Aquatic Ecology

Responses of a native and a recent invader snail to warming and dry conditions: the case of the lower Ebro River

--Manuscript Draft--

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Abstract:	Aquatic habitats have been highly modified by human actions that reduce their native diversity and create conditions suitable for tolerant alien species. Pomacea maculata was detected in 2009 in both the alluvial plain and the final stretch of the Ebro River. Since then, a permanent population has stabilized in the littoral area of the river where the water level fluctuates according to the river discharge. Melanopsis tricarinata is an endemic snail species highly affected by the reduction of its natural habitat. Currently, the two species do not share the same reaches in the river but the possibility exists, as the distribution of the P. maculata is constantly increasing. This study aims to analyse the diets and to assess the responses of both snails to global change. The diet of both species was analysed in the field and their responses to water warming and dryness compared under laboratory conditions. This study includes the calculation of future river water temperatures based on air temperature projections. In addition, based on water discharge management scenarios, the study estimated the increase in dry riverbed area. The diet of both snail species was similar and based on Cladophora. P. maculata better resisted high temperatures and dry conditions than M. tricarinata. The projections of water temperatures showed an increase in daily temperatures, especially in summer. The hydraulic model suggested that a relevant increase in dry riverbed areas will occur. Overall, these results provide insight into the global change factors that could favour P. maculata spread in the river and the reduction of suitable habitat for M. tricarinata and will be useful for future decisions of water discharge management.
Response to Reviewers:	Point-by-point responses to the comments Editor Based on the advice received, we feel that your manuscript could be reconsidered for publication should you be prepared to incorporate major revisions. When preparing your revised manuscript, you are asked to carefully consider the reviewer comments

which are attached, and submit a list of point-by-point responses to the comments. Please also highlighted the revisions using line numbers or color text. In particular, it is very important to set the study standards (objectives, hypotheses etc..) in a firmer footing. Conclusions must match to the data presented and no way much further-reaching than the work conducted.
We have clarified the objectives and predictions and we have changed the discussion to avoid broad interpretations or statements not tested in this study.

Reviewer #1: Line numbers belong to the pdf file AECO-D-18-00108

Tittle
Responses of invasive and native snails within the context of global change in a recently invaded river The tittle perhaps promises more that the study gives. For instance, it suggests that the study involves more than one snail of each type and that the responses to changes and interactions were already observed..
Suggestions:
Potential responses of a native freshwater snail to global environmental changes and to the expansion of a recent invaded Potential responses of a native snail to global environmental changes and the expansion of a recent invaded in the Ebro river

The new title is: Responses of a native and a recent invader snail to warming and dry conditions: the case of the lower Ebro River
We have changed the title according the comments of the reviewer but the final proposal is a little bit different. As the reviewer suggest, we have indicated in the title that only two snail species were included in the study. Including warming and dry conditions, we also focus in these two factors and avoid misinterpretation with species interactions. We consider that the study shows responses and not potential responses to water temperature and desiccation because we have eliminated all parts in the manuscript that refer to potential competition between both species.

Introduction
Especially at the end of this section (lines 100-130) it necessary to do some work regarding objectives, hypothesis and predictions and also the possible applications of the results obtained and conclusions reached. Some sentences are written in a way that seems more adequate for the Results section or for the Abstract (e.g.: The response of both species to two abiotic factors that are related to climate change, water warming and drought, were also compared under laboratory conditions. In addition, the potential increase in dry riverbed area, where P. maculata could potentially resist better the dry conditions in this habitat, was estimated.) There are also some repetitive statements (e. g.: "This type of research could provide the basic framework for the 108 design and implementation of appropriate management plans for the control and eradication of 109 the invasive species"(lines 107-109) and "if recognised by water resources management and governance, the findings could be used to prevent their spread and associated negative ecosystem impacts"(lines 126-127).
The potential applications of the results should wait until the results are presented (i.e. is probably better to me move them to the Discussion section).

We have changed part of this paragraph clarifying objectives and reducing repetitions (Lines 111-121). Last paragraph related to the applications of the results has been moved to the end of the discussion (L 412-15).

Specific comments
47: Evidence that climate change affects biodiversity at the global scale is now unequivocal (e.g., Parmesan & Yohe, 48 2003; Walther, 2010)… These two references seem a little out-dated as to say "now".
We have eliminated “now” in the text and changed Parmesan & Yohe, 2003 by a more recent and general reference: Bellard et al 2012
Bellard, C., Bertelsmeier, C.; Leadley,P., Thuiller, W. & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. Ecology Letters 15: 365–377

57: The species Pomacea maculata Perry, 1810, is a generalist phytophagous gastropod native to South America that has already invaded North America, East and

SE Asia and has recently jumped to southern Europe, first recorded in the continent at 40° N, the northernmost latitude thus far reached by the species (López et al., 2010). This is a too long and awkward sentence. Split it, please.
Done. The text is now: The species Pomacea maculata Perry, 1810, is a generalist phytophagous gastropod native to South America that has already invaded North America, East and SE Asia. It has recently jumped to southern Europe, the first record in the continent at 40° N, the northernmost latitude thus far reached by the species (López et al., 2010).

62 The abiotic factors that determine the successful establishment of P. maculata include temperature, salinity, food availability and desiccation tolerance… "Desiccation tolerance" is not a biotic factor as the sentence implies, it is a trait of the organism. Maybe "desiccation risk" is better.
We have changed the text to reduce references and text: The invasiveness is related to its adaptability to harsh environmental conditions, its ability to invade diverse habitats, its high reproductive rate and voracious appetite and an ability to compete with other native snails and native fauna (Joshi, 2007).

66 The presence of a gill and a lung in Pomacea snails reduces their dependence on dissolved oxygen and thus increases survival in habitats with low humidity
Suggestion: The presence of an aerial lung in addition to a gill in Pomacea snails reduces their dependence on dissolved oxygen and thus increases survival in habitats with low humidity
Done

73 It has been predicted that it could establish in other European wetlands (Gilioli et al., 2017).
On what grounds? Please explain.

The sentence has been extended:
It has been predicted that it could establish in other European wetlands (Gilioli et al., 2017) based on an environmental risk assessment model that describes the area of potential establishment of the apple snails in Europe and predicts their distribution and abundance as a function of spatial variation in the environmental forcing variables.

75 upriver
Upstream?
Ebro is a river more than a stream. Upriver is accepted for the meaning we use and it was suggested by the English grammar corrector service. However, we can change if the editor considers upstream more appropriate.

91 The spread of this non-native snail potentially affects the local biodiversity of the region
Suggestion: The spread of this non-native snail will probably threaten the local biodiversity of the region,
Done
96 This species lives mainly on stones but also in soft sediments. As P. maculata, M. tricarinata disposes of its thick shell, and its corneous operculum closes in response to desiccation or disturbances… What else is known about Melanopsis tricarinata and Pomacea maculata? Besides competition, predation and interference may be significant when Pomacea snails interact with other snails. It has been shown that predation by apple snails on other snails depends on their size, shell thickness and the presence of an operculum but also on their reproductive mode (e.g.: oviparous or oviviparous, gelatinous egg masses or single eggs, etc.). Please provide information about reproductive mode of both species, size at hatching or birth and adult size.

We have widened the description of reproduction, temperature tolerance and size for Melanopsis (lines 99-103) although information for the species is limited. Characteristic for P. maculata varies greatly on the environmental characteristics because of its wide dispersion, for it we include at the introduction (L 63-65) only the most common features and at the discussion we compare the results found in the Ebro with others environments.

97 As P. maculata, M. tricarinata disposes of its thick shell l, and its corneous

operculum closes in response to desiccation or disturbances
Suggestion: As P. maculata, M. tricarinata possess of a thick shell and a corneous operculum that closes in response to desiccation or disturbances
Done the change suggested.
111 The response of both species to two abiotic factors that are related to climate change, water warming and drought, were also compared under laboratory conditions.
The abiotic factor is water temperature. Water warming is the consequence of climate change. Suggestion: The response of both species to two abiotic factors that are related to climate change, water temperature and drought frequency or intensity, were also compared under laboratory conditions.
Thanks for this comment. We have changed the objectives description and now the text is:
In this context, our objectives were: i) to analyse the diets of the native and invasive snail species to determine the potential overlap in resource use if they meet; ii) to analyse the response of both species to water warming and dry conditions; and iii) to provide projections of river water temperature and of the increase in dry riverbed area in order to evaluate the habitat suitability for the two species under future scenarios in the studied area.

