1 **Evidence for physiological niche expansion of an intertidal flatworm:**

2 **Evolutionary rescue in the wild**

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1 **Abstract**

2 Microevolution may enable populations to adapt physiologically to rapid climate

3 change. Where it exists, historical data could provide a rare opportunity to document

4 such adaptation. The intertidal flatworm, *Procerodes littoralis* experiences large

5 changes in salinity throughout the tidal cycle. We investigated whether regeneration

6 performance of *P. littoralis* has changed over the last century. We repeated identical

7 experiments to those published in 1914 on the same species, and from the same

8 location. In the modern experiment, when tested across a range of different salinities

9 (S = 3, 6, 28, 44 and 53) *P. littoralis* could regenerate at lower salinities (S = 3) than

10 reported previously. Also in the modern study, no significant optimum salinity could

11 be identified, whereas in 1914 the optimum salinity (S = 28.5) was clear. The

12 possibility of differences resulting from acclimation or oxygen availability instead of

13 adaptation was investigated and discounted. It would appear, individuals from this

14 population have extended their tolerance performance range further into hyposaline

15 waters in the intervening 104 years since the previous study. Local climate change is

16 suggested to be the microevolution driver, as mean daily precipitation has increased,

17 and the number of days with no/trace precipitation per year has decreased in

18 Plymouth, UK. Climate change is often considered a global phenomenon, but it

19 drives local regime shifts. Here we suggest an evolutionary shift attributable to a

20 century-long local change in precipitation.

21 Key Words: Microevolution, Specialism, Generalism, Historical, Niche expansion,

22 Salinity, Climate Change

23

1   **1. Introduction**

2   Adaptive evolution that prevents the extinction of a population is known as

3   evolutionary rescue (Gomulkiewicz and Holt 1995) and may occur when a population

4   faces rapid climate change (,Rice & Emery 2003; Bell 2013, 2017). Piecing together

5   the chronology of animal evolution and adaptation in the wild is becoming a growing

6   area of research, especially as more evidence of adaptations are emerging, in the

7   wake of extreme environmental pollution and climate change (e.g. Sanford & Kelly

8   2011; Carlson et al. 2014,Bell 2017, Oziolor et al 2019). These studies primarily

9   involve investigating local adaptations and where historical literature or museum

10   specimens exists they can be an important resource, , to test for evolutionary rescue.

11   For example, Higgins et al. (2014) repeated a 40 year old study on the thermal

12   physiology of *Colias spp.* butterfly larvae suggested that their thermal physiology had

13   shifted, and the shift was associated with climate change. The use of historical

14   material in these instances, though currently quite rare, can provide a more refined

15   adaptive evolution timeline for individual populations that are experiencing rapid

16   environmental change, to establish whether or not evolutionary rescue takes place in

17   the wild (Bell 2017 Mills et al. 2018).

18   At the beginning of the 20th Century a series of carefully constructed and described

19   experiments in which the wound-healing and regeneration performance over a range

20   of environmental salinities of a small free-living flatworm from Wembury Bay,

21   (Plymouth, UK) were carried out (Lloyd 1914). The object of those experiments was

22   to '*discover how far marine turbellarians could be made to undergo variation while*

23   *regenerating lost parts*' (Lloyd 1914). *Procerodes littoralis* (Strom 1768) is a common

24   intertidal planarian found where fresh water runs out over gravel and sand under

25   loose rocks, in mid to upper shore intertidal streams where daily, it is subjected to

1   both sea and fresh water (S=35 - 0) (Hayward & Ryland 2012, McAllen et al. 2002,

2   Pantin 1931). They only encounter freshwater during the low tide period as the sea

3   retreats, and are submerged in sea water at high tide so it seems reasonable to

4   assume that during a tidal cycle *P. littoralis* experiences acutely a range of salinities

5   from fresh to full strength seawater depending on the tide height.

6   Regeneration is an evolved mechanism that mitigates the effects of damage by

7   restoring functionality (Reichman 1984). It is well-known that planarians possess

8   pronounced regeneration capabilities (e.g. Reddien & Alvarado 2004). As soft bodied

9   animals, planarians are susceptible to damage in the wild, which reduces

10   functionality. The healing times of animals capable of regeneration provides a

11   quantifiable proxy for fitness under different environmental conditions, allowing us to

12   link Darwinian fitness with physiological performance (Kaack & Pomory 2011).

13   Faithfully reproducing the study of Lloyd (1914) provides a rare opportunity to

14   compare physiological performance in a marine animal roughly 50 years pre- and

15   post- onset of the Anthropocene, an the Epoch which represents humans' influence

16   on Earth, that arguably began around 1950 (Crutzen & Steffen 2003, Steffen et al.

17   2007, Zalasiewicz et al. 2015). This might also provide an example of evolutionary

18   rescue in the wild, if differences in performance can be linked with local

19   environmental changes that have taken place over that same period of just over 100

20   years.

21   Anthropogenic climate change has caused increased precipitation since 1901

22   (medium confidence before and high after 1951) in the mid-latitudes of the Northern

23   hemisphere (IPCC 2014). Concomitantly, the frequency and intensity of precipitation

24   has likely increased in North America and Europe (IPCC 2014). Organisms are

1   restricted to certain locations by a range of abiotic and biotic factors, salinity (osmotic

2   pressure) being a key factor determining the distribution of aquatic species (Remane

3   & Schlieper 1971). Consequently, marine organisms (particularly coastal and

4   intertidal sessile and sedentary species) are subjected to a major physiological

5   challenge when faced with increased precipitation, which in some organisms can be

6   overcome (Lee & Bell 1999). As this species lives at the interface between streams

7   and sea water, individuals are likely to be directly influenced by such a hypothetical

8   increasing freshwater input. This species is also a good model because it does not

9   have a planktonic life stage so cannot migrate. Movement is further restricted by the

10   fact that seaward the stream bed is unsuitable for this species as it becomes a wave

11   cut platform with tidepools instead of loose rocks. Neither can they migrate upstream

12   as they are unlikely to outcompete other more freshwater flatworms that occupy a

13   similar niche, e.g. *Polycelis felina*.

14   Based on these environmental data and observations, there is an opportunity to

15   directly investigate potential physiological changes that have taken place

16   approximately 50 years pre- and post- onset of the Anthropocene (˜1950).

17   Therefore, the aims of our study were two-fold: (1) To look for evidence of

18   physiological differentiation over the past 100 years in a local, wild population in a

19   species where we have good historical data on physiological performance. (2) To

20   identify environmental change over the same period that might explain any detected

21   physiological differences.

22   This was done by repeating the experiment presented in Lloyd 1914 faithfully, using

23   regeneration (wound healing) time for *P. littoralis* (as *Gunda ulvae* in 1914) collected

24   from the exact same location as a quantitative proxy for fitness under a wide range

1   of salinity treatments and comparing their results with our own. The salinities range

2   (S = 6 – 53), was chosen to represent the salinities that *P. littoralis* could experience

3   tolerances for regeneration determined by Lloyd (1914). The rationale for including

4   the lowest salinity (S = 3) is that Lloyd (1914) suggested that flatworms cannot heal

5   at in such hyposaline water, and so including such a low salinity gives an opportunity

6   to investigate whether they have shifted their tolerance over the past century. The

7   rationale for using the higher salinity of S = 53 is that the previous study suggested

8   this was the species 'salinity extreme' and so we can test whether the species has

9   either shifted or expanded the niche window of tolerance. Niche expansion is a key

10 concept for physiology and ecology and has been studied in a wide range of taxa

11 (e.g. Lancaster et al. 2015, Weng et al. 2005) and so this present study provides an

12 excellent opportunity to broaden our knowledge by including poorly represented

13 groups, such as the Platyhelminthes. As acclimation salinity was not recorded in the

14 historic study method and because any difference detected between the historical

15 and present day experiment could potentially be a result of plasticity, we carried out

16 preliminary experiments to test for possible salinity acclimation plasticity in *P.*

17 *littoralis.* When the salinity of sea water changes, so too does the oxygen

18 concentration. And so, to test that what we termed a salinity effect in the core salinity

19 tolerance experiment was not confounded by a potential hypoxic effect, the effect of

20 reduced dissolved oxygen on regeneration time was investigated, keeping salinity

21 and dissolved oxygen concentration constant.

