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Animal behaviour

Ocean warming and acidification may challenge the riverward migration of glass eels

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The dramatic decline of European eel (*Anguilla anguilla*) populations over recent decades has attracted considerable attention and concern. Furthermore, little is known about the sensitivity of the early stages of eels to projected future environmental change. Here, we investigated, for the first time, the potential combined effects of ocean warming (OW; $\Delta + 4°C$; 18°C) and acidification (OA; $\Delta - 0.4$ pH units) on the survival and migratory behaviour of *A. anguilla* glass eels, namely their preference towards riverine cues (freshwater and geosmin). Recently arrived individuals were exposed to isolated and combined OW and OA conditions for 100 days, adjusting for the salinity gradients associated with upstream migration. A two-choice test was used to investigate migratory activity and shifts in preference towards freshwater environments. While OW decreased survival and increased migratory activity, OA appears to hinder migratory response, reducing the preference for riverine cues. Our results suggest that future conditions could potentially favour an early settlement of glass eels, reducing the proportion of fully migratory individuals. Further research into the effects of climate change on eel migration and habitat selection is needed to implement efficient conservation plans for this critically endangered species.

1. Introduction

Increasing carbon dioxide (CO_2) emissions are disrupting the physico-chemical balance of the planet [1]. Rising CO_2 levels trap additional solar energy, with the majority of heat being stored by the ocean, leading to ocean warming (OW) [1]. Furthermore, the ocean absorbs approximately 30% of the additional CO_2 emitted into the atmosphere, changing seawater chemistry. As atmospheric CO_2 rises, more CO_2 dissolves in seawater, reducing seawater pH in a process known as ocean acidification (OA) [1,2]. These physico-chemical changes are expected to have major impacts on marine organisms, with reverberating consequences across marine ecosystems [3,4].

Migratory species are likely to be particularly susceptible to climate change, because they require suitable habitat conditions to be maintained in multiple locations [5]. Moreover, to successfully complete their migration, these animals depend on appropriate environmental cues to guide them and on their own ability to correctly interpret these signals, both of which may be affected by changing environmental conditions [4,6]. Recent research shows that OW can affect larval duration, survivorship and long-distance dispersal [7]. By contrast, OA can interfere with the sensory performance of larval fishes [8–10] leading to altered olfactory, auditory and visual preferences that can affect their homing ability and habitat selection [4].

The European eel (*Anguilla anguilla*) has one of the most remarkable large-scale migrations in the ocean. Hatching in the Atlantic Ocean, leptocephali larvae drift along oceanic currents towards Europe and North Africa, migrating towards continental waters to grow before returning to the ocean to spawn [11]. Successful recruitment and individual migratory strategy is highly dependent on their ability to orient and swim towards riverine cues (e.g. temperature, salinity gradients and inland water odours, such as geosmin) [11]. The dramatic decline in European eel populations over recent decades, associated with their economic and ecological relevance, has attracted considerable attention [12]. Rapid environmental changes predicted for the near future, such as OW and OA, may further challenge this species [4,13–15]. Nonetheless, limited research has addressed this question.

Previous studies suggest changes in the oceanic dispersal of leptocephali larvae [13,14] and adult sex ratio [15] may be induced by OW; however, simultaneous effects of multiple global change stressors on eel migration and recruitment remain understudied. Here, we investigated, for the first time, how OW ($\Delta + 4^\circ$C) and OA ($\Delta - 0.4$ pH units; approx. 900 µatm) may affect the survival and riverward migratory behaviour, including preference towards riverine cues, of *A. anguilla* glass eels.

2. Material and methods

(a) Collection and acclimation

Newly arrived European glass eels were captured at the mouth of the Minho Estuary (salinity 35; January 2017, Portugal) and transported to the experimental aquaculture facilities at Laboratório Marítimo da Guia (Cascais, Portugal). Following a two-week acclimation, the animals were randomly assigned to four treatments: control (C; 14°C, pH 8.0); acidification (A; 14°C, pH 7.6); warming (W; 18°C, pH 8.0) and combined warming and acidification (WA; 18°C, pH 7.6), in a flow-through system with four replicate tanks (15 l) per treatment. Treatment conditions were chosen to reflect present-day conditions at the collection site and an OW/OA predicted scenario [1]. To simulate the salinity gradient associated with riverward migration, two salinity reductions took place after 85 (salinity 15) and 90 days (salinity 0). Temperature was maintained using water chillers and heaters. The acidification set-up followed standard acidification experimental design guidelines [16]. Seawater parameters (electronic supplementary material, table S1) and mortality were monitored daily.