117 We hypothesized that the P. maculata diet would be based on macrophytes because of previous reports on this species (Burlakova et al., 2009) and that its food overlap with the native snail (which has a diet based on algae and detritus) would be limited. However, we also hypothesized that the physiological traits of the apple snail would permit it to survive better than the native species in the event of warming and dryness.
I believe that these are predictions and not hypothesis or at least they are not clearly stated.

WE have changed part of this paragraph clarifying objectives, reducing repetitions and we have change hypothesis by predictions, as the reviewer suggested (L 11-121).

Methodology
174 For the stable isotope analysis, the muscular foot of six individuals of similar size of each species were removed.
Where the six samples pooled and treated thereafter as one? Or they were analysed separately?
Yes, the muscular foot was analysed separately for each animal. We have included it in the text (line 157).

198 Forty-five individuals of both M. tricarinata (17.88±2.1 mm, shell height) and P. maculata (11.86±1.72 mm shell height) were collected.
These sizes indicate that all the P. maculata snails were probably juveniles. But, what about the M. tricarinata specimens? The date of collection (not given) is relevant for estimations of metabolic rate if at least some individuals were sexually mature, especially since the females of M. tricarinata could bear embryos. The juvenile or sexually mature status is also relevant to relative growth estimations, as adults are not expected to grow as fast as juveniles.

The maximum size described for Melanopsis is 47 mm, that means that the individuals used likely are juveniles too. The experiment compare the growth rate between the same population (same size, observe the low SD of the average for both species) of snails at control and experimental conditions for each species separately. Then, the growth rate is related to the differences in the abiotic condition not to the individual size. In addition, we compare the responses for each species separately.

201 Fifteen individuals from each species for each treatment were individually allocated to glass microcosms (8.5 cm diameter and 9 cm height) with 250 ml of dechlorinated water.
250 ml/15 snails= 16.66 ml/snail. This seems too little water for each snail. Although water was changed quite often the snails were probably crowded, especially in the case of the native snail, whose individual were bigger on average.

These 15 individuals were individually allocated to glass mesocosms (Line 184). It was already in the text.

224 Metabolism
Metabolism is a too broad and complex concept. Should be replaced here and thereafter by metabolic rate or, even better, by oxygen consumption.
Done, we have changed by oxygen consumption or oxygen consumption rate along the text.

225 Metabolism was measured as the oxygen consumption corrected by individual dry weight
Suggestion: Metabolic rate was measured as the oxygen consumption relative to individual dry weight
Done

241 The effects on metabolism in both experiments were analysed by two-way ANOVA (temperature and time as factors) after checking for normality and homogeneity of variance.
Normality and homoscedasticity were OK?
Yes, if these characteristics were not accounted we had not used a parametric test.

Results
293 Diet analyses: Based on the gut content analysis, the diet of P. maculata consists of 59 % algae and vegetal material, 39 % detritus and 0.49 % fungi. The food items for M. tricarinata were 64 % detritus, 36 % algae and other vegetal material, and 0.02 % fungi. The SIAR model based on stable isotopes showed that the most important carbon source for the two snails was Cladophora (90 % for P. maculata and 98 % for M. tricarinata), while the other sources did not provide a significant contribution of carbon (Table 2).
I believe that these results are not properly shown and could be analysed with greater detail. For instance, is not clear if the data of each snail were pooled together (as I guess in view of the lack of error measures) or were analysed as replicates (a much better option). Some index of resource use overlap could be calculated for the gut content and isotopic data. The overlap could be shown graphically trough a bidimensional plot of all gut contents, perhaps after a principal components analysis. If the stable isotopes were determined individually for the six snails of each species the degree of overlap could also be plotted in a similar way.

According to the suggestions from the reviewer 3 we have eliminated the section of the gut content to avoid confusion between food availability (or ingested) and assimilated food.
Dietary proportions of assimilated food items were determined using 'SIAR', an R package that solves mixing models for isotopic data within a Bayesian framework [Parnell et al 2010]. SIAR estimates probability distributions of resource contributions to a primary consumer diet by accounting for variation and uncertainties in the input data. As mentioned before the snails were analysed separately. These data were included in the model (input data) which output is the proportion of the different sources within a 95% of confidence (data in Table 2) for all the individuals (snails) included. The model use the replicates (individual samples) to calculate the probability distribution of each source in the diet of the consumer.
We cannot obtain results of the diet for each individual separately using stable isotopic analyses and SIAR.

Discussion
349 Our study shows that P. maculata and M. tricarinata of the lower Ebro River share the same food preferences To estimate preferences some estimation of resource availability is needed.
Yes , the reviewer observation is correct. We have changed the sentence:
Our study shows that P. maculata and M. tricarinata of the lower Ebro River feed on the same food sources according to the Carbon assimilation…

410-422 A decrease in precipitation for the Ebro Basin is projected, especially ….. is present.
The changes predicted will increase the areas where dessication risk would be a relevant ecological factor, not the suitable habitats for P. maculata. The fact that this will probably reduce the suitable habitat for M. tricarinata is not stressed here.

We have included a sentence at the end of the paragraph to stress the additional effects on Melanopsis:. At the same time, these changes may also reduce the suitable habitat for M. tricarinata.

Tables and figures
635 Table 2. Results of the SIAR model for the diet of M. tricarinata and P. maculata. Values represent the 95 % confidence intervals for each basal resource (in percentage).
Suggestion: Table 2. Results of the SIAR model for the diet of M. tricarinata and P. maculata. Values represent the limits of 95 % confidence intervals for each basal resource (in percentage).
Done

657 Figure 3: Daily mean river water temperature (RWT) modelled for the lower Ebro Basin for the selected climate model ensemble for the reference periods 1981 - 2010 (grey), RCP 4.5 (2036 -2065, blue) and RCP 8.5 (2036 - 2065, red). Horizontal black lines refer to the two temperatures tested in the experiments. DOY: day of year.
According to the caption only the means of RWT are plotted for three different scenarios, so there should be three lines. However, there are at least seven different lines (probably nine, but the image quality is not good in the pdf version). Perhaps the minimum and maximum temperatures were also plotted. Besides, the inside legend is very small and cryptic.
Yes, each group of lines represents the mean, maximum and minimum values of water temperature. We have included it in the figure legend.

References
There are six pages of references in the 30 pages of the ms. However, the references could be a little more updated and focused on snail-snail interactions.

References have been reduced (from 64 to 49) and updated.

Reviewer #3: The manuscript was well written, with clear methods and easily interpretable results. While I appreciate the authors' focus on water system management in the context of protecting native biodiversity, and limiting invasive species spread, this work only looks at individual species' response to two specific parameters (drought, and increased water temperature). Because these two species do not currently overlap, and there is no available information on how they each perform in the presence of the other (i.e. competition), the authors may be best advised to move the focus away from invasive species, and instead focus on changing climate. Alternatively, if time and resources are available, a couple of additional experiments specifically focusing on competition between these two species would greatly strengthen the argument that P. maculata is, in fact, a problematic invader, that could negatively impact M. tricarinata. Do the authors have pre-invasion community data? If P. maculata is a strong competitor, one might expect to see changes in the community structure (including nutrient cycling) that can be directly linked to the presence of the non-native species (as compared to changes at other sites where P. maculata is not found). It would also be helpful to know if these two species differ in their ability to assimilate resources. If the native snail is more efficient at processing nutrients, there may be less of an effect if resources are reduced by the non-native snail. It would also be helpful to know what P. maculata's salt tolerance is, since M. tricarinata is in a reach with high saline content, P. maculata is not. While P. maculata may be more tolerant to drought and increased temperature, if M. tricarinata is better adapted to higher salt loads, it may decrease potential competition. An additional set of experiments could clarify this, as well. My main suggestion is that the authors either focus on water system management in the context of overall ecosystem health (potentially focusing on maintaining the population of M. tricarinata, irrespective of P. maculata), or conduct a couple of addition experiments establishing that P. maculata has the potential to negatively compete with M. tricarinata. In its current form, this manuscript is drawing broad-reaching conclusions from a very narrow set of parameters.