22 Finally, a climatic link to the broadened salinity tolerance (particularly at low

23 salinities) observed was suggested. As a consequence, local precipitation data were

24 analysed to test the prediction that there has been an increase in the daily mean

25 precipitation and a decrease in the number of days with no/trace precipitation per

1   year in Plymouth, from 1931 until present, to marshal support for the hypothesis that

2   a broadened salinity tolerance evolved in response to increased freshwater

3   exposure.

4

5   <div align="center">**2. Materials and methods**</div>

6   **2.1 Collection and maintenance of flatworms**

7   Using a clean artists' paint brush, *P. littoralis* were collected from beneath rocks in

8   the stream running down Wembury Bay, Devon, UK (50°19'03.3"N 4°05'03.8"W)

9   during Spring/Summer 2016 and Summer 2017. Flatworms were removed to the

10   laboratory within 60 min of capture and kept in continuously aerated, dilute sea water

11   (S = 22, T = 15⁰C) for 7 days where they were distributed between a number of small

12   aquaria (vol. = 8 L). This salinity was chosen as it represents the mid-range salinity

13   they experience in the wild (McAllen et al. 2002). During the holding period,

14   flatworms were fed *ad libitum* on chopped defrosted cattle liver prior to a water

15   change each day.

16   **2.2 Regeneration performance at different salinities**

17   To compare regeneration (wound healing) performance and tolerance over a range

18   of different salinities the method presented in the previous study was followed as

19   closely as possible (Lloyd 1914). Each 5 mL well in a 12-well plate was randomly

20   assigned a treatment of either S = 3, 6, 28, 44 or 53, identical salinities to those used

21   in the previous study. The desired salinities were constructed by carefully mixing

22   deionised water with natural sea water (S = 3, 6 and 28) or by dissolving Instant

23   Ocean (Aquarium Systems) in natural sea water (S = 44 and 53). Any loss due to

1   evaporation was replaced by topping up with deionised water and the salinity

2   checked using a hand-held refractometer (Atago Master-One, Kyoto, Japan).

3   Individual flatworms ($n > 15$ per treatment) were starved for 3 d and then bisected

4   along the transverse axis using a sharp scalpel blade, exactly as the previous study.

5   The anterior section of the individual was placed into separate treatment wells using

6   a clean, fine paintbrush and posterior ends were discarded, the same as the

7   previous study. To prevent the treated flatworms escaping, mesh (0.5 mm) was

8   secured over the wells on the well plate and a lid was placed over the mesh to

9   reduce evaporative water loss.

10   After the flatworms were bisected, the degree of regeneration of the posterior end of

11   the anterior section from each individual was assessed visually every 24 h under low

12   power magnification (EZ4, objective 8-35X, Leica Microsystems). Regeneration was

13   defined as when no tissue protruded out of the bisected area and the wound was

14   replaced with a smooth posterior, see Fig S1 for a photographic visual aid. Worms

15   were not fed after being bisected in accordance with Lloyd 1914's methodology.

16   Water was replaced in each well every 24 h. The experiment was carried out twice

17   with a fresh batch of flatworms. There was no significant difference in wound healing

18   time at the same salinity treatments between the first experiment and the repeat data

19   sets (Kruskal-Wallis test; $P = 0.961$), and so data were pooled ($n \leq 30$ per treatment)

20   before analysis.

21   In the Lloyd 1914 study wound healing time was expressed as a range in days. We

22   simulated the data from this range using a random number generator in R Studio

23   corresponding to the same sample size employed in the current study. These

24   generated data were subsequently used for statistical analysis.

1   To formally test for any temporal difference (Loyd 1914 vs current study) in the

2   relationships between wound healing time and salinity , an estimation statistics

3   approach (Ho et al., 2019) was adopted To quantify any differences detected

4   Gardner-Altman plots were generated using an open source website

5   (https://www.estimationstats.com/#/analyze/two-independent-groups) The 95%

6   bootstrap confidence intervals (bias-corrected and accelerated) were calculated from

7   a nonparametric sampling of the data. P-values from two-sided permutation t-tests

8   were also calculated and are reported alongside confidence intervals in the following

9   format (mean difference = x (95 % confidence intervals—upper limit, lower limit), p =

10   ). For each permutation P value, 5000 reshuffles of the control and test labels were

11   performed.

12

13   **2.3 Plasticity and salinity acclimation**

14   To test the possibility that the difference between the previous study (Lloyd 1914)

15   and the present-day study could be accounted for by acclimation, and was therefore

16   a result of plasticity, the following experiment was carried out. Flatworms ($n = 12$ per

17   treatment) from a stock aquarium (S = 22) were transferred to another aquarium and

18   were kept at S = 22 or acclimated to 35 for 48 h before being used in the plasticity

19   experiment described below. This acclimation period was relatively short because

20   the flatworms experience large salinity fluctuations in the wild and so, it was

21   assumed that they did not require a protracted period to adjust to changing ambient

22   salinities.

23   Bisection, husbandry and wound healing determination were carried out exactly as

24   described above (Sect.. 2.2), except that flatworms were kept at only one test salinity

1   (S = 53). The rationale behind the use of such high salinity was that it should,

2   potentially produce the most sensitive results compared to choosing a lower salinity

3   that the worms may be more suited to. This is because of higher mortalities and

4   longer healing times that were exhibited in the S = 53 treatment compared to the S =

5   3 treatment and it was important to ensure we included a salinity treatment that was

6   used in the main experiment.

7   This experiment was conducted twice, the repeat experiment used flatworms

8   collected from the same location but at a different time. There was no significant

9   difference between the resultant data sets (Kruskal-Wallis test; $P = 0.961$), so they

10   were combined ($n = 24$). These data were treated as non-normally distributed

11   following a Shapiro-Wilk test and the effect of salinity acclimation tested using the

12   non-parametric Wilcoxon Rank Sum test with continuity correction.

13   **2.4 Regeneration after pre-acclimation to low oxygen tensions**

14   Here, the experiment described in section 2.2 was repeated using two extreme

15   salinity treatments S = 3 and 44. Flatworms ($n = 8$ per treatment) from the stock

16   aquaria (acclimated to S = 22, T = 15 ºC) were bisected along the transverse axis

17   and the anterior section of the individual was placed using a clean, fine artist's

18   paintbrush into separate air-tight glass jars to keep oxygen concentration as constant

19   as possible (each vol. = 11 mL) containing one of four experimental treatments: low

20   salinity-normoxia, high salinity-normoxia, low salinity-hypoxia and high salinity-

21   hypoxia. Seawater solutions of different salinity were prepared exactly as described

22   above. Normoxic conditions (nominally $O_2 = 8$ mg L^{-1}) were created by gently

23   aspirating the sea water solution in the jar with scrubbed atmospheric air supplied,

24   *via* an air pump. Hypoxic conditions (nominally $O_2 = 3$ mg L^{-1}) were produced by

1   aspirating the sea water solution in the jar with a scrubbed air: nitrogen mix.

2   Regeneration time was determined exactly as described for the experiment outlined

3   in section 2.2. These data were treated as non-normally distributed and

4   heterogenous according to the Shapiro-Wilk test and Levene's test, therefore any

5   effects of hypoxia were tested for using the non-parametric Kruskal-Wallis test.

6   **2.5 Local precipitation data**

7   Daily precipitation data collected from the Mount Batten Weather Station, Plymouth,

8   Devon, UK between 1931 and 2016 (except 1946, 1947 and 1948) were obtained

9   from the Met Office National Meteorological Archive, Exeter. The mean daily rainfall

10   from each year between 1931 and 2016 (except 1946-48 due to missing values, $n =$

11   83) were determined non-seasonal after decomposing the data and a Holt-Winters

12   exponential smoothing with trend and without seasonal component was performed.

13   Raw data had less than 10% missing values and those missing values were repaired

14   using the Seasonally Decomposed Missing Value Imputation function. A 15 year

15   forecast of mean daily rainfall in Plymouth from the Holt-Winters filtering with a lag of

16   20 was produced. To ensure that these data met the assumptions of the Holt-

17   Winters model, further analyses were conducted and included: Pearson's correlation

18   coefficient and a KPSS test for correlation and stationarity, an autocorrelation plot

19   and Ljung Boxplot analyses to test for autocorrelation, a histogram of forecasted

20   errors was produced and a Shapiro-Wilks test to identify normality and, a plot was

21   produced to identify homogeneity in the variance of the residuals. Number of days

22   with no/trace precipitation from each year between 1931 and 2016 (except 1946,

23   1947, 1948 and 1969) ($n = 82$) were extracted and treated as normally distributed

24   following a Shapiro-Wilk test and were tested for a correlation with time (year) using

25   a Pearson's correlation coefficient.

