“greater” ?). Consider re-writing and add reference to Fig.2c.

Changed to: “higher than”
Added reference to “figure 2c”

Line 287ff and Fig.3: It seems that anthropogenic disturbance has most impact on growth rates at intermediate Ts (11-15°C) and is lower at 10°C and lowest at 18°C. This aspect should be addressed in more detail than just comparing 10 and 18°C data.

In absolute terms the impact on growth time decreases linearly as temperature increases. Even in percentage terms the general trend is in this direction. We have now added ‘e.g.’ before the bracketed data for 10°C (as was present for the bracketed data for 18°C) to further clarify that these temperatures are just examples pulled out to support Figure 3. We have a lot of results and we are striving to strike a balance between presenting and discussing all important findings while also presenting a clear and relatively simple overall message.

Line 326f: “…typical metabolic rate-temperature curve….lower metabolic rates at relatively low and high temperature…”. Be more specific which metabolic rate, because RMR rises with warming, whereas Total VO2 shows an asymptote. Please check throughout the ms.

Clarified: “typical total MR-temperature curve” and “lower total MR”

Line 330: “..the activity metabolic rate of scallop activity decreases”. Do you mean the difference between slopes with rising activity level (lower slope at 24°C compared to 6°C)? Please consider re-writing for better clarity.

The text has been revised:

“Interestingly, as temperature increases, the activity metabolic rate of the scallops to perform a given level of activity decreases (figure 1). To our knowledge this relationship between activity-specific energy efficiency and temperature has not been reported in any animal previously, perhaps because relationships presented between metabolic rate and temperature do not usually partition resting and active costs [44]……”

Line 132f: The authors should check the literature for “temperature-dependent aerobic scope” which describes the relationship between AMR-RMR and temperature. See e.g. review by Farrell, Comp Biochem Physiol A, 2002.

We believe the referee is referring to line 332. In light of the comments of other referees we have adjusted this paragraph to improve clarity. The point we are making here is that at higher temperatures, for a given level of activity the metabolic rate of king scallops specifically to power that activity is less. Note that this is not the same as stating that at higher temperatures scallops exhibit a lower metabolic rate specifically associated with their activity – our statement is effectively about the scallops’ energy efficiency at any given level of activity, as illustrated in figure 1.

Line 331ff: How does this fit to findings by Schalkhausser et al. (citation #17) who observed similar AMR but lower clapping force of scallops at 20°C than at 10°C?
Schalkhausser et al. did not measure AMR as defined in our study and unlike in the present study Schalkhausser et al. measured valve clapping until fatigue. A higher clapping force applied by the scallop would result in a faster acceleration of the shell and thus a higher VeDBA. In our study maximum VeDBA was lower at 21°C than at 12°C which is similar to the lower clapping force of scallops at 20°C than at 10°C in Schalkhausser et al. We make the point (citing Schalkhausser et al.) in our manuscript:

“Less activity by the scallops at the highest temperatures is perhaps most likely due to a reduced level of force generated by the muscles [17, 49]”

Figures:
In general:
Please add “O2” after mg.

O_2 has been added after mg

“0.5” should be replaced by “0.52”, shouldn’t it?

Now g^{-0.52}

Fig.1.
In the text it is “AMR” (line 259), in figure it is “VO2” (axe title) and in the legend it is “activity metabolic rate”. Please be more consistent.

For better clarity use consistently RMR, AMR or total MR (as defined in M&M) throughout the ms and figures.

For better clarity we now use consistently RMR, AMR and total MR (as defined in M&M) throughout the manuscript, including Figure 1.

AMR decreases with warming (e.g. at 0.2g it is ~17 mgO2/min/g at 6°C vs. ~4 mgO2/min/g at 24°C). Furthermore, when extrapolating VO2 values to 0g to estimate RMR (see e.g. Lurman et al. J Exp Biol 2007) RMR is higher in the cold than in the warmth – contrasting to warming-induced rise in RMR shown in Fig 2b. Please comment.

