nature portfolio

Double blind per review submission

Corresponding author(s): COMMSBIO-22-1650A

Last updated by author(s): Dec 7/2022

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

$\overline{}$					
Š	+-	٦t	IC.	tι	CS
٠,		71	1		1 >

n/a	Confirmed
	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
	A description of all covariates tested
	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Pictures from the dorsal head were converted to binary white/black images using NIH ImageJ public domain software. The density of positive pixels was used to determine the pigmentation index. The free online software, Kinovea version 0.8.15, was used to track the movement of tadpoles to determine the locomotor performance.

Data analysis

The statistical significance (p < 0.05) between treatments was determined with GraphPad Prism 9 by using multiple ANOVA followed by Bonferroni's post hoc test or t-test ($n \ge 8$; $N \ge 3$).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data have been deposited in the Dryad repository site with an assigned unique digital object identifier (DOI): doi:10.5061/dryad.6q573n61f. Cloned sequences were deposited in Gene Bank with access numbers provided in Supplementary table 1

Human research participants

Policy information about studies involving human research participants and Sex and G	ander in Bacaarch

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

ease select the one b				

Life sciences	Behavioural & social sciences	Ecological, evolutionary & environmental science	ces
---------------	-------------------------------	--	-----

 $For a \ reference \ copy \ of \ the \ document \ with \ all \ sections, see \ \underline{nature.com/documents/nr-reporting-summary-flat.pdf}$

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

Using Xenopus laevis as a model system, we study two responses in ectotherms that contribute to temperature regulation: 1) A skin coloration response where dorsal pigmentation of the tadpole is adjusted to the environmental temperature to change the light refractory conditions, and 2) A behavioural response where the locomotor performance of the organism aligns with the ambient temperature. We explore both the molecular thermosensors, and the relationship between skin colour change and the locomotor preference in response to an ethologically-relevant cold temperature (24 and 6 Celsius degrees).

Research sample

Data were collected in a laboratory using Xenopus laevis tadpoles at two developmental time, stage 42/43 and stage 45/46. For all experiments, sample size in each group (control or treated) was between 8 and 12 tadpoles ($n \ge 8$); The experiments were performed three times (N = 3).

Sampling strategy

Twelve tadpoles were set in a 60 mm dish at 24 degrees for 24/48 h before exposure to cooling temperatures. Individuals with developmental malformations (e.g. twisted tails, edemas etc) were removed before data collection (skin pigmentation or locomotor performance). Thus a minimum of 8 tadpoles were analyzed.

Data collection

Data were collected by Mr. Hannan Malik and Dr. Gabriel Bertolesi. For skin pigmentation studies, pictures of the dorsal head were taken with identical conditions of light, exposure time and diaphragm aperture, and converted to binary white/black images using NIH ImageJ public domain software. Pigmentation level (arbitrary units) was automatically converted by the software. For locomotor performance, a tray containing 9-12 tadpoles set individually in 35 mm dishes were recorded for one minute, every 5 minute during a total time of 30 minutes. The free online software, Kinovea version 0.8.15, was used to track the movement (mm) of each tadpole.

Timing and spatial scale

Skin pigmentation data were obtained between November 2018 and September 2021, unless correlation with locomotor performance was required. Locomotor performance data were obtained between July 2021 and March 2022.

Data exclusions

 $Tadpoles\ showing\ development al\ malformation\ (e.g.\ twisted\ tails,\ edemas\ etc)\ were\ excluded\ for\ data\ collection.$

Reproducibility

Skin pigmentation level changes considerably between hatches, therefore a representative experiment from at least three independent experiments (hatches) is shown. Also common is variation in the locomotor performance between hatches. Importantly cooling the temperature produced the same trend in all the experiments.

Randomization	Tadpoles were randomly grouped.	
Blinding	Pigmentation level provided by the densitometry of the head picture and the locomotor performance measured as the movement into the dish were generated automatically by the Image J and Kniovea software respectively, therefore blinding the samples was no necessary.	
Did the study involve field	d work? Yes No	
Donorting fo	r specific materials systems and methods	
.	r specific materials, systems and methods uthors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each materia	
	evant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.	
Materials & experime	ntal systems Methods	
n/a Involved in the study	n/a Involved in the study	
Antibodies	ChIP-seq	
Eukaryotic cell lines		
Palaeontology and a		
Clinical data	riganisnis	
Dual use research o	f concern	
,		
Antibodies		
Antibodies used	rabbit polyclonal anti-human TRPM8 (1:200 dilution; antibodies-online.com, ABIN351227) and rabbit polyclonal against human Tyrp-1 (1:200 dilution; Thermo Scientific, IL; PA5-81909)	
Validation	ABIN351227: Reactivity in Xenopus laevis by immunohistochemistry and Western blot (showed in supplementary figures). Lack of	
	staining in the absence of primary antibody. PA5-81909: Provided by the supplier. Advanced validation; This antibody was verified by relative expression to ensure that the	
	antibody binds to the antigen stated	
Eukaryotic cell lin	es	
Policy information about <u>ce</u>	ell lines and Sex and Gender in Research	
Cell line source(s)	The melanophore (MEX) cell line was originally generated from stage 35 Xenopus laevis embryos (ref #40) at Dr. Validimir Rodionov's laboratory, University of Connecticut.	
Authentication	Immunoreactivity against Tyrp-1 confirmed the nature of the melanophore cell line.	
Mycoplasma contaminati	on Lack of mycoplasma contamination was confirmed by DAPI staining.	
Commonly misidentified (See <u>ICLAC</u> register)	lines The cell line obtained from Xenopus melanophores (MEX) is not registered in ICLAC	
Animals and othe	r research organisms	
Policy information about <u>st</u> <u>Research</u>	udies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in	
Laboratory animals	Xenopus laevis embryos at stage 42/43 and 45/46 were used. Embryos were staged according to Nieuwkoop and Faber using Xenbase (http://www.xenbase.org) RRID:SCR_003280).	
Wild animals	ild animals N/A	

The Animal Care and Use Committee, University of Calgary, approved procedures involving frogs and embryos, which was signed by Dr Derrick Rancourt.

N/A

N/A

Reporting on sex

Ethics oversight

Field-collected samples

Note that full information on the approval of the study protocol must also be provided in the manuscript.