(b) Behavioural trials

Migratory response and cue preference were determined through a binary-choice test. Two water flows, leading to eel traps, delivered treatment water (sham) or test water into a choice chamber (cue; freshwater (FW-test), or geosmin (10^{-10} mg l^{-1}; Geo-test, >97%, Sigma-Aldrich)). The experimental device (electronic supplementary material, figure S1) and protocol were based on previous studies with this species [17–19]. The proportion of animals in the traps at the end of each trial, regardless of cue preference, was used as a proxy for migratory activity [19]. The proportion of animals collected in the cue side was used to address cue preference. The FW-test was only performed at salinities of 35 and 15.

(c) Statistical analyses

Generalized linear and mixed models (GLM, GLMMs) were used to analyse the effect of temperature and pH on survival and behaviour. Salinity was included as a fixed effect when relevant. Replicate and cue side were considered as random factors and included if rendering a better model fit. Models were validated and analyses performed using R software (R Development Core Team, 2016).

A detailed description of rearing conditions, experimental procedures and statistical analysis is available in the electronic supplementary material.

3. Results

The survival variation over the 14-week exposure period is represented in figure 1*a*. The proportion of surviving eels over the first 85 days, before salinity reduction, was reduced by OW ($p = 0.007$), regardless of pH conditions ($p > 0.05$; statistical analyses are summarized in table 1). The proportion of migrating eels in the FW-test (figure 1*b*(i)) was higher under OW ($p < 0.001$), but not under OA. Furthermore, there was a significant interaction between OW and OA ($p = 0.005$), such that migration was not higher than controls in the combined OW/OA treatment. A similar relationship was observed for temperature in the Geo-test (figure 1*c*(i); $p < 0.001$), although in this case OA resulted in a decrease in migratory activity regardless of temperature conditions ($p < 0.001$). Moreover, in both the FW ($p < 0.05$) and geosmin ($p < 0.001$) trials, the proportion of individuals in the riverine cue side was lower in OA treatments (figure 1*b*(ii),*c*(ii); approx. 50% probability for both choices).

4. Discussion

Despite the European eel's challenging life history and intrinsic resilience towards extreme conditions [13], few studies have empirically addressed the impacts of near-future climate change conditions on migration behaviour. Environmental variables, namely temperature, have a paramount influence over the magnitude and periodicity of eel migration [19]. Indeed, our results indicate a reduction in glass eel survival under a 4°C temperature increase. Although considered an eurythermal species, increasing temperature lowered glass eels' survival. Decreased survival likely reflects lower body condition, which is considered a major cause for eels not to venture to full riverward migration [11,19]. Nevertheless, our results show an increased migratory response of OW-acclimated animals. Increased activity levels with warming are ubiquitous in ectotherms and widely documented in eels (e.g. [19]). Hence, we argue that the foreseen warming of coastal waters may both (i) reduce the number of recruits and (ii) induce an earlier colonization of continental waters, due to the increased migratory response.

Most research regarding the effects of acidification in eels has focused on aquaculture-related hypercapnia, to which they present a remarkable physiological tolerance (e.g. [20]). Accordingly, the levels tested here did not affect survival rates. Indeed, few fish species have shown reduced survival under OA-relevant conditions [21]. Nonetheless, OA