Thank you for your positive comments and suggestions. Unfortunately, we cannot do additional experiments to test the real competition of these two species. We thank the suggestion and we try to get fund to continue studying the potential effects of P. maculata in this river and on the other native snails and grazers.
We have eliminated all parts in the manuscript that refer to potential competition between both species and have described the results of the experiments separately for both species.
The results of the scenarios have been related to both species favoring the spread of the non-native and reducing habitat for the native species.
We have changed the title and the abstract to be more focused on the objectives of the paper. We have clarified the objectives and predictions and we have changed the discussion to avoid broad interpretations or statements not tested in this study.
Available information of M. tricarinata is really limited and most of the details the reviewer required are not in the bibliography. There is no data about the efficiency of the nutrient assimilation of this species. However, we have included information about size, habitat and feeding.
P. maculata is located downstream (site 2) the reach with M. tricarinata (site 1). Conductivity, TDS, Cl- , SO4-, NO3- concentrations (Table 1) were higher in site 2 where P. maculata is present. That means that P. maculata is living in the site with higher conductivities/salinities than the native species. It seems a misinterpretation of the data in table 2 by the reviewer.
Pomacea can tolerate a wide range of conductivities (Joshie, 2007) and the same is observed in the Ebro where this snail is present in rice fields, irrigation channels and river. However, the range for Melanopsis tricarinata is likely narrower although not tested or described yet.

Line comments:

60: The reference of a "closely related-species,…" means very little, unless there is documentation that they perform similarly, and that both have negatively impacted non-native habitats. If there is strong evidence that P. maculata causes measurable damage in any of the invaded range, the authors should describe, and cite.
We have eliminated “closely related species” by a new text with a more recent reference: The freshwater Rice Apple Snail (Pomacea spp) is considered one of the 100 worst invasive species in the world (GISD, 2017),…

63: The authors should more specifically describe the parameters that P. maculata can tolerate, particularly since they later describe a difference in salinity between the sites where P. maculata and M. tricarinata were collected.
We have changed the text and eliminate that referent to abiotic factors. In this part we have described the abilities for invading new habitats of P. maculata. Later we have describe the tolerance to environmental parameters. We think this new text distribution improves the information about this snail.
The text is now: The invasiveness is related to its adaptability to harsh environmental conditions, its ability to invade diverse habitats, its high reproductive rate and voracious appetite and an ability to compete with other native snails and native fauna (Joshi, 2007). The presence of an aerial lung in addition to a gill in Pomacea snails reduces their dependence on dissolved oxygen and thus increases survival in habitats with low humidity (Seuffert & Martin, 2009 a, b). In addition, P. maculata is pre-adapted to periodic dryness, an evolutionary life trait that may allow it to take advantage of unpredictable environments such as floodplains (Burlakova et al., 2009).

101: The authors need to define the common name "apple snail". Context here suggests that they are referring to P. maculata, but is less clear elsewhere in the manuscript.
We have reviewed the use of apple snail along the text. Now, we only use this term to refer at the group Pomacea spp, especially when we use references from bibliography that refers to P. maculata but also to other species of the group.

125: The first appearance of the non-native snail was over a decade ago, so why are the authors only discussing "potential damage to native diversity."? If pre-invasion survey information exists, it should be compared to more recent data. While P.

maculata may not currently overlap with M. tricarinata, they certainly interact with other native grazers, and may negatively impact them.
We have eliminated this paragraph from the introduction. There is no data of interaction of P. maculata and other grazers in the river. The studies in this area have been focused on rice fields where the snail is affecting rice production with important economic consequences (Joshi R.C. & Vélez-Parera, X., 2017).

158: Were any of the "potential food resources" actually found associated with animals collected, or are they just assumed?

We wrote potential because we collected all the resources present in the river that the snail could feed on: biofilm, detritus from different places (on fine sediment, accumulated between stones, on river side, in the middle…), and macrophytes. A priori we did not know which of the resources would be used by the snail. We only had the information from the bibliographic references.

165: Did you collect these animals from a variety of microhabitats (rocks, sand, edge habitat, etc…)? If so, did you document specific details associated with each animal used for the diet analysis? This is important, because if all of the animals used for the gut content portion were collected from the same general spot, it could result in biased diet results.
Yes, we collected the animals from different habitats in the river, where the snails were observed. However, according to the next comment, we have eliminated the section of the gut content analysis.

293-299: Are these diet percentages similar to the distribution of resources in the stream? These values may be a stronger representation of what's available, rather than what the animals prefer. Since you did not directly address food preference, you should avoid conflating observed diet and preferred food resources.
According to this comment we have eliminated the section of gut content analysis. We think that it is more important to show the preferred food, that is the sources where C is assimilated.

350: As previously mentioned, you did not explore preference, only observed diet.
Yes, the reviewer observation is correct. We have changed the sentence:
Our study shows that P. maculata and M. tricarinata of the lower Ebro River feed on the same food sources according to the Carbon assimilation… (L 329-30).

358-360: Do you have data on primary production in this stream, or general information on the amount of biomass consumed by P. maculata? If this is a particularly productive stream, it may be able to support both of these species (keeping in mind that primary productivity may increase or decrease with warming temperatures…). It would also be helpful to know how they interact with each other. You seem to be making the assumption that if they co-occur, it will negatively impact the native snail, but it entirely possible that the opposite is true. A simple set of competition experiments would help address this question. If existing data or time are unavailable, authors should be wary of drawing any conclusions about the impact the non-native snail would have on the native snail.
It is impossible to do an extra experiment to test the real competition between both species and maintaining the data of our current experiments. For this, we have eliminated the text related to competition. We only refer to the changes in the habitat in future scenarios that could favor the establishment and spread of the non-native snail and at the same time the reduction of habitat suitability for the native snail.

369: Reference to "invasive apple snail", but it is unclear whether this is referring to P. maculata, or the previously mentioned species. Please clarify.
In this case, we have included in brackets: (Pomacea spp.) to clarify that the data is referred to different spp in the group of the apple snails. In the rest of the text, its use has been revised.

371-376: This is only one specific case of competition (different habitat, different species), with no specific information on how the non-native reduced growth rate of native (food competition, increased overall animal density, etc..), so authors should be

	very cautious about using it to support any conclusions about how M. tricarinata would respond. Again, the addition of simple competition experiments would strengthen this argument. We agree with the reviewer about we have to be cautious in this conclusion. We have change the text and now is: It is difficult to ensure that M. tricarinata may be susceptible to interspecific food competition with P. maculata in the lower Ebro because our experiment did not test this statement but the described dietary flexibility and voracious behaviour of the different apple snail species (Lach et al., 2000) could suggest this hypothesis (L353-56). 423: Broad discussion of harm on "diverse ecosystem", but the authors only ever discuss interactions with other snails. If there is data addressing negative effects to other species, the authors should include them, otherwise, they should focus on the species addressed in the paper. 426-427: "…conservation of the native diversity and the avoidance of additional ecological impacts." It is unclear what the authors are driving at, here. If there is additional information regarding the broader-reaching negative impacts of P. maculata, the authors need to discuss them. For both comments: We have change the final text. Now, we only refer to the deleterious effects of this apple snail group on different ecosystem services highlighted by the cited references. We have eliminated broad statements like native diversity and additional ecological impacts (lines 401-15). 437: This paper does not adequately establish that P. maculata is a truly "harmful species", so the authors should use caution with this wording. Not all non-native species are problematic, and not all problematic species rise to the established definition of an invasive species (causing economic or ecological harm). It is true that this paper do not stablish harm effects of this species in the river but the effects of P. maculata in the Ebro river wetland and rice fields is extensively documented. Several studies alarm about the risk of the spread of this species in Europe and the consequently effects on ecosystem services provided by these wetlands, mainly in the South of Europe. Of course, non all the non-native species in stablished as invasive species but Pomacea maculata has “everything to gain”. New references included Joshi R.C. (2007). Problems with the management of the golden apple snail Pomacea canaliculata: an important exotic pest of rice in Asia. In: Vreysen M.J.B., Robinson, A.S., Hendrichs, J. (Eds), Area-wide control of insect pests (pp. 257-264). Springer, Netherlands Joshi R.C. & Vélez-Parera, X. (2017). The rice apple snail in Spain: a review. Focus on agriculture, March/April, 106-108. Retrieved from: www.international-pest-control.com
Author Comments:	We prefer the first option: free online colour. Thank you. This manuscript has been been revised and edited for English language by native experts (American Journal Experts, certificate verification: 5857-EC9E-1BFD-BBE1-ACCC).