In response to the point raised elsewhere by the referee, we have now clarified in the manuscript that figure 1 represents relationships between AMR and VeDBA. As stated in the ms, “AMR is the rate of energy expended specifically to undertake activity, and thus does not include the RMR component of MR during active periods.” Thus RMR cannot be estimated by extrapolating back to VeDBA = 0 g. The 95% CIs (not shown in figure 1) of each regression line (i.e. for each temperature) includes AMR = 0 when VeDBA = 0.
Fig. 2.
Information of \( N \) should be added to legend.

Added: (\( n=10 \) to \( 12 \) at each temperature)

Supplement file:
Please add units where missing.

We have added units where missing. Scallop AFDTM (g) changed to Scallop ash-free dry tissue mass (g). Clarified that “Detailed behaviour: cough, dig, turn, 180° flip, spin and swim)” \( O_2 \) use is activity metabolic rate (i.e. does not include routine metabolic rate).
Appendix B

Feet, heat and scallops: what is the cost of anthropogenic disturbance in bivalve aquaculture?

Robson et al.

Response to reviewer

Dear Dr Rands,

Thank you for the opportunity to respond to further referee comments, and in turn to further develop our manuscript. We are grateful to Professor Guderley for her additional comments. We provide our responses (in bold) beneath the original comments (in italics). We hope you will agree that the manuscript has been suitably improved as a result.

Reviewer: 1

Comments to the Author(s)

The authors have made many revisions that have improved the paper, but I feel that the central problem that bothered me in the preceding version has not been adequately addressed. The authors present an unusual finding: that the energetic efficiency of activity increases with temperature. The data that support this are not shown directly, rather some regression lines supporting this idea are shown, but without the data points. Furthermore, the authors have acknowledged the fact that scallop activity is anaerobic by removing consideration of aerobic metabolic pathways from the discussion, but MANY statements remain that make it sound as though the authors feel that scallop activity is aerobic (see below). This needs to be corrected.

Specific concerns:

1) In the definition of the metabolic rate measurements (line 83 onward), AMR is defined as the rate of energy expended specifically to undertake activity and does not include the RMR component of MR during active periods. The reader that knows that scallop activity is anaerobic, needs to know what the authors considered the active period. If the period is only that when the scallops were moving, then the data do not show the aerobic cost of activity as this is only apparent as the repayment of the oxygen debt after the movement is finished. The explanation of how scallop RMR, AMR and total MR were calculated (line 160 onward) does not indicate how long measurements of AMR continued. This crucial information is only given at Line 188 – 196 and then the reader is referred to another paper and the supplementary data. While I understand that the calculations were made using R and Excel, the criteria used to decide what VO2 values were associated with activity need to be clear, since this is such a central point in this study.

We have edited the text in several places to make clear that in measuring oxygen consumption to quantify energy expenditure, we have accounted for the substantial component of anaerobic metabolism exhibited my scallops by measuring both VO2
during the activity and the subsequent EPOC period (where \( \dot{V}O_2 \) above resting levels due to ‘payback’). This includes some information on how the EPOC was defined.

The Materials and Methods section now includes the following statements:

Related to the definition of AMR near line 83: “Because scallop metabolism includes a substantial anaerobic component, the repayment of oxygen debt after a period of activity had finished was also measured.”

And

“Most importantly, \( \dot{V}O_2 \) above RMR immediately after the cessation of activity until it decreased to within + 1 standard deviation of RMR was included in the calculations of AMR; this period of raised \( \dot{V}O_2 \) typically lasted between 0.5 and 30 minutes.”

Line 164: were the different individuals of radically different sizes, justifying the use of mass-corrected values?

Yes. The ash-free dry tissue mass size range of the scallops was 1.97-18.83 g. This has always been stated in the manuscript.