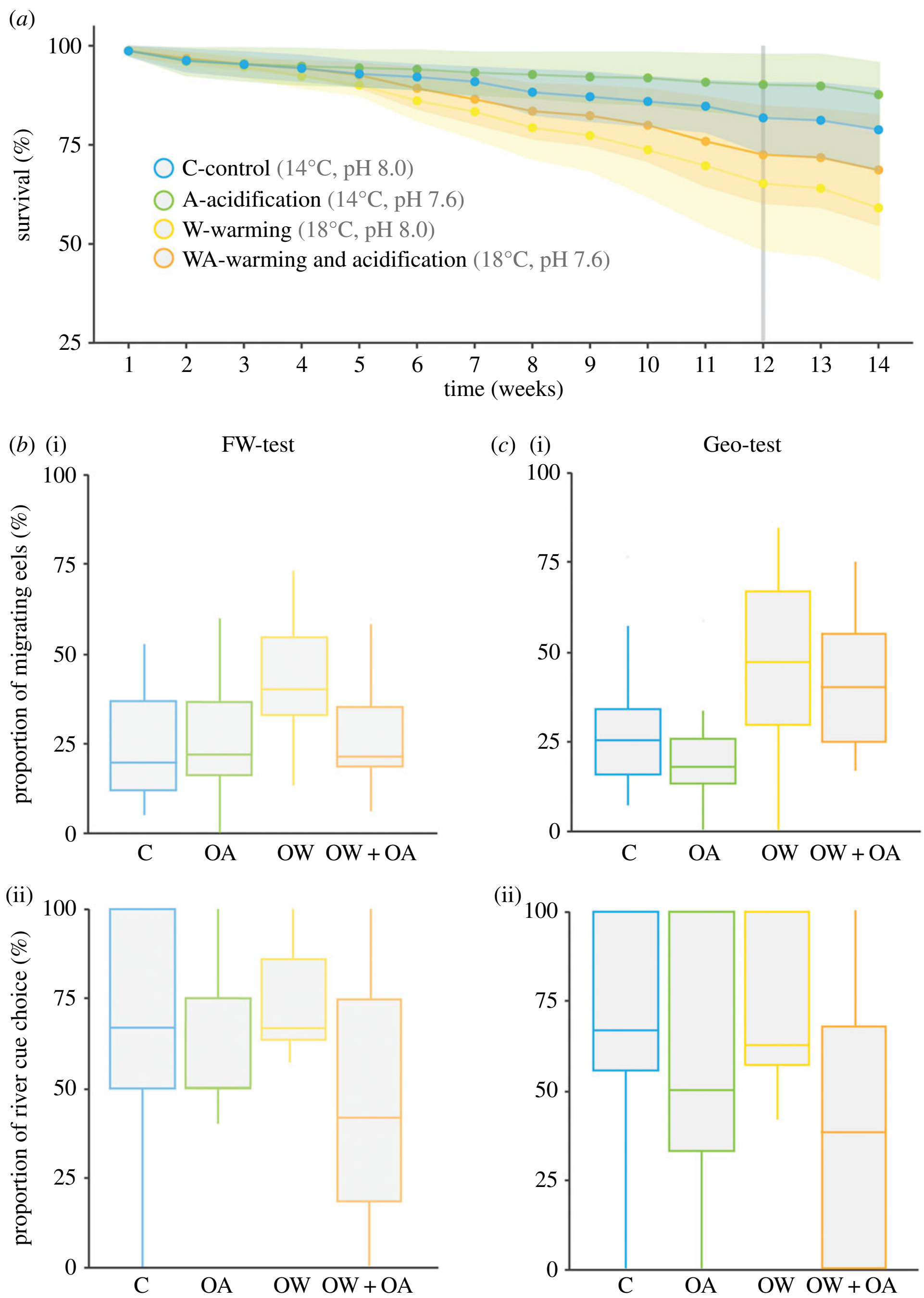

Figure 1. (*a*) Proportion of surviving eels (%) over the exposure period (in weeks). Shaded areas represent the standard deviation at each week point. The grey line at the 12th week marks the first salinity reduction. (*b*) Proportion of eels exhibiting upstream migratory activity (%) in the freshwater (*b*(i)) and geosmin (*b*(ii)) tests. (*c*) Proportion of eels choosing riverine cue side in the freshwater (*c*(i)) and geosmin (*c*(ii)) tests. Horizontal lines indicate the median, boundaries indicate the 25th and 75th percentiles, and the whiskers indicate the highest and lowest values of the results.

significantly affected the glass eels' migratory response: (i) dampening the OW effects in the FW trials and (ii) reducing the proportion of migrating animals, regardless of temperature, in the Geo-test. Additionally, OA (iii) reduced the overall preference towards both environmentally relevant riverine cues. These results align with literature, which reports preference changes towards a wide array of ecologically relevant cues [22], including reduced salinity [23]. In fact, high pCO_2 and associated pH reduction is thought to impair sensorial information perception, constraining behavioural responses [4]. These behavioural changes have been associated with both an impaired olfactory system [10] and disrupted neurological pathways, e.g. the malfunction of $GABA_A$ receptors [9], including in diadromous species such as the pink salmon (*Oncorhynchus gorbuscha*) [6]. Although behavioural changes were observed under OA conditions in the present study, the underlying mechanisms remain unaddressed, and it is worth mentioning that these may differ between freshwater and marine habitats [22]. Future research should also consider the influence of natural CO_2 cycles, characteristic of estuarine habitats, over glass eel's behaviour in an OA context [24].