Responses of a native and a recent invader snail to warming and dry conditions: the case of the lower Ebro River

Running head: Invasive and native snail responses to global change

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Abstract

Aquatic habitats have been highly modified by human actions that reduce their native diversity and create conditions suitable for tolerant alien species. *Pomacea maculata* was detected in 2009 in both the alluvial plain and the final stretch of the Ebro River. Since then, a permanent population has stabilized in the littoral area of the river where the water level fluctuates according to the river discharge. *Melanopsis tricarinata* is an endemic snail species highly affected by the reduction of its natural habitat. Currently, the two species do not share the same reaches in the river but the possibility exists, as the distribution of the *P. maculata* is constantly increasing. This study aims to analyse the diets and to assess the responses of both snails to global change. The diet of both species was analysed in the field and their responses to water warming and dryness compared under laboratory conditions. This study includes the calculation of future river water temperatures based on air temperature projections. In addition, based on water discharge management scenarios, the study estimated the increase in dry riverbed area. The diet of both snail species was similar and based on *Cladophora*. *P. maculata* better resisted high temperatures and dry conditions than *M. tricarinata*. The projections of water temperatures showed an increase in daily temperatures, especially in summer. The hydraulic model suggested that a relevant increase in dry riverbed areas will occur. Overall, these results provide insight into the global change factors that could favour *P. maculata* spread in the river and the reduction of suitable habitat for *M. tricarinata* and will be useful for future decisions of water discharge management.

Key Words: *Pomacea maculata*, *Melanopsis tricarinata*, climate change, apple snail, stable isotopes, temperature, river, discharge management.

INTRODUCTION

Rivers and streams are highly sensitive to climate change not only because of shifts in the thermal regime or runoff levels but also because of other increasingly confounding or complex environmental pressures (Ormerod et al., 2010). Evidence that climate change affects biodiversity at the global scale is unequivocal (e.g. Walther, 2010, Bellard et al., 2012) and shows that changes affect organism physiology and may alter the distribution and ranges of species. In addition, most of the world's aquatic habitats have been highly modified by human activities, which reduce native diversity and create conditions that are suitable for the spread of tolerant non-native species. In this way, non-native species invasions are among the primary leading causes of biodiversity loss worldwide, especially in freshwater ecosystems (Dudgeon et al., 2006). Some invasive species cause decline or extinction of native species through predation or competition and may also affect communities and ecosystems by, among other impacts, changing inter-species interactions or altering nutrient cycling (Sampaio & Rodil, 2014; Fausch & García-Berthou, 2013).

The species *Pomacea maculata* Perry, 1810, is a generalist phytophagous gastropod native to South America that has already invaded North America, East and SE Asia. It has recently jumped to southern Europe, the first record in the continent at 40° N, the northernmost latitude thus far reached by the species (López et al., 2010). The freshwater Rice Apple Snail (*Pomacea* spp) is considered one of the 100 worst invasive species in the world (GISD, 2017), posing risks to crops and human and wildlife health (Carlson et al, 2017). The invasiveness is related to its adaptability to harsh environmental conditions, its ability to invade diverse habitats, its high reproductive rate and voracious appetite and an ability to compete with other native snails and native fauna (Joshi, 2007). The presence of an aerial lung in addition to a gill in *Pomacea* snails reduces their dependence on dissolved oxygen and thus increases survival in habitats with low humidity (Seuffert & Martin, 2010). In addition, *P. maculata* is pre-adapted to periodic dryness, an evolutionary life trait that may allow it to take advantage of unpredictable environments such as floodplains (Burlakova et al., 2009). *Pomacea maculata* was detected in 2009 (specimens matched with its morphological variability and mitochondrial DNA variation, Lopez et al., 2010) in both the alluvial plain and the final stretch of the Ebro River (NE Spain), the largest Iberian river flowing into the Mediterranean. It has been predicted that it could establish in other European wetlands (Gilioli et al., 2017) based on an environmental risk assessment model that describes the area of potential establishment of the apple snails in Europe and predicts their distribution and abundance as a function of spatial variation in the environmental forcing variables. Since the initial colonization, a permanent population has been established in the river from its mouth to 20 km upriver (Joshi & Vélez-Parera, 2017), with specimens mainly located in the littoral area where the water level fluctuates according to the river discharge. The lower course of the river (110 km) is highly regulated by dams, and its flow is managed according to the water demands of the basin, which are mainly irrigation and hydropower needs.

Climate change scenarios project an increase in the frequency and magnitude of extreme events such as floods and droughts (IPCC 2013, Dankers & Feyen, 2008). The Mediterranean region is particularly sensitive to these changes. Longer and more severe droughts exacerbate the periodic occurrence of low-flow or even no-flow periods in permanent and temporary rivers. High water stress is already a common characteristic of the region (Gallart & Llorens, 2004, Skoulikidis et al., 2009) because of changes in climate, land use and water abstraction and regulation (Barceló & Sabater, 2010). Human responses to climate change are an additional major source of ecological change, via habitat degradation or the alteration of the flow regime. Non-native species that are tolerant to dryness or flow intermittency could benefit from these new conditions, which may increase the probability of their survival and allow them to invade regions that sometimes have or develop into more favourable conditions than their natural habitat (Richardson et al., 2000).

The spread of this non-native snail will probably threaten the local biodiversity of the region, including native snail species such as *Melanopsis tricarinata* (Bruguière, 1789), an endemic snail of the Iberian Peninsula. This species is sensitive to strong anthropomorphic pressures (pollution and flow reduction) and is especially affected by the reduction of its natural habitat (Martínez-Ortí & Robles, 2003). It is classified as a Least Concern species in the IUCN Red List but as an Extinction Risk species in the Catalonian region. This species lives mainly on stones but also in soft sediments. Its maximum size is 47 mm, eggs are laid individually or in clutches and parthenogenesis has been described. It can feed on algae and organic matter and as P. *maculata*, *M. tricarinata* possess of a thick shell and a corneous operculum that closes in response to desiccation or disturbances. Temperature range tolerance is between 13 and 27 ºC (Martínez-Ortí & Robles, 2008).

Currently, the two species do not share the same reaches in the river or the springs of the Ebro delta, but the possibility exists, as the distribution of the *P. maculata* is constantly increasing. In the lower Ebro, the native gastropod lives approximately 6 km upriver of the section of the river that is currently invaded by *P. maculata*. It is frequently emphasized that one of the most valuable types of research that can be conducted when an invasion occurs is the assessment of potential damage that the invasive species may cause to the native species, particularly if the spread of the invasive species is likely to occur in the future (Fausch & García-Berthou, 2013). In this context, our objectives were: i) to analyse the diets of the native and invasive snail species to determine the potential overlap in resource use if they meet; ii) to analyse the response of both species to water warming and dry conditions; and iii) to provide projections of river water temperature and of the increase in dry riverbed area in order to evaluate the habitat suitability for the two species under future scenarios in the studied area. We predicted that the *P. maculata* diet would be based on macrophytes because of previous reports on this species (Burlakova et al., 2009), and that its food overlap with the native snail (which has a diet based on algae and detritus) would be limited. We also predicted that the physiological traits of *P. maculata* would permit it to survive better than the native species in the event of warming and dryness. Future scenarios of higher river water temperatures and reduction of discharge downstream from dams would provide niche opportunities for the non-native snail to spread.