1 **2.6 Statistical analyses**

2 Non estimation statistics analyses were conducted in R (version 3.2.4.) using

3 RStudio (version 1.1.383 RStudioTeam, 2016). Non-parametric tests (Shapiro-Wilk

4 and Levene's Test) were used to ensure statistical uniformality between treatments

5 as some data had non-normal distribution and were heterogenous, even after being

6 transformed logarithmically and using square root transformation. The estimation

7 statistics were conducted using an open source website

8 (https://www.estimationstats.com/#/analyze/two-independent-groups) (Ho et al.

9 2019)

10 <div align="center">**3. Results**</div>

11 **3.1 Regeneration performance at different salinities**

12 While in the modern experiment flatworms healed in all treatments, including the

13 lowest salinity used (S = 3), there was no healing at S = 3 as reported in the previous

14 study (Lloyd 1914) (Fig. 1A).

15 Mortality was greatest at the salinity extremes (S = 3 and 53) and decreased through

16 to the mid salinity (Fig. 1B). All flatworms in S = 3, 6 and 28 either healed or died

17 within 11 d. At S = 44 three individuals did not heal and at S = 53 seven individuals

18 did not heal within 11 d.

19 Simulated data generated from the results presented in Lloyd (1914) indicated that

20 there would have been significant differences across the salinity range examined.

21 We detected a significant difference between the modern data and the historic

22 (simulated) data at S = 6 (unpaired mean difference = 4.82 d (95 % CI 4.14, 5.29), P

23 < 0.05), S = 28 (unpaired mean difference = -2.57 d (95 % CI -3.47, -1.83), $P <$

24 0.05), S = 44 (unpaired mean difference = 1.74 d (95. % CI 0.474, 2.58) $P < 0.05$),

1   but not at S = 53 (unpaired mean difference = 0.444 d (95 % CI -1.56, 1.56) P =

2   0.499) (Fig. 2).

3   **3.2 Salinity acclimation plasticity**

4   Flatworms exhibited no significant salinity acclimation plasticity under the conditions

5   tested. There was no significant difference in regeneration times between

6   differentially acclimated individuals (S = 22 and 35) (Wilcoxon Rank Sum test with

7   continuity correction, P = 0.572). Wound healing took approximately 8 d in each

8   case. However, an increase in mortality (10 more deaths) was recorded at S = 22

9   compared with S = 35 acclimated flatworms. The number of unhealed individuals by

10   11 d was two from S = 22-acclimated and four from S = 35-acclimated flatworms.

11

12   **3.3 Wound healing performance and pre-acclimation to low oxygen tensions**

13   Dissolved oxygen concentration did not affect wound healing time of flatworms.

14   There was no significant difference in wound healing time of flatworms exposed to

15   hypoxia or normoxia at either salinities (Kruskal-Wallis test, P = 0.195, Fig. 3).

16   Mortality was low in all treatments, one death in S = 3 and S = 44 at O_2 = 3 mg L^{-1} in

17   both cases.

18

19   **3.4 Precipitation in Plymouth since 1931**

20   The Holt-Winters Exponential smoothing with trend and without seasonal component

21   model was generated with the following smoothing parameters and coefficients and

22   the sum of squared errors was calculated. A forecast from the Holt-Winters

1   smoothing model was predicted for 15 years into the future (Fig 4.) This suggests an

2   overall upward trend in mean daily rainfall.

3   Mean daily precipitation in Plymouth showed a significant positive correlation with

4   time between 1931 and 2016. The mean daily rainfall data was described as non-

5   stationary with a trend following significant results from a KPSS test (P=0.0361) and

6   Pearson's correlation (R^2 (adj) = 0.04663, P < 0.05, n = 83) (Fig 5.A). An

7   autocorrelation plot was produced from the forecast and the residuals were touching

8   the confidence interval line (0.2) and so a Ljung-Box Test was conducted to test for

9   non-zero autocorrelation. This test was non-significant (P>0.05) with a lag maximum

10   of 20 and so there is little evidence of non-zero autocorrelations in the sample with

11   forecast errors at lags 1-20 (Fig 5.B). A constant variance within the residuals was

12   assumed (Fig 5.C). A histogram was then generated to visualise the forecast errors

13   which suggests that the errors are roughly centred on zero but slightly skewed to the

14   left. A Shapiro-Wilks test for normality was then conducted which was non-significant

15   (P>0.05) so the data can be considered normally distributed (Fig 5.D).

16   Number of days with no/trace precipitation in Plymouth showed a significant negative

17   correlation with time between 1931 and 2016 (Pearson's correlation, R^2 (adj) =

18   0.2433, $P < 0.005$, $n = 82$) (Fig. 6).

19

20   **4. Discussion**

21   Since 1914, a key aspect of physiological performance, namely regeneration, in

22   waters of different salinities of the Wembury Bay population of *P. littoralis* has

23   broadened. Although we cannot fully discount the suggestion that methodological

24   variations could still play a role in the results, we suggested that we have presented

1   good evidence that these flatworms now perform and survive better in hyposaline

2   waters than they did over century ago. The lowest salinity limit for healing in 1914

3   was S = 5.7 and currently is at least S = 3 (Lloyd 1914). Furthermore, the Wembury

4   population in 1914 displayed an optimum performance salinity of S = 28.5 (Lloyd

5   1914) whereas in our study, performance was better across a wider salinity range

6   with no obvious optimum. Physiologically, this suggests that *P. littoralis* has adapted

7   further into hyposaline waters whilst simultaneously maintained the ability to heal in

8   hypersaline conditions. Therefore, we suggest that our study provides evidence that

9   the population has experienced a microevolutionary niche expansion, shifting from

10   specialism to generalism in terms of salinity tolerance performance, over

11   approximately 100 years. In this study microevolution is defined as a change in a

12   measurable trait in individuals of a population over a short time period. This

13   conclusion is supported by the performance curve across the salinity treatments from

14   this study fits a linear model (where there is a positive correlation between mean

15   wound healing time and salinity). Lloyd's 1914 data do form a 'typical' environmental

16   tolerance normal distribution curve, whereby performance is best in the mid-range of

17   the salinities which tails off towards the extremes (S = 3 and 53). This change does

18   not seem to be explained by acclimation, but is possibly attributable to environmental

19   salinity change *per se*, rather than accompanying physico-chemical changes in

20   oxygen solubility affecting oxygen availability.

21   Performance curves can be used to qualitatively model physiological tolerance

22   against energy expenditure. Although commonly used to characterise thermal

23   tolerances, performance curves provide a useful tool to visualise the response of an

24   organism to environmental stress (Chown et al. 2010). The regeneration

25   performance of *P. littoralis* at a range of salinities from the historical and modern

1 study can be presented together (Fig. 4A). This visualisation is comparable to and

2 supports the specialism versus generalism model proposed by (Huey & Hertz 1984)

3 (Fig. 4B). In this microevolutionary process, the population of *P. littoralis* has gained

4 breadth and lost maximum performance in terms of salinity tolerance. During

5 environmental change, specialism can be disadvantageous and a strategy

6 associated with extinction (Colles et al. 2009, McKinney 1997). Whereas in thermal

7 performance models, it has been suggested, that those with broader tolerance

8 ranges (generalists) are favoured in variable environmental conditions (Gilchrist

9 1995). Therefore, we suggest that increasing salinity variability over 100 years due to

10 increased precipitation in Plymouth along with usual tidal cycles, has favoured

11 individuals with broader salinity tolerance ranges (generalists). According to the

12 theory of evolutionary rescue (Gomulkiewicz & Holt 1995), these generalists could

13 potentially evolutionarily 'rescue' the Wembury Bay, Devon population of *P. littoralis*

14 from the effects of the projected precipitation increase in the mid latitudes. This type

15 of occurrence is rare but not unknown, as a shift in performance curves from a

16 specialist curve to a generalist curve in 40 years, associated with climate change has

17 also been documented previously in a terrestrial species (Higgins et al. 2014).