2) I would like to help the authors substantiate the validity of their central message concerning the decrease in the activity associated metabolic rate with the increase in temperature. Firstly, it would be much more convincing to have data points associated with the regression lines that are shown in Figure 1. Providing the R2 values is a minimal gesture towards convincing readers of the validity of the data. The individual data points in Figure 2 show a wide spread in activity levels between what looks like 10C and 18C, whereas the activity for the two lower temperature (6 and 8) and the higher temperatures (21 and 23) falls in a much smaller range of values. This indicates that the range of VeDBA values in Figure 1 should be much smaller at the low and high temperatures, but this is not the case. Only the three intermediate temperatures have a greater range of VeDBA values. Providing the individual VeDBA points associated with the AMR values would be MUCH more convincing. Since the increase in energetic efficiency with an increase in temperature is a surprising finding, the authors should provide as much support as possible for it. Perhaps the data for Figures 1 and 2 were taken under different conditions, making the comparisons I undertook inappropriate, but the basic problem that the regression lines are unaccompanied by the data upon which they are based remains.

We now present a revised Figure 1, in colour, which enables us to clearly present both the raw data points and the lines of best fit through the data for each temperature condition (as requested by Professor Guderley). Presenting the raw data underscores evidence of an interaction between temperature and VeDBA, where the slope of the AMR-VeDBA relationship becomes steeper as temperature decreases. Thus, at any given level of activity (i.e. any given VeDBA value), AMR is higher at progressively lower temperatures. And we back this up with inferential statistical analysis. The results now include the following statements: “A full factorial general linear model
including VeDBA and temperature with scallop ID as a random factor confirmed this, providing strong evidence that the interaction term was an important predictor of AMR ($F_{1, 681} = 484.96$, $P < 0.001$).

As an aside, Figures 1 and 2 cannot be compared directly because whereas Figure 1 presents measures of AMR, Figure 2(b) presents estimates based on conversions of recorded VeDBA.

3) I am surprised that the authors state that studies concerning the relationship between temperature and metabolism do not partition resting and active metabolic rates. The seminal studies of Fry (1949, I believe) examine this question, considerations of aerobic power budgeting do as well, Wolfgang Wieser’s studies of metabolic partitioning also examine this central question.

We stand by our statement that the literature is presently lacking empirical data on RMR and AMR as separate constituents of total MR, as well as the potentially differing effects of temperature on them. N.B. Except our recent study Halsey et al. 2015 cited appropriately in this manuscript.

RE: Fry and Hart (1948) Swimming speed of goldfish at different temperatures. J. Fish. Res. Board Can

Unfortunately, this seminal study does not show how temperature affects chosen activity levels but rather how temperature affects possible exertion levels.

Wolfgang Wieser’s studies (e.g. Mehner and Wieser (1994) Effects of temperature on allocation of metabolic energy in perch (*Perca fluviatilis*) fed submaximal rations. Journal of Fish Biology) do not show how temperature affects chosen activity levels but rather ‘scope for spontaneous activity’ i.e. not total MR – routine MR = Activity MR as defined in our study.

4) Lines 366–373, lines 381–388 The arguments in these paragraphs ignore the anaerobic nature of scallop activity. The term activity needs to be changed to “recuperation from activity”, otherwise their terminology is erroneous.

We have edited these arguments, including adjustments as suggested by Professor Guderley, to fully recognise the anaerobic element of scallop activity.

Line 413: The statement that the absolute energy costs to move are greater in larger individuals needs to be justified, either with data or with a reference.

Section 3.3: “ANOVA provided no evidence for an effect of temperature (10 to 18°C) or scallop ash-free dry tissue mass on swim VeDBA or single swim duration: all $P \geq 0.60$.” This has always been stated in section 3.3 of the manuscript.
Large scallops have larger muscle masses, which requires more energy to move. This is shown in equation 2 of the present manuscript, whereby activity metabolic rate (AMR) is described both by activity level (VeDBA) and also (ash-free dry) mass. This evidence has been included in the sentence flagged up by Professor Guderley.

I'm sure that the authors have guessed who I am, I can't remember whether I identified myself the last time.... Helga Guderley is my name.

Professor Guderley, thank you very much for your input to our manuscript.