METHODOLOGY

Study area

The Ebro Basin in the NE of the Iberian Peninsula covers 85,362 km^2. The human population in the basin is approximately 3 million, with a density of 33 inhabitants km^{-2}. The main land use is agriculture, with more than 1 million hectares under irrigation (90% of the water usage in the basin). The main stem of the Ebro is 910 km long and has been strongly affected by the construction of reservoirs. The most important reservoirs are in the lower part of the catchment and regulate water discharge downstream (the minimum discharge has been 80 $m^3 s^{-1}$ since 2014). The average water temperature in the studied reach was 18.2±6.1 °C, with a maximum temperature of 29.2°C in July and a minimum temperature of 6.8°C in January (Tortosa gauging station, data from 2002 to 2017, Confederación Hidrográfica del Ebro, 2017).

Field sampling and laboratory analysis

A permanent population of *Pomacea maculata* is established at site 1 (40° 42' N, 0° 38' E), which is 25 km upstream of the river mouth and the site where we collected the invasive animals. Individuals get into the river through the outflow of an irrigation channel from the delta. The apple snail has also been seen occasionally 15 km upstream from site 1 (40° 47' N, 0° 30' E). The maximum range of *Melanopsis tricarinata* is from 3 km to 16 km upstream of the city of Tortosa. The native snails were collected at site 2 (40° 55'N, 0° 29' E), 56 km from the river mouth (Figure 1).

The snails were collected by hand from both sites during spring 2015 when their abundances were relatively high. Dissolved oxygen, temperature, pH, conductivity and total dissolved solids were measured in situ with a portable multiparameter (YSI Pro Plus, Yellow Springs, USA). Water samples were collected and immediately filtered through 0.7 µm glass fibre filters (Whatman GF/F, Kent, UK). Samples were kept frozen until their analysis on a 761 compact ion chromatograph (Metrohm, Herisau, Switzerland).

Three to five replicates of each potential food resource for the snails were collected at each site. The fine particulate organic matter (FPOM, superficial layer) was sampled with a small core. The epilithic biofilm was collected from cobbles taken directly from the streambed. *Cladophora* sp. and macrophyte (*Myriophyllum* sp) samples were collected by hand.

In the laboratory, the shell height (distance between the tip of the apex and the edge of the bottom lip, in mm) of all snails collected was measured with a digital calliper.

Stable isotopes

For the stable isotope analysis, the muscular foot of six individuals of similar size of each species were removed and analysed separately. Based on the presence of resource at the sites, we collected FPOM, biofilm and *Cladophora* to represent the basal resources of both snail species. *Myriophyllum* was present only at the site where *P. maculata* was present. All materials were dried at 60°C to constant weight and ground using a mortar and pestle to ensure homogeneity, and 0.5-3 mg of each material was placed in tin capsules. The carbon (C) and nitrogen (N) contents and stable isotopes of C and N were analysed on a Flash 1112 elemental analyser connected to a Delta C isotopic ratio mass spectrometer with a Conflo III interphase (Thermo Scientific, Inc., Spain). Stable isotope data are expressed as the relative difference between the ratios (*R*) of samples and standards (Pee Dee Belemnite limestone for δ^{13}C and atmospheric nitrogen for δ^{15}N) [i.e., $\delta^{15}N=(R_{sample}/R_{standard})$ - 1) x 1000]. The analytical precision of multiple runs was 0.1 ‰ for δ^{13}C and 0.2 ‰ for δ^{15}N.

The δ^{13}C values were corrected to avoid the effects of high lipid levels, as indicated by high C:N ratios, according to the equations of Post et al. (2007).

Experimental setup

To evaluate the effect on the snails of temperature and dryness on the snails, *P. maculata* and *M. tricarinata* individuals were collected in the field and maintained in aquaria under laboratory conditions for a week. The water temperature in the aquaria was 20 °C (average temperature of the river reach during the spring) under a 12 h light photoperiod. The snails exposed to higher temperatures (see below) were acclimated during this week (1.5°C increase every 2 days). Fresh lettuce and supplementary fish food were provided *ad libitum*, and additional calcium was added. Dechlorinated water was used in the aquaria and was changed every two days.

Experiment 1: Temperature

Forty-five individuals of both *M. tricarinata* (17.88±2.1 mm, shell height) and *P. maculata* (11.86±1.72 mm shell height) were collected. Fifteen individuals of each species were dried without the shell at 70 °C until constant mass to calculate their initial dry weights. The effects of two controlled temperatures, 20 °C and 25 °C, were tested on the living snails. Fifteen individuals from each species for each treatment were individually allocated to glass microcosms (8.5 cm diameter and 9 cm height) with 250 ml of dechlorinated water. Water from the microcosms was oxygenated throughout the experiment and was replaced three times a week. The snails were fed a pre-weighed quantity of lettuce and fish food at each water change. The water temperature and conductivity (WTW electrode) and levels of nitrate, nitrite, ammonium and pH (colorimetric kit, Tera test) were measured twice a week.

At the end of the experiment (15 days), individuals were freeze-dried and weighed (without their shells).

Experiment 2: Simulation of dry conditions

Twelve aquaria were filled with pre-combusted wet sand (grain size 0.25-0.5 mm, 60% of water content at the beginning of the experiment). Fifteen individuals of each species (same shell size than experiment 1) were placed in the aquaria and distributed in two temperature treatments (20°C and 25°C, 3 aquaria per temperature and species). The survival rate was recorded after 7 and 15 days for *M. tricarinata* and after 7, 15, 30 and 60 days for *P. maculata*. Survival was evaluated by placing individuals in water for 24 hours and observing any movement (Wada & Matsukura, 2007).

End points

Snail growth

The relative snail growth rate (RGR) was estimated in experiment 1 (effects of temperature) as RGR=DWf-DWi / (DW x day), where DWf and DWi are the final and initial dry weights, DW is the mean weight of all the individuals between the start and the end of the test, and day is the number of days the test lasted.

Oxygen consumption rate

Oxygen consumption rate was measured as the oxygen consumption relative to individual dry weight (mg O_2 L^{-1} g PS^{-1} min^{-1}). Measurements were made with an optical oxygen microsensor adapted to a 20 ml glass vial (Fibox 4 PreSens, Regensburg, Germany) filled with the oxygen-saturated water into which the snail had been placed. The oxygen concentration was recorded every 5 seconds for 10 minutes. In experiment 1, it was measured at the beginning and end of the test (1 and 15 days, 4 individuals per treatment and species). For the second experiment, the measurements were made at the end of the test (3 *P. maculata* individuals and the two temperature treatments).

Experimental data analysis

Dietary proportions were determined using SIAR, an R package that solves mixed models for isotopic data in a Bayesian framework (Parnell *et al.* 2010). SIAR estimates the probability distributions of resource contributions to a primary consumer diet by accounting for variations and uncertainties in the input data. The trophic enrichment factor (TEF) used was 1.3 (±0.3) ‰ for $\delta^{13}C$ and 2.2 (±0.3) ‰ for $\delta^{15}N$ (McCutchan et al., 2003).

The effect of temperature on the growth rate of the snails was analysed by one-way ANOVA. The effects on oxygen consumption rate in both experiments were analysed by two-way ANOVA (temperature and time as factors) after checking for normality and homogeneity of variance. A post hoc Tukey test was applied when significant differences were found. These analyses were performed with IBM-SPSS statistics 22 for Windows (SPSS Inc., Chicago, USA).