18 Evolutionary rescue in response to rapid climate change has been supported by

19 studies of experimental evolution (Bell & Gonzalez 2009), but models suggest that

20 this may lead to overestimations of evolvability in wild, locally adapted populations

21 (Schiffers et al. 2012). Transferring information from evolvability models and applying

22 it to natural populations is challenging (Gomulkiewicz & Shaw 2012). Caution is

23 required when using evolutionary rescue in terms of this study, as factors other than

24 healing time may influence fitness.

1   An alternate interpretation of our data is that what we are witnessing is not niche

2   expansion or evolutionary rescue of one species, but the consequences of

3   speciation. While in the current experiment more individuals died at the salinity

4   extremes, healing was quicker at S = 6 compared with S = 28. It could be suggested

5   that two species are living in this habitat but occupy different salinities. One species

6   could have exhibited greater healing performance at the higher salinities (S ≥ 28)

7   and so, died more frequently at the lower salinities (S = 3 and 6). The second

8   species could have a greater healing performance at the lower salinities (S ≤ 28) and

9   so, died more frequently at the higher salinities (S = 44 and 53). It is also worth

10   noting that the mortality data follow a similar pattern to Lloyd 1914 healing time data,

11   this adds to the evidence that a sub section of the population has diverged into

12   hyposaline tolerance and the other has remained physiologically similar to the

13   population from 1914. In Plymouth there could be a second congeneric species

14   named *Procerodes irenae* (Hartog 1968)*.* This suggestion received little support and

15   it was concluded that the morphological differences observed were not enough to

16   warrant both phenotypes being recognised as separate species (Ball & Reynoldson

17   1981). That said, given the recent advancement in molecular techniques in

18   phylogenetic analysis, and as generalists are favoured when faced with climate

19   change, it may be interesting to revisit this 'two species' hypothesis.

20   It is important to recognise that repeating this experiment does introduce some

21   variables that cannot be mitigated against. These include inconsistencies with the

22   method, for example the mixture of salts used to make the high salinity seawater

23   would be different as well as the water chemistry in the freshwater used to make the

24   lower salinity treatment. The acclimation conditions could be different, but it is

25   assumed this would not be the case due to the results from the acclimation plasticity

1   experiment. In this experiment the flatworms were fed to ensure they all were

2   starting from the same energy budget, but in Lloyd they were starved. It is not

3   thought that this would have an effect on the results as the previous study states

4   "*When bisected, [the worms] restored their normal form as rapidly as did fully fed*

5   *specimens*" (Lloyd 1914). Simulating Lloyd's data could introduce bias but the

6   difference observed is large enough that this would not cause a large enough effect.

7   The evolution of niche expansion has been shown to be attributed to biotic factors

8   such as, intraspecific competition (Bolnick 2001) and predation (Seeley 1986) as well

9   as abiotic factors such as, physical environmental shifts (Scott et al. 2003). A

10 potential driver for the evolutionary shift reported in this study, could be a change in

11 local precipitation. The average increase of daily precipitation in Plymouth since

12 1931 is in accordance with reports (IPCC 2014), that state that precipitation has

13 increased in the mid-latitudes of the Northern hemisphere since 1901 (IPCC 2014).

14 As previously stated, *P. littoralis* is directly at risk from increased freshwater input as

15 it inhabits streams that run into the sea (McAllen et al. 2002, Pantin 1931) and as

16 this species has no planktonic life stage it is unlikely to be capable of migrating to

17 another area. This increase in precipitation offers a potential explanation as to why

18 *P. littoralis* has developed wound healing ability at $S = 3$ and improved healing

19 performance at $S = 6$. Maintaining an ability to heal at higher salinities is also

20 important as warming also threatens mid-latitudes too (IPCC 2014). Increasing

21 temperatures could cause coastal areas to experience increased salinities due to

22 evaporation, and so the nature of this microevolutionary niche expansion buffers

23 against increased precipitation and temperature.

24 *P. littoralis* has physiologically expanded into hyposaline waters and, now has

25 potential to invade freshwater. In the past 200 years, this phenomenon has been

1   facilitated by humans, usually by transplantation or habitat modification (Lee & Bell

2   1999). To our knowledge, there is no evidence suggesting that marine planarians

3   have invaded freshwater over a microevolutionary time frame. As cryptic species

4   though, planarians may often be overlooked in terms of investigating freshwater

5   invasions. *P. littoralis* is usually restricted to the stony substratum of the intertidal

6   zone (Ball & Reynoldson 1981). At Wembury Bay the stony substratum extends up

7   to the fully freshwater stream, so in this case, substratum is not the limiting factor.

8   The other restriction is, that *P. littoralis* cannot reproduce in freshwater, although it

9   needs to be confirmed whether or not individuals from the Wembury Bay population

10   can complete a life cycle in hyposaline water (Ball & Reynoldson 1981). Finally, all

11   triclad flatworms are carnivorous and niche competition between *P. littoralis* and the

12   freshwater planarian *Polycelis felina* (found further upstream at Wembury Bay) may

13   also be a barrier to this population of *P. littoralis* extending further upstream into

14   freshwater.

15   To conclude, it is suggested that a microevolutionary niche expansion has occurred

16   within the same population of *P. littoralis* over the past 100 years. Comparing

17   historical findings with modern data provide an intermediate method between

18   experimental evolution and palaeontological analysis of the fossil records to explore

19   evolutionary physiology. The former may not be ecologically relevant (Carlson et al.

20   2014).The latter can be described as saltational or discontinuous evolution, lacking

21   resolution to investigate transitionary periods in evolution on the scale presented in

22   this study (Kirkpatrick 1982). But we can now start to utilise historic data more widely

23   to better understand how organisms have changed and are changing in response to

24   the Anthropocene.

25   **Author Contributions**

15

16 **Literature Cited**

17 Ball IR, Reynoldson TB (1981) British planarians, Platyhelminthes, Tricladida : keys
18 and notes for the identification of the species. Cambridge University Press, New
19 York

20 Bell G, Gonzalez A (2009) Evolutionary rescue can prevent extinction following
21 environmental change. Ecol Lett 12:942–948

22 Bell G, (2013) Evolutionary rescue and the limits of adaptation. Phil Trans R Soc B:
23 Biol Sci *368*: 20120080

24 Bell G, (2017) Evolutionary rescue. Annu Rev Ecol, Evol Syst, 48: 605-627

25 Bolnick DI (2001) Intraspecific competition favours niche width expansion in
26 *Drosophila melanogaster*. Nature 410:463–466

1 Carlson SM, Cunningham CJ, Westley PAH (2014) Evolutionary rescue in a
2 changing world. Trends Ecol Evol 29:521–530

3 Chown SL, Hoffmann AA, Kristensen TN, Angilletta MJ, Stenseth NC, Pertoldi C
4 (2010) Adapting to climate change: A perspective from evolutionary physiology. Clim
5 Res 43:3–15

6 Colles A, Liow LH, Prinzing A (2009) Are specialists at risk under environmental
7 change? Neoecological, paleoecological and phylogenetic approaches. Ecol Lett
8 12:849–863

9 Crutzen PJ, Steffen W (2003) How long have we been in the Anthropocene era? An
10 Editorial Comment. Clim Change 61:251–257

11 Gilchrist GW (1995) Specialists and Generalists in Changing Environments. I.
12 Fitness Landscapes of Thermal Sensitivity. Am Nat 146:252–270

13 Gomulkiewicz R, Holt RD (1995) When does Evolution by Natural Selection Prevent
14 Extinction? Evolution (N Y) 49:201

15 Gomulkiewicz R, Shaw RG (2012) Evolutionary rescue beyond the models. Philos
16 Trans R Soc B Biol Sci 368:20120093–20120093Hartog C Den (1968) Marine
17 Triclads from The Plymouth Area. J Mar Biol Assoc United Kingdom 48:209–223

18 Higgins JK, MacLean HJ, Buckley LB, Kingsolver JG (2014) Geographic differences
19 and microevolutionary changes in thermal sensitivity of butterfly larvae in response
20 to climate. Funct Ecol 28:982–989

21 Ho J, Tumkaya T, Aryal S, Choi H. and Claridge-Chang A. (2019). Moving beyond P
22 values: data analysis with estimation graphics. Nat. Methods 16:565–566.

