A model-based assessment of adaptation in embryonic life histories of annual killifish

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Abstract

Annual killifish embryos can arrest development in different diapauses and survive desiccation of temporary ponds in soil egg banks. They seem to implement diversified bet-hedging, generally seen as an adaptation to uncertain environments. Using a pragmatic eco-evolutionary model tailored to annual fish, it is investigated whether rates of embryonic survival and development and hatching could be adapted to pond filling regimes with within-year variation only.

In the model, ponds fill and dry gradually. Locations within a pond where embryos are buried can therefore differ in seasonal dryness patterns. Results from lab experiments on the Argentinian pearlfish *Austrolebias bellottii* are used to model embryonic life histories and hatching probabilities. Maturation, survival and reproduction of fish are modelled in agreement with lab experiments and field data on the same or related species.

Invasion fitness sensitivities of four stage-specific rates of development, five mortality rates and two hatching probabilities were estimated. Faster development rates and lower mortalities were always favoured. Only trade-offs or constraints could keep them at their current values in the simulated environments. For hatching probabilities, pond filling regimes were found which made their invasion fitness sensitivities zero or, for hatching at the first rewetting, with different signs in different pond filling regimes. Between-year variability might render development rates adapted if faster rates would increase population size fluctuations which would be selected against. For hatching at first rewetting, an alternation between years with positive and negative selection will contribute to their adaptedness.

Keywords: Adaptation, environmental change, annual fish, diapause, pond life cycle, eco-evo-devo, cumulative hazards, longitudinal studies, Adaptive Dynamics.

Introduction

Evolutionary ecology has a long tradition of studying adaptation to uncertain environments (Cohen 1966, Ellner 1997, Stearns 2000, Evans & Dennehy, 2005). This has occurred conjointly with the development of modelling methods for stochastic population and evolutionary dynamics (Tuljapurkar 1990a) and has repeatedly inspired to relate trait variation to magnitudes of environmental unpredictability (e.g., Venable 2007, Pinceel et al. 2017). In recent years, there have been efforts to understand what the driving forces of evolution in uncertain environments are (Starrfelt & Kokko 2012), to understand whether and how plasticity and maternal effects evolve given uncertainty (de Jong & Behera 2002, Dey et al. 2016, King & Hadfield 2019, Van Dooren 2022). More realistic and less strategic modelling is also called for (Childs et al. 2010). On top of that, due to trends in environmental change, the stationary regimes we assume to determine what is adaptive, might not occur anymore and adaptational lags might prevail (Van Dooren 2022).

The study of evolution in uncertain environments tends to focus on so-called risk-spreading (Den Boer 1968) or delaying strategies (Tuljapurkar 1990b), of which diapause is probably the most studied in various organisms such as crustaceans, plants, worms and vertebrates (Evans & Dennehy 2005). Diapause, where development is arrested or interrupted, is the emblematic trait used to explain that evolution can favour reduced average growth rates and random bet-hedging (Simons 2011). Life history demographic processes and evolutionary demography are essential to understand adaptation in uncertain environments. A developmental diapause strategy which is adaptive in a certain environmental regime generates a distribution of individuals over diapausing and non-diapausing states. Demographic processes project individuals from there on forward in time to follow their fates and that of their descendants. In the long term, an adapted strategy is expected to prevail in a population as there then is no long-term directional selection in the given environmental regime giving rare alternative strategies a systematic advantage (e.g., Ellner 1997). Van Dooren &

Varela-Lasheras (2018) focused on the determination of embryonic life histories of annual killifish to understand the consequences of developmental decisions involving diapause, i.e., the numbers of embryos present when an opportunity to reproduce presents itself and the fraction remaining in an early diapause. If these developmental life histories can be embedded into a demography across the entire life cycle (Childs et al. 2010), we come a bit closer to an eco-evo-devo which is predictive and can start to relate environmental regimes to adaptation.