Prediction of dry bed areas in the lower Ebro River

The riverbed area that will potentially dry out was estimated using the HEC-RAS hydraulic model (v. 2.2, US Army Corps of Engineers, USA), an integrated system of software that performs one-dimensional, steady flow water surface profile computations. The model was used to calculate the loss of the river water surface area for a given scenario of flow reduction, which we assumed was equivalent to the increase in the riverbed area exposed to the atmosphere. For the model geometric data, a high-resolution dataset on river morphology was used which was provided by the Ebro River authorities (Confederación Hidrográfica del Ebro, Spain). The dataset consists of cross-sectional profiles every ca. 50 m along the course of the lower Ebro. Each cross-section has a resolution of less than 3 m. The model was run under steady flow conditions. On the first step, the wet perimeter of each cross-section was estimated for the different discharge values, which were chosen based on recent past, current, and potential future flow regulation plans. Then, for each pair of cross-sections, the area of the polygon delimited by the distance between the cross-sections and the difference in the wet perimeter for the given flow reduction scenario were calculated. Although the lower Ebro has some pronounced meanders, the defined polygons seldom presented curvature due the proximity between the consecutive cross-sections (ca 50 m). Therefore, to simplify the calculations, the area of the polygons was calculated as that of a scalene trapezoid (i.e., the arithmetic mean of the bases multiplied by the height).

River water temperature under future climate

The regression approach presented by van Vliet et al. (2011) and adapted to the Ebro Basin by Vigiak et al. (2017) was applied to estimate the river water temperature (RWT) for the lower Ebro. To evaluate the habitat suitability of the area under a future climate scenario for the two species, the calculation of RWT was based on air temperature projections for the 2050 horizon, i.e., the period between 2036 and 2065. Projections for the future climate were made available through the EURO-CORDEX initiative (Kotlarski et al., 2014). These simulations were generated with different regional climate models (RCMs) that dynamically downscale various general circulation models (GCMs) at different representative concentration pathways (RCPs 4.5 and 8.5) (Gampe et al., 2016). A clustering approach (Wilcke & Bärring, 2016) was applied to reduce the number of GCM-RCM combinations while conserving most of the spread of the original climate model ensemble. The selection led to three GCM-RCM combinations that are the basis for the “sustainable” (RCP4.5) and “myopic” (RCP8.5) scenarios within the GLOBAQUA European project (Navarro-Ortega et al., 2015). Climate model simulations are prone to biases on the catchment and regional scales (Dosio, 2016), and a bias correction is needed before the simulated series can be applied to further impact models. Therefore, a distribution-based scaling approach (Yang et al., 2010) was applied to the selected simulations for bias correction. To account for local topography and better represent observations, in the bias-corrected data sets, the downscaling algorithm SCALMET (Marke, 2008) was applied to disaggregate the simulations from their native resolution of 0.11° (~12 km) to a 1 km grid.

RESULTS

The water physicochemical variables of the sampling sites are shown in Table 1. Conductivity, TDS and nitrate were higher in site 1. The higher chloride values in site 2 are related to the influence of marine water intrusion in the Ebro estuary (Muñoz & Prat, 1989).

Diet analyses

The SIAR model based on stable isotopes showed that the most important carbon source for the two snails was *Cladophora* (90 % for *P. maculata* and 98 % for *M. tricarinata*), while the other sources did not provide a significant contribution of carbon (Table 2).

Experiment 1. Temperature

Pomacea maculata showed a significantly higher growth rate (mg DW mg^{-1} day^{-1}) under higher temperatures (one-way ANOVA, F=7.04, *p*=0.013, N=15) with a mean value of 47.0±17.3 at 20°C and 71.0±30.1 at 25°C. In contrast, for *M. tricarinata* the growth rate differences were not significant (F=1.45, *p*=0.24, N=15) between treatments with a mean growth rate of 26.8±23.4 at 20°C and of 17.2±20.6 at 25°C.

The oxygen consumption of *M. tricarinata* was higher at higher temperature (two-way ANOVA, temperature effect: F=134.2 p<0.001) and decreased in the second week of the experiment (time effect: F=37.9, p<0.001): differences between treatments were higher at the end of the experiment (temperature x time, F=6.16, p=0.025). In contrast, *P. maculata* oxygen consumption was lower at higher temperature (F=10.03, p=0.006), and although oxygen consumption was lower in the second week (time: F=22.11, p<0.001), the differences between the treatments were similar (temperature x time: F=0.42, p=0.53) (Figure 2).

Experiment 2. Dryness effect

After 7d under dry conditions, survival of *M. tricarinata* was 80 % at 20°C and 73.3 % at 25°C. After 15 d, all individuals were dead. However, all individuals of *P. maculata* survived after 60 d of desiccation at both temperatures. There were no significant differences (one-way ANOVA, $p>0.05$) in the oxygen consumption of *P. maculata* under different temperatures at the end of the experiment.

Prediction of dry bed areas in the lower Ebro River

In 2014, the basin water authorities approved a reduction in the minimum discharge of the lower Ebro from 100 to 80 $m^3 s^{-1}$, which has implied the emergence and drying of riverbeds. Since the distribution in the field and our results of the resistance to desiccation point to dry riverbeds as a potential habitat for *P. maculata*, we quantified how recent and potential future changes in water flow management may increase the area of these habitats. According to our results, the recent reduction from 100 to 80 $m^3 s^{-1}$ resulted in an increase of 12 ha of dry riverbed from Flix to Xerta, the reach that is just below the dams (Table 3; Figure 1), and 4 ha in the reach where the two species are present. Potential future reductions (e.g., a minimum discharge of 70 and 60 $m^3 s^{-1}$) will further increase the dry riverbed areas (Table 3), thus favouring the habitat for the invasive snail species.

River water temperature under future climate

The climate projections show increased temperatures throughout the year with a more pronounced increase during the summer months and lower changes during the winter (Gampe et al. 2016). The resulting mean daily RWT is presented in Figure 3. In accordance with air temperature projections, the RWT increases for the projected period in both scenarios, being slightly more pronounced for the myopic scenario and particularly elevated during the summer. There is a shift of approximately one month towards an earlier onset of RWTs>20°C to the end of April and a general prolongation of the period with RWTs>20°C. As summarized in Table 4, this extension of the period with elevated temperatures is projected by all three simulations and both RCPs and is slightly more pronounced in the myopic scenario, RCP 8.5. While the occurrence of high RWTs>25°C is rare during the reference period (5-14 days), these high RWTs are projected throughout the summer months by all future simulations under both scenarios and may last 55 – 83 days.

DISCUSSION

Our study shows that *P. maculata* and *M. tricarinata* of the lower Ebro River feed on the same food sources according to the Carbon assimilation (stable isotope analyses). The laboratory experiments demonstrate that *P. maculata* is physiologically better able to resist high temperatures and dry conditions than the native snail species. Our hydraulic model predicts an increase in the lower Ebro dry riverbed areas according to current and future scenarios of water flow management. And, the projections of water temperature models show an extreme increase in high temperatures during the summer. These results have a straightforward implication. The dried littoral area of the river and its water level fluctuations due to water flow regulations, and the resistance of the non-native species to high temperatures and dry conditions, will favour the establishment and spread of the invasive snail in areas where the native snail species is currently present.

Contrary to our hypothesis, the diet preference of the two snails was dominated by the algae *Cladophora* sp. Apple snails are capable of grazing on submersed and floating macrophytes and can produce changes in the natural vegetation of the wetlands they invade (Glasheen et al., 2017). Although macrophytes (e.g., *Myriophyllum*) are the preferred food for the *P. maculata* (Burlakova et al., 2009), they can consume other resources when macrophytes decline or are less palatable, including filamentous algae (Fang et al., 2010). López-van Oosterom et al. (2016) described a generalist diet for a *P. canaliculata* in their native area, with detritus, vascular plants and algae representing common food resources. Moreover, in their study, Horgan, Stuart & Kudavinadage (2014) reported that the capacity of invasive apple snails (*Pomacea* spp.) to deplete or eliminate other aquatic snails is often associated with notable declines in aquatic vegetation. Posch et al. (2013) studied interspecific competition between introduced *P. maculata* and the native apple snail *Pomacea paludosa* in Florida and observed that the growth rates of the native snails were lower in regions where invasive snails were dominant. It is difficult to ensure that *M. tricarinata* may be susceptible to interspecific food competition with *P. maculata* in the lower Ebro because our experiment did not test this statement but the described dietary flexibility and voracious behaviour of the different apple snail species (Lach et al., 2000) could suggest this hypothesis.