As a facultative catadromous species, newly arrived individuals may embrace alternative migratory tactics—leading to the colonization of rivers or early settlement in marine or estuarine habitats [11,19]. Previous research suggests that glass eel preference for freshwater cues is important when selecting between these habitats [25]. In addition to OA-related

Table 1. Summary of statistical analyses for survival, migratory response and cue preference in glass eels after cross-factorial acclimation to present-day (14°C; pH 8.0) and predicted ocean warming (18°C) and acidification conditions (pH 7.6). FW, freshwater; RV, response variable; Temp, temperature; SE, standard error; GLM, generalized linear model; GLMM, generalized linear mixed model. Bold values indicate $p < 0.05$.

fixed effects		estimate	s.e.	*Z*-value	*p*-value
survival	RV: proportion of surviving individuals			*GLM. Family: negative binomial*	
(intercept)	final model terms: RV ~ pH + temp	−0.208	0.075	−2.756	0.006
pH		0.104	0.087	1.201	0.230
temp		−0.235	0.087	−2.719	0.007
migratory response					
FW-test	RV: proportion of migratory individuals			*GLMM. Family: binomial. Random factor: replicate*	
(intercept)	final model terms: RV ~ pH * temp + sal	−0.983	0.166	−5.918	<0.001
pH		0.123	0.216	0.574	0.566
temp		0.824	0.199	4.147	<0.001
salinity		−0.260	0.152	−1.710	0.087
pH * Temp		−0.864	0.303	−2.857	0.005
geosmin test	RV: proportion of migratory individuals			*GLMM. Family: binomial. Random factor: replicate*	
(intercept)	final model terms: RV ~ pH + temp;	−0.958	0.151	−6.333	<0.001
pH		−0.486	0.136	−3.574	<0.001
temp		1.075	0.136	7.884	<0.001
cue preference					
FW-test	RV: proportion of riverine cue choice			*GLMM. Family: binomial. Random factor: test side*	
(intercept)	final model terms: RV ~ pH;	0.861	0.345	2.495	0.013
pH		−0.793	0.262	−3.024	0.002
geosmin test	RV: proportion of riverine cue choice			*GLM. Family: binomial*	
(intercept)	final model terms: RV ~ pH	0.610	0.144	4.251	<0.001
pH		−0.729	0.217	−3.357	<0.001

preference disruption, body condition is implicated in migratory strategy selection [19]. Thus, future environmental conditions could potentially favour an early settlement, reducing the proportion of fully migratory individuals. Shifts in habitat use and migratory strategies can potentially escalate into marked demographic disturbances, influencing pivotal life-history traits such as growth, sex ratio, maturation time, migratory capacity and reproductive potential [11]. Additionally, fully migratory individuals are already more vulnerable to exploitation, habitat degradation and anthropogenic pressure [13], which may further exacerbate their critically endangered population status. The present study illustrates how changing ocean conditions may trigger unexpected changes in European eel population dynamics, which emphasize the need for increasing efforts to implement efficient conservation plans.

Ethics. Faculdade de Ciências da Universidade de Lisboa animal welfare organization (ORBEA, Statement 5/2016) and Direção-Geral de Alimentação e Veterinária (DGAV) approved procedures in the context of the project CLIMATOXEEL (PTDC/AAG-GLO/3795/2014), fulfilling requirements imposed by the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Data accessibility. Data are available online from the Dryad Digital Repository at: http://dx.doi.org/10.5061/dryad.7jv4466 [26].

Authors' contributions. T.F.G., R.R. and J.R.P. conceived and designed the experiment. C.A. ensured animal collection. F.O.B., E.S., C.F. and T.F.G. performed the experiment. E.S., F.O.B. and C.P.S. analysed and interpreted the data. F.O.B. and C.S. wrote the manuscript. All authors reviewed and approved the final manuscript, agreeing to be held accountable for the content herein.

Competing interests. No conflicts of interest declared.

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References

1. Hartmann DL, Tank MGK, Rusticucci M. 2013 Climate change 2013: the physical science basis. Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Geneva, Switzerland: IPCC. See https://www.ipcc.ch/report/ar5/wg1/.