Annual killifish use embryonic diapauses to persist in temporary ponds in Africa and South America that dry out seasonally (Berois et al. 2015). They are oviparous cyprinodontiform fish species from the suborder Aplocheiloidei which evolved "annualism" repeatedly (Furness et al. 2015b, Helmstetter et al. 2016,Thompson et al. 2021). An embryonic life history characterization cannot just use diapausing and non-diapausing as individual states, because diapause in annual killifish can occur in three different developmental stages (Wourms 1972a-c). Diapause I can occur between epiboly and the onset of embryogenesis. Diapause II can be observed in the long-somite embryo and can coincide with a delay in the development of anterior structures relative to the rest of the body (Furness et al., 2015a; Podrabsky et al. 2010). Embryos which are completely developed and seem ready to hatch can decide not to do so when wetted. This is Diapause III, which seems the most constitutive among the three diapauses across all killifish (Wourms 1972c). Different adaptive explanations have been given for patterns observed in embryonic life histories of annual fish. Data patterns have repeatedly been interpreted as in agreement with adaptive bet-hedging (Furness et al. 2015a, Pinceel et al. 2015, Polačik et al. 2017, Van Dooren & Varela-Lasheras 2018). Furness et al. (2015) proposed that diapause II occurs less or not at high temperatures because these predict the probability that another generation can be completed before the cool dry season starts. Similarly, Polačik et al. (2017) proposed that eggs from younger mothers develop faster to be ready when an extra opportunity to hatch and reproduce within a season presents itself. Both these explanations assume that such opportunities occur. However, in *Austrolebias* annual fish (Alonso et al. 2023), for

example, embryos will not hatch and grow when they have not experienced a dry period yet. These explanations therefore require an environmental regime with two periods where reproduction can occur within a year. An alternative explanation for the same observations could be that developing fast is the best option given the expected mortality rates in the different stages (Van Dooren & Varela-Lasheras 2018). A more solid demonstration that observed responses are adaptive requires a demographic model and fitness calculations of the entire life cycle (Childs et al. 2010, de Jong et al. 2011).

In this paper, such a model is presented. It is used to investigate whether observed patterns are adaptive or not in given environmental regimes. A non-historical phenotypic definition of adaptation is used where one assumes that the traits most commonly seen are the ones which maximize fitness and therefore tend to persist (Reeve & Sherman 1993). The model makes two important assumptions: it is assumed that a developmental strategy is adapted when it is potentially at an evolutionarily "optimal" stationary state of an adaptive dynamics model, i.e., when there is no persistent directional selection (Coulson 2021) and the gradient of invasion fitness as a function of the mutant strategies is negligible. Second, data observed on individual development in lab conditions are assumed to be representative of what individuals do in the field. The model structure is tailored to specifics of annual killifish life histories and is integrating data mostly from *A. bellottii*, the Argentinian pearl killifish (Steindachner 1881). To facilitate the integration of new data and falsification of the model, it is capable of iterating population dynamics of fish and embryos from day to day, with environments varying on the same time scale. Population census points can vary between years. Pond filling regimes can be non-constant over time and are less simplified within years than for example growth seasons in the model of ten Brink et al. (2020). To determine invasion fitness and adaptiveness, it is necessary to assume stationarity of the environment in the long run (Metz et al. 1992, Van Dooren 2022). This study is primarily investigating effects of within-year environmental variation and annual regimes are therefore repeated across years. An algorithm

changes environmental parameters to find pond filling regimes where observed data patterns are adapted, i.e., the invasion fitness gradient is minimized.

The results demonstrate that most traits of observed embryonic life histories are never completely adapted to just within-year variability. Selection in each annual environment is always towards less mortality and faster development, except for hatching probabilities. It is discussed how between-year variation could render the observed embryonic life histories adapted.

Methods

In the model, annual fish individuals can find themselves in different pond compartments and environments (Figure one). The state of the pond is described by the amount of water it contains. A pond can be in a dry state, where no pond soil is having water above. In the wet state, at least some water is present and fish can hatch, grow and reproduce. Eggs produced in a wet period are recruited to the egg bank, as adults lay them in the wet soil. In agreement with available data (Van Goor 2009, Polačik et al. 2021, 2023), eggs are not developing unless they experience dry, less hypoxic conditions. This can occur with different starting times within the same wet period depending on the location of an egg within the pond extent. The model does not contain explicit temperature effects. The data on *Austrolebias bellottii* (Van Dooren & Varela-Lasheras 2018) used to parameterize the embryonic life history were collected at 19.5C and 20.5C, while the average annual temperature for their site of origin was 17.9C (Ingeniero Maschwitz Argentina, Worldclim value used in Helmstetter et al. 2020). The model is coded in R (R Core Team 2020) and the scripts available will become available on https://zenodo.org/ when the manuscript is accepted for publication. In the supplementary information the explanations below are translated into equations for the population dynamics.

The pond environment

The temporary pond is described by a disc with a fixed center of which the size is defined by the maximum radius r_{max} which it can obtain in a wet season (Table 1). Until this distance from the pond center, eggs can be deposited and an egg bank is therefore present. The pond is not always filled to its full extent with water. Over time, the pond radius $r_{pond}(t)$ can change per day, where t can be any date. For simulation, non-negative radiuses within [0, r_{max}] are allowed but to assign individuals to locations, radiuses are rounded down giving discrete location categories. Half emptying and refilling of ponds was not considered for now. Between-year variability was not considered. In longer simulations, patterns within a year were repeated across years. In leap years, $r_{pond}(t)$ of the previous year was copied into January 1 to December 30 and December 31 of the previous year was duplicated.