Warming increases the metabolic rates of aquatic poikilothermic organisms. Surprisingly, water warming did not affect the oxygen consumption of *P. maculata* but did increase its growth rate. In contrast, the *M. tricarinata* respiration rate increased with temperature, and it seems that the corresponding difficulties in metabolism maintenance might translate into lower growth rates. According to Ramakrishnan (2007), gastropods increase their oxygen consumption rates 2- to 3-fold with a water temperature increase of 10°C. Our experiment was performed within the optimal range of temperature for several ampullariids (Estebenet & Martín, 2002). *Melanopsis tricarinata* is especially sensitive to low temperatures (<13°C, Martínez-Ortí & Robles, 2008) but well adapted to warm conditions. However, the continued exposure to a high water temperature (25°C) seemed to affect *M. tricarinata* more than *P. maculata*. Currently, the maximum temperature in the lower Ebro is only occasionally higher than 28°C (a few days in the summer). Future projections of the river water temperature (RWT) show an increase in the length of the period with temperatures exceeding 20°C and a shift towards an earlier onset of these conditions, which favour *P. maculata*. The magnitude of the increase in high temperatures (RWT>25°C) will be more pronounced, and these conditions are likely to occur throughout the summer period from June to September and favour the growth of *P. maculata* while negatively affecting *M. tricarinata*.

Our results provide evidence that *P. maculata* from the Ebro River can resist dryness for more than two months. These results are similar to those of other studies reporting that adult P. *maculata* snails could survive a dry period of 28 days to almost one year (Ramakrishnan, 2007, Bernatis, Mcgaw & Cros, 2016). *Melanopsis tricarinata* survived only approximately a week. The ability of freshwater snails to tolerate dry conditions for extended periods is an evolutionary trait. *Pomacea* species are amphibious (Hayes et al., 2015), with the ability to exchange gas across their gill and across the walls of their mantle cavity lung. These abilities allow them to feed in the water interface or just above the water level, maintain aerobic metabolism during periods of aquatic hypoxia by extending their siphon to the water surface, oviposit egg masses on emerged surfaces (if female), and tolerate prolonged emersion during the desiccation of their habitats. Some species of *Pomacea* aestivate or overwinter during dry and cold periods (Hayes et al., 2015) by burrowing in emerged or submerged soils (Estebenet & Martín, 2002). These tolerances of abiotic stressors contribute to their development in their native habitats and likely confer the traits of a successful invader (Kolar & Lodge, 2001) in the lower Ebro, thus allowing them to thrive more than other species.

The frequency and intensity of droughts is increasing in the Mediterranean region (IPCC, 2013). A decrease in precipitation for the Ebro Basin is projected, especially during the summer month. This decrease in precipitation combined with increased evapotranspiration due to higher temperatures will led to less surface runoff, thus favouring dry periods in the area (Gampe et al. 2016). In parallel, the water demand in the Ebro Basin is currently growing mainly because of the increase in agricultural activities. In 2014, the water authorities of the basin approved a reduction from 100 to 80 $m^3 s^{-1}$ in the minimum discharge of the dams that feed the lower part of the river and its delta (Confederación Hidrográfica del Ebro, 2014). As our results show, this reduction increased the dry surface area in the littoral zone of the river, the area most affected by water level fluctuations. According to Byers et al. (2013), the habitats that are most vulnerable to apple snail colonization are those with an intermediate level of water permanence. Hence, the littoral edges of the Ebro River are an ideal habitat for the spread of *P. maculata* and may contribute to it reaching areas where the native snail species is present. At the same time, these changes may also reduce the suitable habitat for *M. tricarinata*.

Considering the estimated deleterious effects that the apple snail could have on the diverse ecosystem services provided by freshwater regions in Europe (Carlsson, 2017, Gilioli et al., 2017), the control of the *P. maculata* population in the lower Ebro River is essential. In fact, several actions to control its population in the river have been conducted since 2010. Manual eradication has been the most effective method to control new settlements in the river, but it does not produce complete extermination and is only effective at the early stages of invasion. Our results provide insights on the factors that could favour *P. maculata* dispersal and present a spatial basis for the prediction of its future spread. Furthermore, our estimation of the potential increase in dry riverbed areas might be a useful visualization tool to convince managers that the potential dispersal capacity of this species is related to discharge management. We also hope that this research will be of particular interest to nature conservation, environmental management, and public education sectors and emphasize the risk associated with this non-native species in the Ebro River.

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Table 1. Physicochemical parameters of the Ebro River water at site 1 (location of the *M. tricarinata* collection) and site 2 (location of the *P. maculata* collection). TDS: total dissolved solids.

	Site 1	Site 2
Temperature (ºC)	15.1	17.2
Conductivity (µS cm^{-1})	578.0	708.0
TDS (mg L^{-1})	442.0	630.5
pH	8.17	8.45
Cl^- (mg L^{-1})	61.90	329.58
NO_3^- (mg L^{-1})	8.95	16.92
SO_4^- (mg L^{-1})	148.26	229.45

Table 2. Results of the SIAR model for the diet of *M. tricarinata* and *P. maculata.* Values represent the limits of 95 % confidence intervals for each basal resource (in percentage).

Species	***Cladophora***	***Myriophyllum***	**Biofilm**	**FPOM**
Pomacea maculata	90-91	0.5-0.6	0.8-1.0	0.9-1.0
Melanopsis tricarinata	98	-	0.2-0.3	0.6-0.8

Table 3. Results of the HEC-RAS model in two reaches of the lower Ebro under different scenarios of flow reduction. The first raw (flow reduction from 100 to 80 $m^3 s^{-1}$) was established in 2014 for the water authorities.

	Flix-Xerta		**Xerta-Amposta**	
Flow reduction (m^3 s^{-1})	**Reduction in wet area (%)**	**Absolut increment in dry river bed (Ha)**	**Reduction in wet area (%)**	**Absolut increment in dry river bed (Ha)**

100 to 80	1.3	11.9	0.7	3.9
100 to 70	2.0	19.2	1.1	5.8
100 to 60	2.9	26.9	1.5	8.0

Table 4: Average number of days with river water temperatures above 20°C and 25°C for the three climate simulations of the reference periods 1981 – 2010, RCP 4.5 and RCP 8.5 (2036 – 2065).

	ICHEC-EC-EARTH_ KNMI-RACMO22E	**ICHEC-EC-EARTH _CLMcom-CCLM4**	**MOHC-HadGEM2-ES _SMHI-RCA4**
Reference period	115 / 14	116 / 5	117 / 9
RCP 4.5 (sustainable)	138 / 62	138 / 55	147 / 75
RCP 8.5 (myopic)	145 / 60	140 / 61	153 / 83

Figure 1: Distribution of *M. tricarinata* (blue) and *P. maculata* (red) in the lower Ebro River and location of the sampling sites. Red dots show sporadic apple snail outbreaks observed in the river. Apple snail has invaded the rice fields and irrigation channels of the left side of the river delta since 2009 (red shading).

Figure 2. Temperature effect on the oxygen consumption (mg O_2 L^{-1} g PS^{-1} min^{-1}) of *M. tricarinata* (left) and *P. maculata* (right) in experiment 1.

Figure 3: Daily mean, maximum and minimum river water temperature (RWT) modelled for the lower Ebro Basin for the selected climate model ensemble for the reference periods 1981 – 2010 (grey), RCP 4.5 (2036 – 2065, blue) and RCP 8.5 (2036 – 2065, red). Horizontal black lines refer to the two temperatures tested in the experiments. DOY: day of year.