23 Huey RB, Hertz PE (1984) Is a Jack-of-All-Temperatures a Master of None?
24 Evolution (N Y) 38:441–444

25 IPCC 2014 Climate Change 2014: Synthesis Report. Contribution of Working Groups
26 I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate
27 Change (eds Pachauri RK, Reisinger A). IPCC, Geneva, Switzerland: IPCC

28 Kaack KE, Pomory CM (2011) Salinity effects on arm regeneration in *Luidia clathrata*
29 (Echinodermata: Asteroidea). Mar Freshw Behav Physiol 44:359–374

30 Kerfoot WC, Weider LJ (2010) Experimental paleoecology (resurrection ecology):
31 Chasing Van Valen's Red Queen hypothesis. Limnol Oceanogr 49:1300–1316

32 Kirkpatrick M (1982) Quantum evolution and punctuated equilibria in continuous
33 genetic characters. Am Nat 119:833–848

34 Lancaster LT, Dudaniec RY, Hansson B, Svensson EI (2015) Latitudinal shift in
35 thermal niche breadth results from thermal release during a climate-mediated
36 range expansion. J Biogeogr 42:1953–1963

37 Lee CE, Bell MA (1999) Causes and consequences of recent freshwater invasions
38 by saltwater animals. Trends Ecol Evol 14:284–288

1 Lloyd D (1914) The Influence of osmotic pressure upon the Regeneration of *Gunda*
2 *ulvae*. Proc R Soc London 88:1–20

3 McAllen R, Walker D, Taylor A (2002) The environmental effects of salinity and
4 temperature on the oxygen consumption and total body osmolality of the marine f
5 latworm *Procerodes littoralis*. J Exp Mar Bio Ecol 268:103–113

6 McKinney ML (1997) Extinction vulnerability and selectivity: Combining ecological
7 and paleontological Views. Annu Rev Ecol Syst 28:495–516

8 Mills LS, Bragina EV, Kumar AV, Zimova M, Lafferty DJ, Feltner J, Davis BM,
9 Hackländer K, Alves PC, Good JM, Melo-Ferreira J (2018) Winter color
10 polymorphisms identify global hot spots for evolutionary rescue from climate
11 change. Science 359: 1033-1036

12 Pantin CFA (1931) The adaptation of *Gunda ulvae* to salinity III. The electrolyte
13 exchange. J Exp Biol 8:82–94

14 Reddien PW, Alvarado AS (2004) Fundamentals of planarian regeneration. Annu
15 Rev Cell Dev Biol 20:725–757

16 Reichman OJ (1984) Evolution of regeneration capabilities. Am Nat 123:752–763

17 Remane A, Schlieper C (1971) Biology of Brackish Water, 2nd edn. John Wiley &
18 Sons, Inc., New York

19 Rice KJ, Emery NC (2003) Managing microevolution: restoration in the face of global
20 change. Front Ecol Evol 1:469–478

21 RStudioTeam (2016) RStudio: Integrated development environment for R.

22 Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. Ann Rev Mar
23 Sci 3:509–35

24 Schiffers K, Bourne EC, Lavergne S, Thuiller W, Travis JMJ (2012) Limited
25 evolutionary rescue of locally adapted populations facing climate change. Philos
26 Trans R Soc B Biol Sci 368:20120083–20120083

27 Scott SN, Clegg SM, Blomberg SP, Kikkawa J, Owens IPF (2003) Morphological
28 shifts in island-dwelling birds: The roles of generalist foraging and niche expansion.
29 Evolution (N Y) 57:2147–2156

30 Seeley RH (1986) Intense natural selection caused a rapid morphological transition
31 in a living marine snail. Proc Natl Acad Sci 83:6897–6901

32 Steffen W, Crutzen PJ, McNeill JR (2007) The Anthropocene: Are humans ow
33 overwhelming the great forces of Nature. AMBIO A J Hum Environ 36:614–621

34 Strom H (1768) Beskrivelse over Norske Insecta. Kongl. Norske Videnske. Selsk.
35 Skr. Trondheim IV:313–371

36 Weng KC, Castilho PC, Morrissette JM, Landeira-Fernandez AM, Holts DB, Schallert
37   RJ, Goldman KJ, Block BA (2005) Satellite tagging and cardiac physiology
38   reveal niche expansion in salmon sharks. Science (80-) 310:104–106

1   Zalasiewicz J, Waters CN, Williams M, Barnosky AD, Cearreta A, Crutzen P, Ellis E,
2   Ellis MA, Fairchild IJ, Grinevald J, Haff PK, Hajdas I, Leinfelder R, McNeill J, Odada
3   EO, Poirier C, Richter D, Steffen W, Summerhayes C, Syvitski JPM, Vidas D,
4   Wagreich M, Wing SL, Wolfe AP, An Z, Oreskes N (2015) When did the
5   Anthropocene begin? A mid-twentieth century boundary level is stratigraphically
6   optimal. Quat Int 383:196–203

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8   **Figure Legends**

9   Fig. 1. Effect of different salinity treatments on the regeneration (wound healing)

10   time (A) and mortality (B) of bisected *Procerodes littoralis.* A. Wound healing times

11   from the modern study are displayed as grey bars. Simulated wound healing times

12   taken from a range presented in Lloyd 1914 are displayed as black bars. Values are

13   expressed as means ± 1 S.E.

14   Fig. 2. The mean difference between the modern data and historic (simulated) data

15   for S = 6, 28, 44 and 53 is shown in this Gardner-Altman estimation plot. Both groups

16   are plotted on the left axes; the mean difference is plotted on a floating axes on the

17   right as a bootstrap sampling distribution. The mean difference is depicted as a dot;

18   the 95% confidence interval is indicated by the ends of the vertical error bar (Ho et

19   al. 2019).

20   Fig. 3. Effect of high (O_2 = 8 mg L^{-1}) and low (O_2 = 3 mg L^{-1}) dissolved oxygen on the

21   regeneration time of *Procerodes littoralis* in S=3 and S=44 salinity treatments.

22   Values are expressed as means ± 1 S.E (S = 3 at 3 mg L^{-1} n= 7; S = 3 at 8 mg L^{-1} n = 8;

23   S = 44 at 3 mg L^{-1} n = 7; S = 44 at 8 mg L^{-1} n = 8). Note: there are no error bars on the S-

24   3 8 mg L^{-1} bar because all individuals healed on the same day.

25

26   Figure 4. Mean daily rainfall data collected from Mount Batten, Plymouth (black line).

27   Holt-Winters exponential smoothing of the mean (red line) and the forecast 15 year i

1 nto the future (blue line). The dark blue shaded area represents the 80% prediction i

2 nterval and the grey shaded area is the 95% prediction interval.

3

4 Figure 5. (A) Average daily precipitation (mm) per year at Mount Batten, Plymouth, U

5 K between 1931 and 2016. Line of best fit shows a significant positive relationship. (

6 P<0.05, n=83). Data courtesy of the Met Office National Meteorological Archive, Exet

7 er.

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9 Fig 6. Number of dry days (no precipitation/trace) per year at Mount Batten, Plymout

10 h, UK between 1931 and 2016. Line of best fit shows a significant positive relationshi

11 p (P<0.005, n=82). Data courtesy of the Met Office National Meterological Archive, E

12 xeter.

13 Fig. 7. (A) Performance curves for regeneration ability of *P. littoralis* across a range o

14 f salinities from Lloyd's 1914 study and from the present study. The present study val

15 ues are a mean from each salinity treatment, Lloyd's performance curve is generated

16 from medians from the ranges recorded in Lloyd's results. Performance indicates the

17 inverse of wound healing time, the higher the performance the quicker the wound he

18 aling time, where the curve meets the x axis this indicates that the worms cannot hea

19 l at that salinity. (B) The specialism versus generalism model proposed by Huey and

20 Hertz (1984).

21

22 Fig. S1 (A) A *P. littoralis* that has been bisected and is in the process of healing, note

23 the protrusions at the posterior end which indicates an open wound. (B) The posterio

24 r end of a *P. littoralis* that has been bisected and is now healed, B.1 highlights the s

1   mooth posterior, this was at the point where individuals would be marked as healed.