2. Caldeira K, Wickett ME. 2003 Oceanography: anthropogenic carbon and ocean pH. *Nature* **425**, 365. (doi:10.1038/425365a)
3. Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso JP. 2013 Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob. Chang. Biol.* **19**, 1884 – 1896. (doi:10.1111/gcb.12179)
4. Nagelkerken I, Munday PL. 2016 Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. *Glob. Chang. Biol.* **22**, 974 – 989. (doi:10.1111/gcb.13167)
5. Robinson RA *et al.* 2009 Travelling through a warming world: climate change and migratory species. *Endanger Species Res.* **7**, 87 – 99. (doi:10.3354/esr00095)
6. Ou M *et al.* 2015 Responses of pink salmon to CO_2-induced aquatic acidification. *Nat. Clim. Chang.* **5**, 950 – 957. (doi:10.1038/nclimate2694)
7. O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP, Weiss JM. 2007 Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proc. Natl Acad. Sci. USA* **104**, 1266 – 1271. (doi:10.1073/pnas.0603422104)
8. Munday PL, Leis JM, Lough JM, Paris CB, Kingsford MJ, Berumen ML, Lambrechts J. 2009 Climate change and coral reef connectivity. *Coral Reefs* **28**, 379 – 395. (doi:10.1007/s00338-008-0461-9)
9. Nilsson GE, Dixson DL, Domenici P, McCormick MI, Sørensen C, Watson S-A, Munday PL. 2012 Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nat. Clim. Chang.* **2**, 201 – 204. (doi:10.1038/nclimate1352)
10. Porteus CS, Hubbard PC, Uren Webster TM, van Aerle R, Canário AVM, Santos EM, Wilson RW. 2018 Near-future CO_2 levels impair the olfactory system of a marine fish. *Nat. Clim. Chang.* **8**, 737 – 743. (doi:10.1038/s41558-018-0224-8)
11. Edeline E, Dufour S, Elie P. 2009 Proximate and ultimate control of eel continental dispersal. In *Spawning migration of the European eel*, pp. 433 – 461. Dordrecht, The Netherlands: Springer.
12. Stone R. 2003 Freshwater eels are slip-sliding away. *Science* **302**, 221 – 222. (doi:10.1126/science.302.5643.221)
13. Knights B. 2003 A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. *Sci. Total Environ.* **310**, 237 – 244. (doi:10.1016/S0048-9697(02)00644-7)
14. Bonhommeau S, Chassot E, Planque B, Rivot E, Knap AH, Le Pape O. 2008 Impact of climate on eel populations of the Northern Hemisphere. *Mar. Ecol. Prog. Ser.* **373**, 71 – 80. (doi:10.3354/meps07696)
15. van Ginneken V. 2017 Is global warming the cause for the dwindling European eel population? *Oceanogr. Fish. Open access J.* **2**. 2017. (doi:10.19080/OFOAJ.2017.02.555597)
16. Cornwall CE, Hurd CL. 2016 Experimental design in ocean acidification research: problems and solutions. *ICES J. Mar. Sci. J. du Cons.* **73**, 572 – 581. (doi:10.1093/icesjms/fsv118)
17. Tosi L, Sola C. 1993 Role of geosmin, a typical inland water odour, in guiding glass eel *Anguilla anguilla* (L.) migration. *Ethology* **95**, 177 – 185. (doi:10.1111/j.1439-0310.1993.tb00468.x)
18. Edeline E, Dufour S, Elie P. 2005 Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. *Mar. Ecol. Prog. Ser.* **304**, 191 – 199. (doi:10.3354/meps304191)
19. Edeline E, Lambert P, Rigaud C, Elie P. 2006 Effects of body condition and water temperature on *Anguilla anguilla* glass eel migratory behavior. *J. Exp. Mar. Biol. Ecol.* **331**, 217 – 225. (doi:10.1016/j.jembe.2005.10.011)
20. Mckenzie DJ, Piccolella M, Valle AZD, Taylor EW, Bolis CL, Steffensen JF. 2003 Tolerance of chronic hypercapnia by the European eel *Anguilla anguilla*. *J. Exp. Biol.* **206**, 1717 – 1726. (doi:10.1242/jeb.00352)
21. Heuer RM, Grosell M. 2014 Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *AJP Regul. Integr. Comp. Physiol.* **307**, R1061 – R1084. (doi:10.1152/ajpregu.00064.2014)
22. Leduc AOHC, Munday PL, Brown GE, Ferrari MCO. 2013 Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: a synthesis. *Phil. Trans. R. Soc. B* **368**, 20120447. (doi:10.1098/rstb.2012.0447)
23. Pistevos JCA, Nagelkerken I, Rossi T, Connell SD. 2017 Ocean acidification alters temperature and salinity preferences in larval fish. *Oecologia* **183**, 545 – 553. (doi:10.1007/s00442-016-3778-z)
24. Jarrold MD, Humphrey C, McCormick MI, Munday PL. 2017 Diel CO_2 cycles reduce severity of behavioural abnormalities in coral reef fish under ocean acidification. *Sci. Rep.* **7**, 10153. (doi:10.1038/s41598-017-10378-y)
25. Mccleave JD, Edeline E. 2009 Diadromy as a conditional strategy: patterns and drivers of eel movements in continental habitats. *Am. Fish. Soc. Symp.* **69**. 97 – 119.
26. Borges FO, Santos CP, Sampaio F, Figueirado C, Paula JR, Antunes C, Rosa R, Grilo TF. 2018 Data from: Ocean warming and acidification may challenge the riverward migration of glass eels. Dryad Digital Repository. (doi:10.5061/dryad.7jv4466)