Figure 1

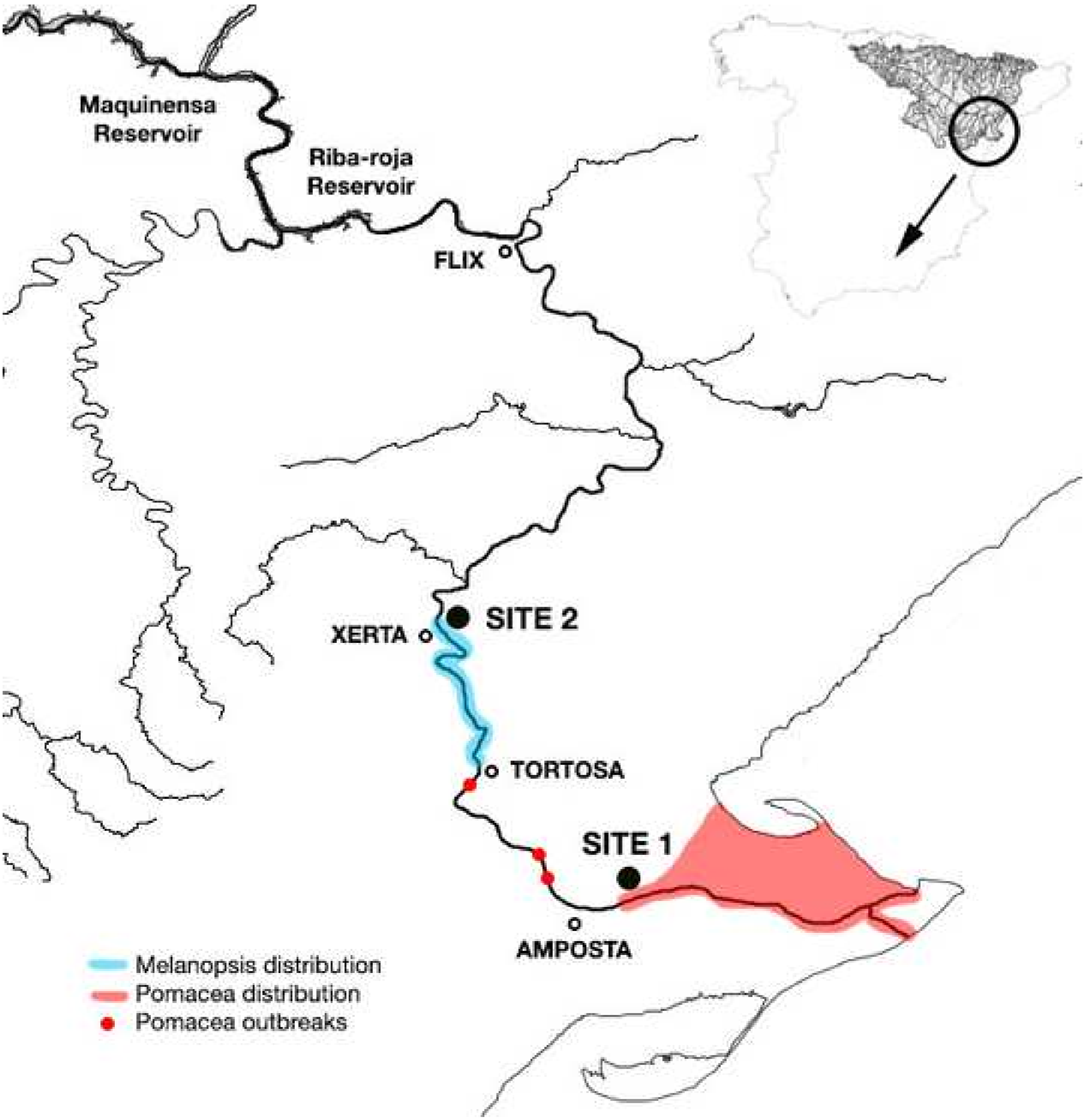
Maquinensa
Reservoir
Riba-roja
Reservoir
FLIX
SITE 2
XERTA
TORTOSA
SITE 1
AMPOSTA
Melanopsis distribution
Pomacea distribution
Pomacea outbreaks

Figure 2

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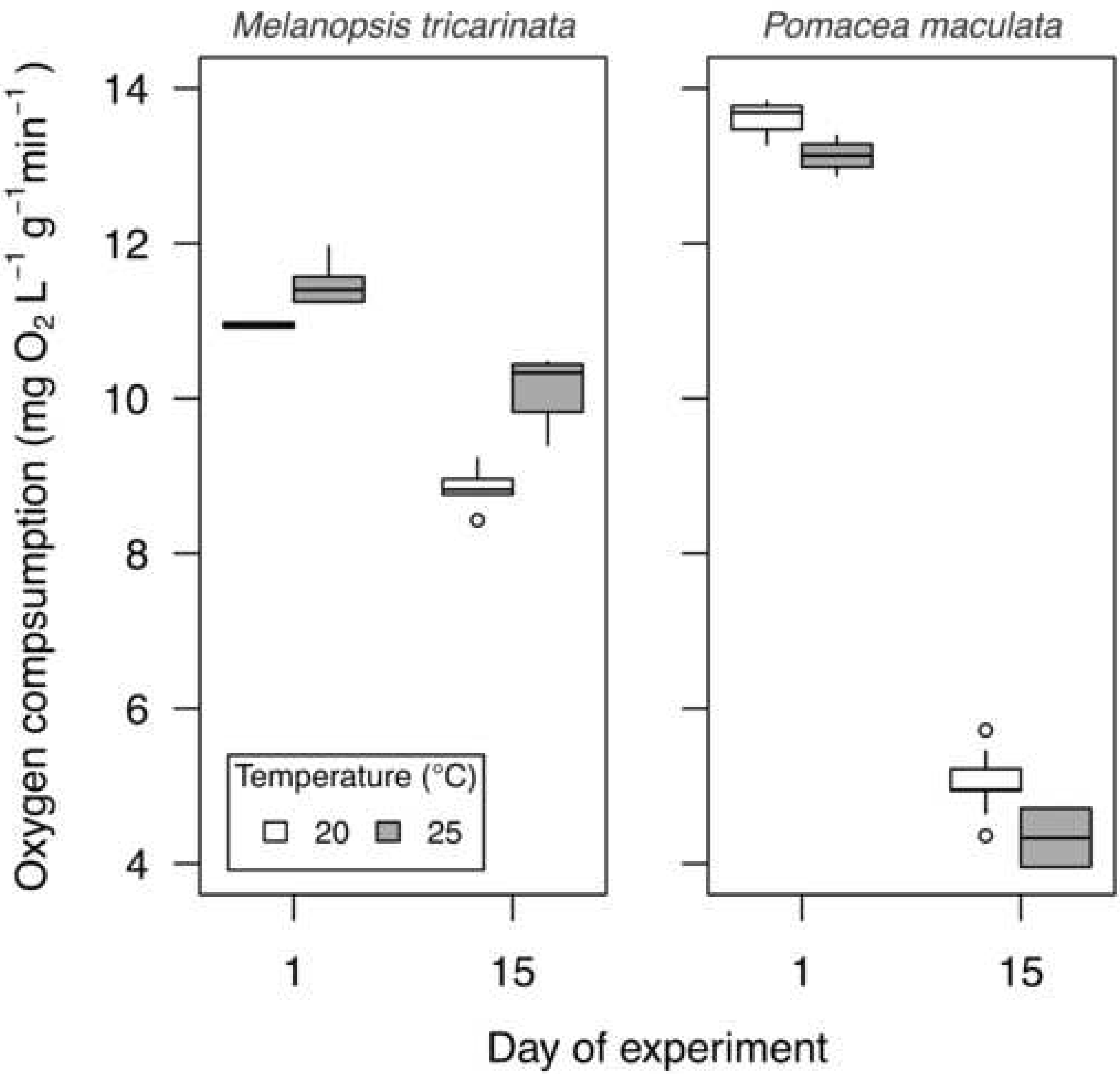

Figure 3

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