2   Scale bars indicate approximately 1 mm.

3   Fig. S2. (A) An autocorrelation plot of the forecasted residuals generated from the H

4   olt Winters forecast, blue dashed line indicates the confidence interval line. B) Plotte

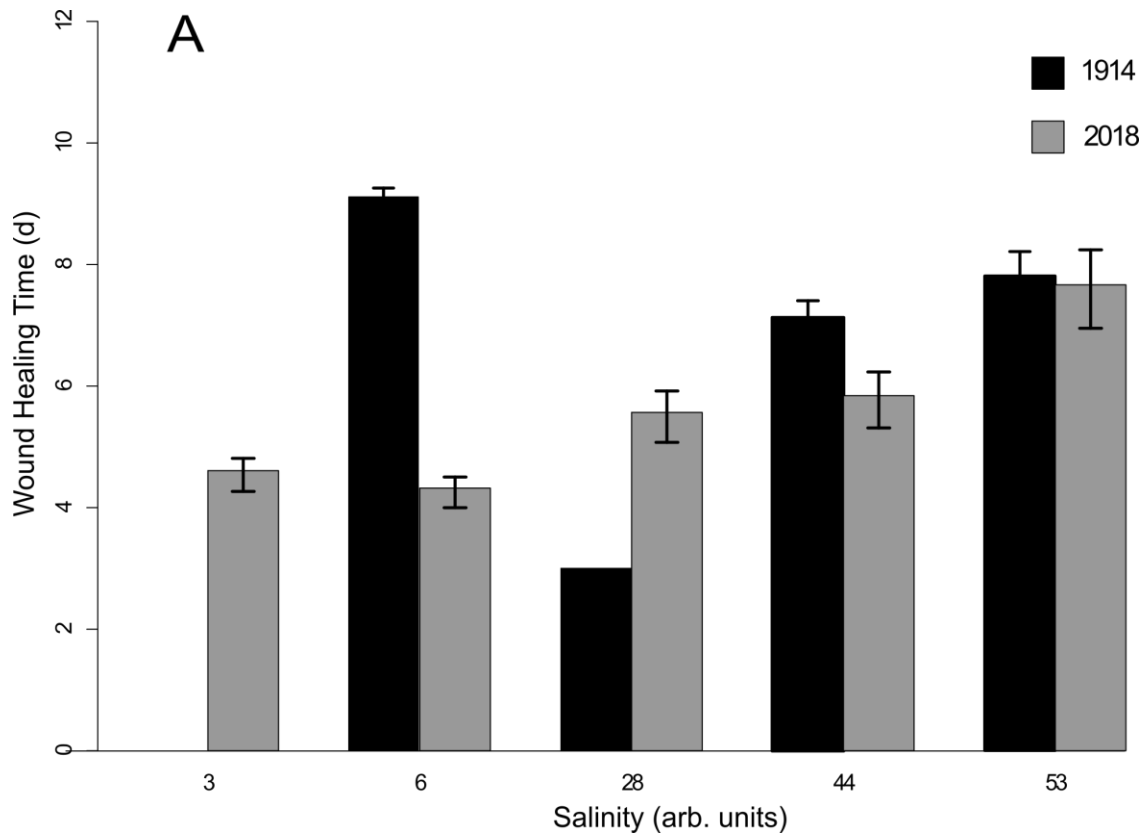5   d residuals of the mean daily rainfall data that have been detrended showing a rough

6   constant (homogenous) variance. (C) A histogram of forecasted errors from the Holt-

7   Winters model indicating a normal spread around zero with a slight skew to the left.

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11   Clayton & Spicer, Figure 1

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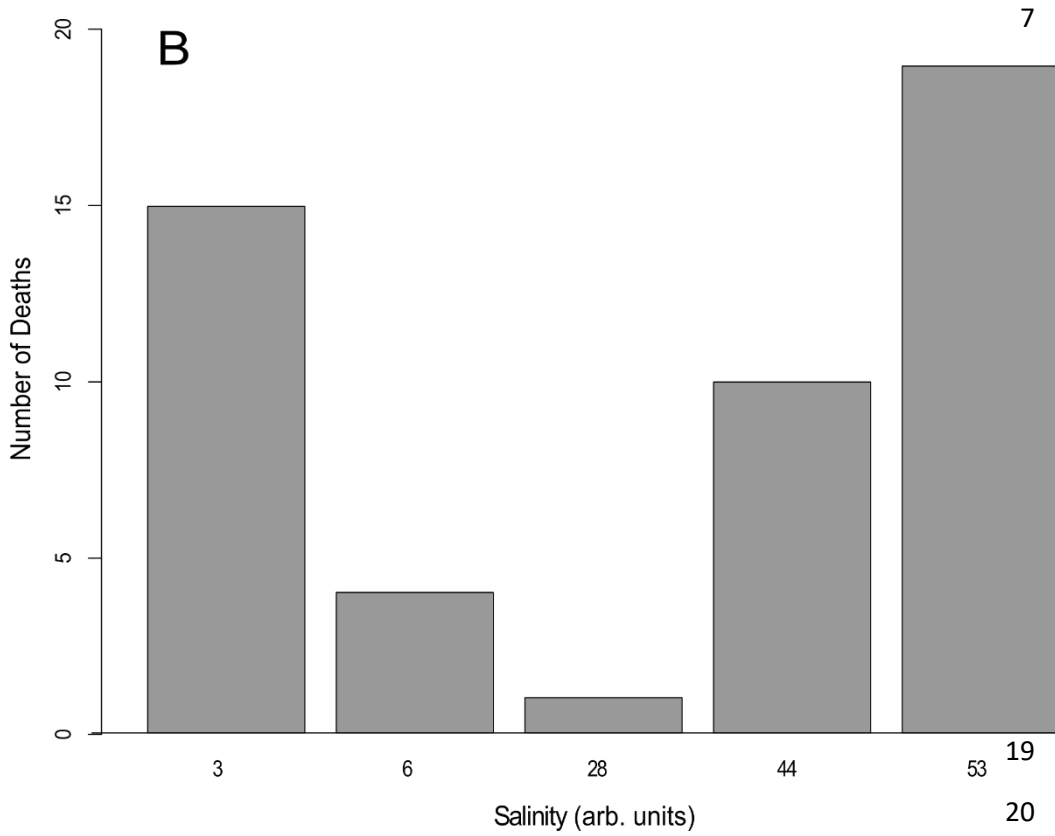
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23 Clayton & Spicer, Figure 2

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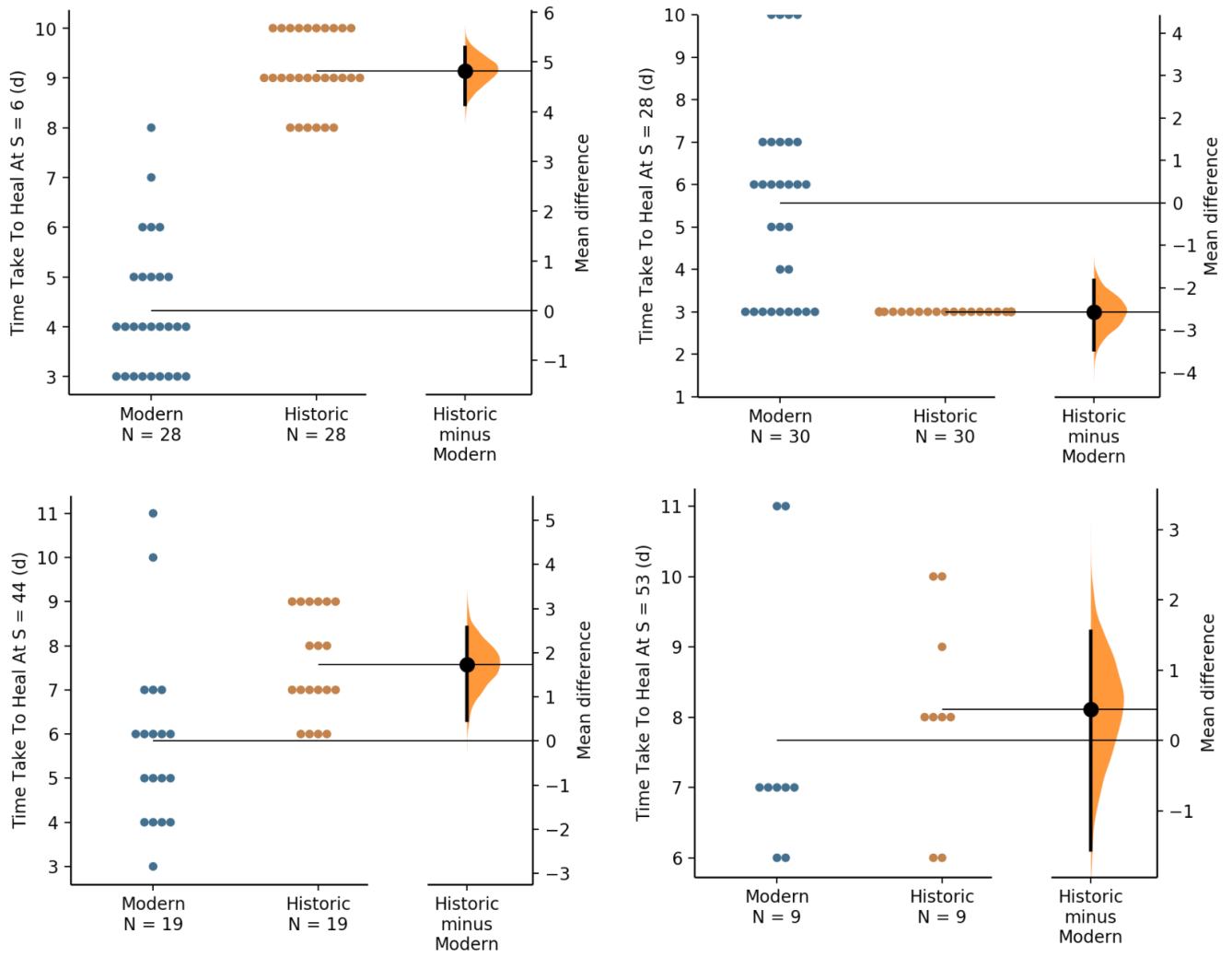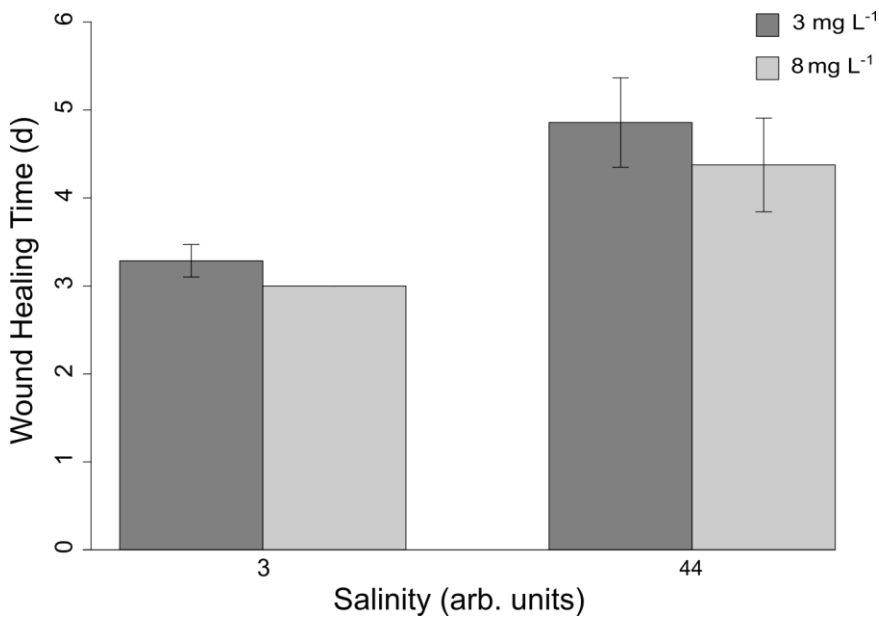
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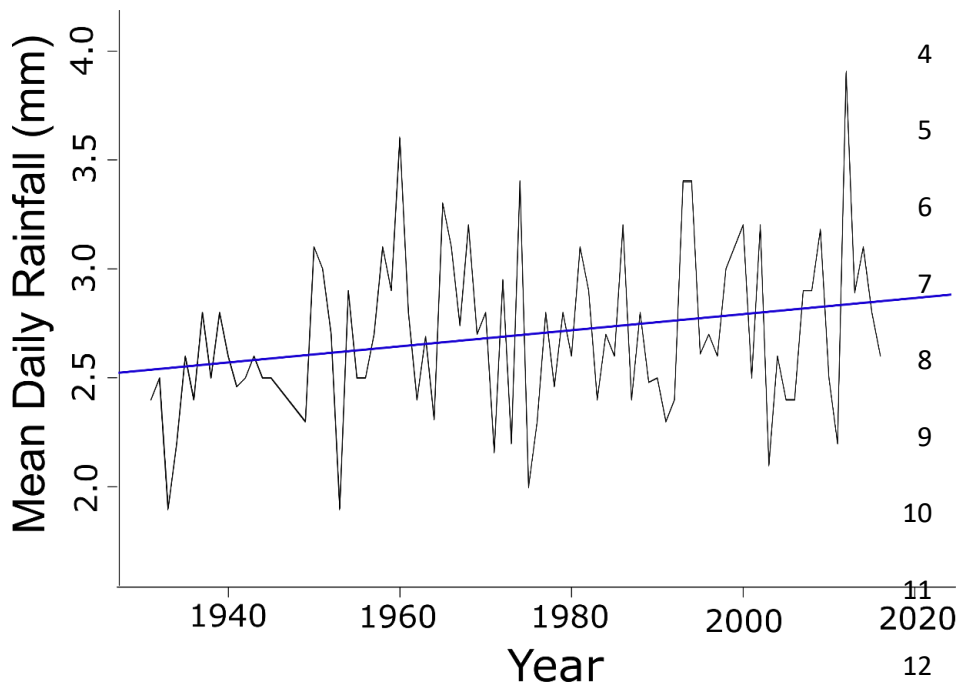
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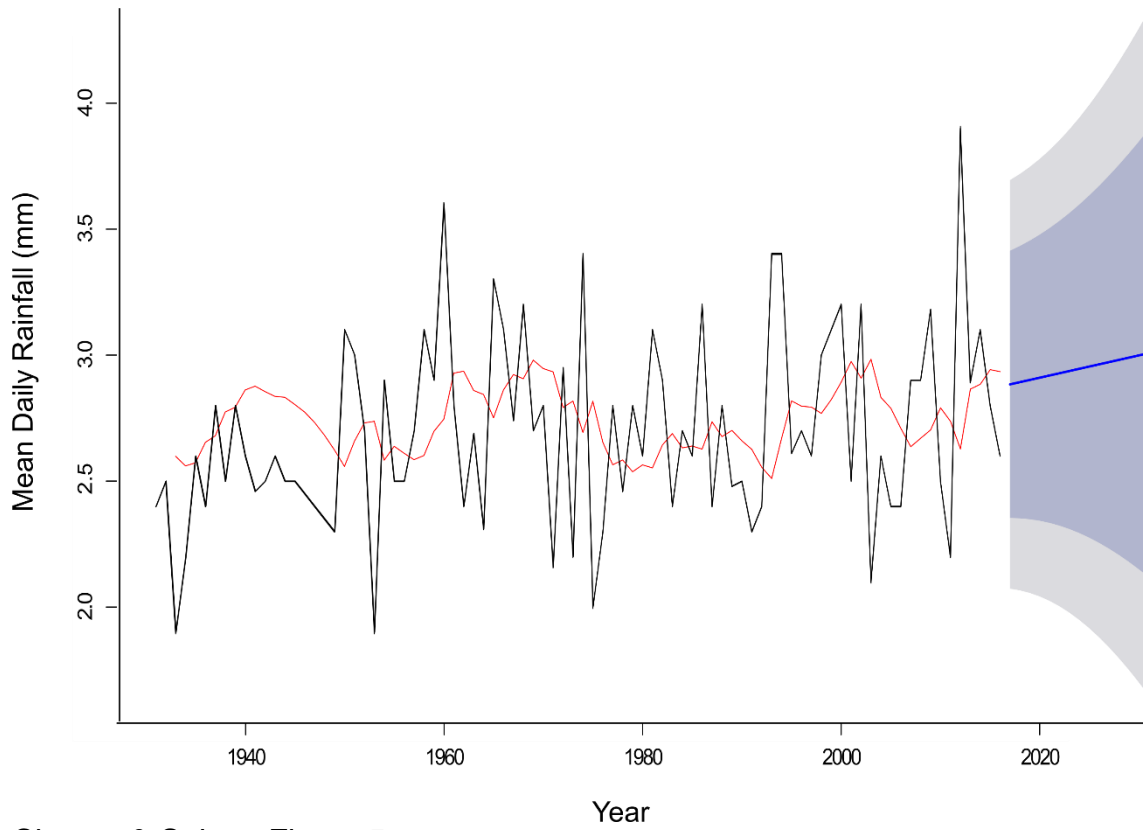
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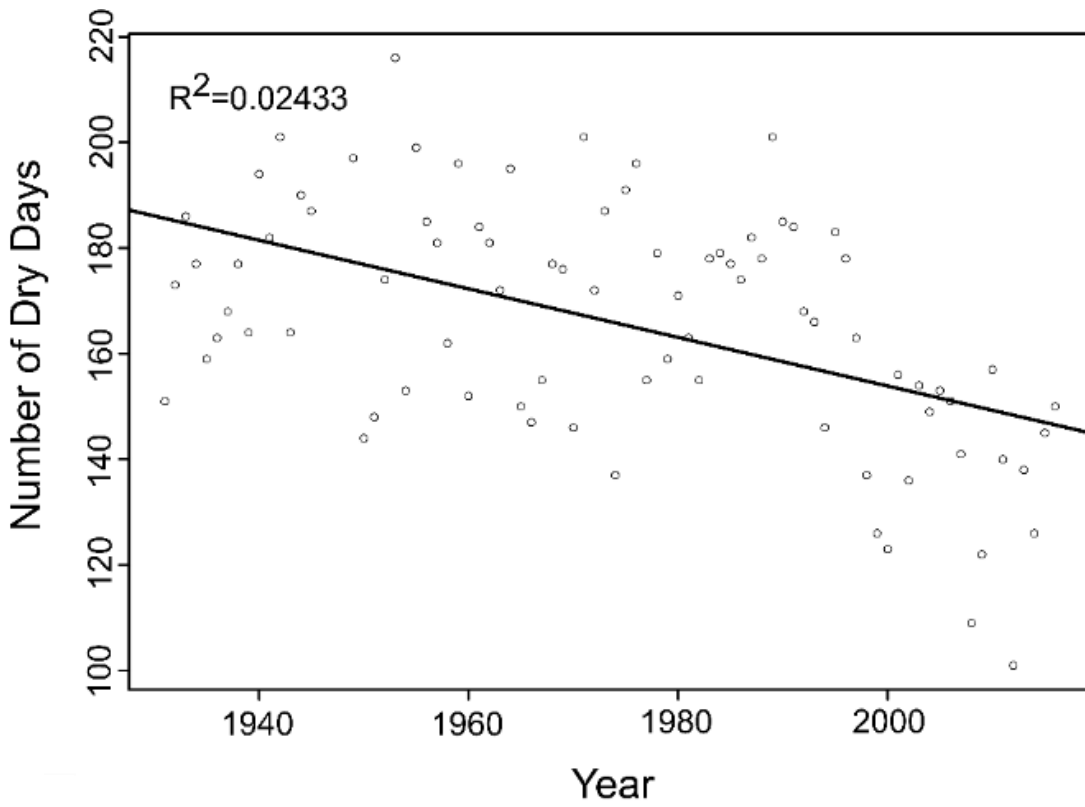
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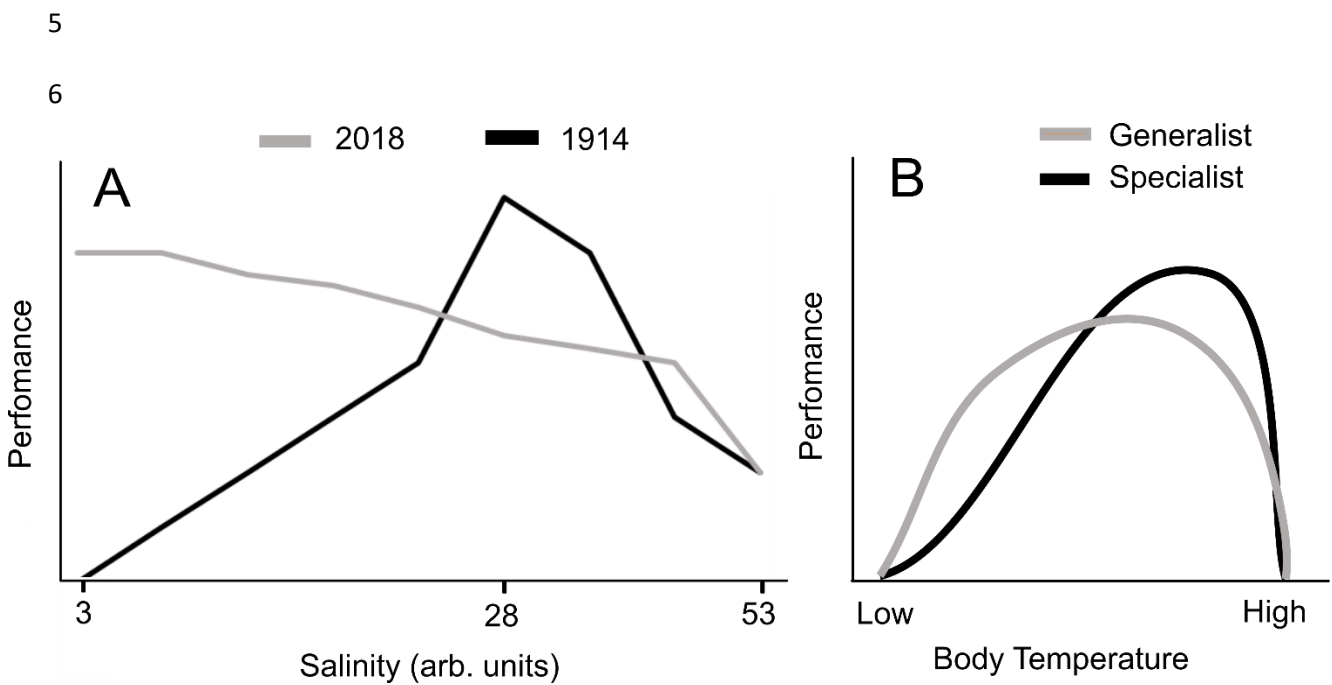
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9   Clayton & Spicer, Figure S1

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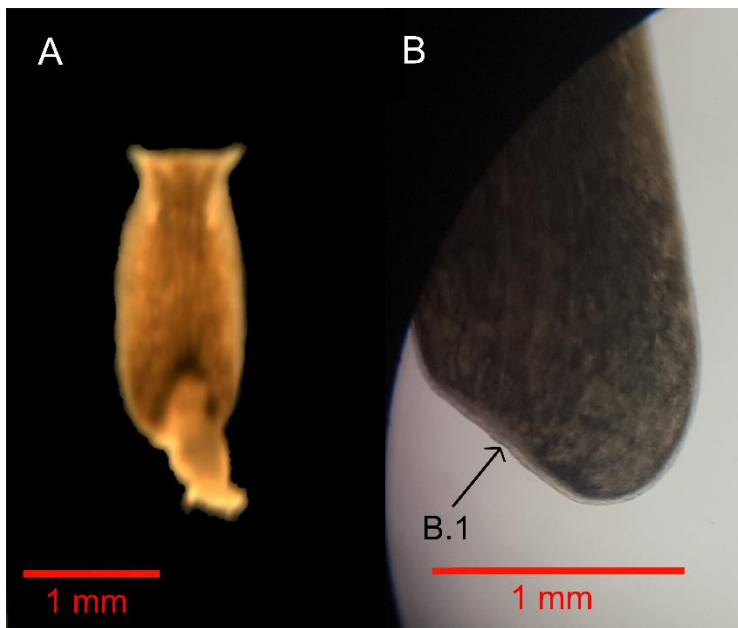
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1   Clayton & Spicer, Figure S2