Pond radius time profiles were kept as simple as possible, with linear increases and decreases in radius in a limited number of time intervals within a wet period and up to two wet periods within a year. Radiuses r_{pond}(t) are therefore piecewise linear continuous functions when time is given as a real number. First a limited number of seasonal scenarios were explored (see Figure two for examples). In the simplest one (Y0), the pond had water starting on April 11 (day 101), increased to maximum size r_{max} on July 19 (day 200) and became dry again on October 28 (day 301). From this scenario the period from April 11 to July 19 where the radius increased was then modified. In scenario Y0, the pond covers $0.3r_{max}$ on day 130 and $0.7r_{max}$ on day 170. Scenarios were constructed with decelerating filling where $0.7r_{max}$ would be reached on days 163 (YD1), 150 (YD2) and 135 (YD3) respectively. Scenarios with accelerated filling where $0.3r_{max}$ would be reached on days 136 (YA1), 148 (YA2) and 163 (YA3). In a second category of modifications of Y0, a short wet period was inserted from days 71 to 90, with the maximum pond radius of that period reached on day 80 at values $ms_{\max}$. Scaling parameter m varied from 0.2 to 1 in steps of 0.2, yielding scenarios YS1-YS5. Furthermore, in order to be able to numerically minimize fitness gradients, a parametric description of pond radius profiles was chosen. The piecewise linear function s_{pond}(t) specified by a specific

parameter vector $\boldsymbol{\rho}$ is detailed in Supplementary Figure one. A parameter vector $\boldsymbol{\rho}$ contains ten parameters ρ_i affecting interval lengths and maximum pond radiuses within intervals.

Embryonic life histories

We based the embryonic life histories on data collected and hazards estimated on the cohort in Van Dooren & Varela-Lasheras (2018) which started incubation in wet and oxygenated conditions and which was further incubated after some time in air with 100% relative humidity (code provided as Supplementary Material). In the model, five distinct developmental stages are considered (Figure one, Van Dooren & Varela-Lasheras 2018, simplified from Wourms 1972a), each with a specific age-dependent mortality rate pattern and for the first four developmental stages, an age-dependent pattern of the risk to enter the next developmental stage. Embryos are assigned to stage one at age zero. In stage two, a neural keel and somites have appeared. In stage three, optic cups are visibly present. In stage four, the eyes have become pigmented. In the fifth stage the embryo completely surrounds the yolk sac. Diapause has been observed in *Austrolebias bellottii* and in these conditions in stages one (DI) and five (DIII). From stage five, embryos can hatch when they are rewetted. The age-dependent patterns for transitions in these stages are derived from age-dependent multi-state modelling with competing risks (de Wreede et al. 2011). Hatching probabilities were estimated using logistic regressions (Van Dooren & Varela-Lasheras 2018). In this study it was noted that after some time, the rate of events (deaths or developmental transitions) decreases to almost zero and a similar observation was made by Polačik & Vrtilek (2023). This slowdown is more prominent even after the first rewetting of eggs and we incorporated that into the life history as explained below.

Embryonic life histories are modelled differently when embryos are in wet conditions (e.g., their location covered by water and conditions hypoxic), when they are in dry conditions (less hypoxic) and haven't been rewetted yet (first dry period) and in subsequent dry periods.

For the first dry period embryos experience, estimated cumulative hazards are used to obtain transition probabilities between developmental stages or from any developmental stage to the dead

state. Fifteen different such transitions can be observed in total across a time interval (from each developmental stage to all more advanced ones and to the dead state), with some transitions more probable than others.

Cumulative hazards are measures of the summed probabilities across all observation times that individuals have made a specific transition by a specific time (de Wreede et al. 2011) and are represented using time-dependent matrices $\boldsymbol{A}(a)$, here of age a, with a ranging from zero to the maximum age considered or observed. $\boldsymbol{A}(0)$ equals zero. Each entry A_{ij}, with i denoting the row and j the column of each matrix element represents the cumulative hazard at that time for the transition from state i to state j. Note that this is different from standard practise in matrix population dynamics models, where columns denote departure states and rows arrival states (Caswell 2000). Six states are used for an embryonic life history, the five developmental stages and the dead state. All sub-diagonal elements A_{ij} are zero. In a statistical analysis, non-parametric estimates of matrix function $\boldsymbol{A}$ can only change at ages where individuals have been observed. If we give observation times a_τ an index τ, then the estimated changes in matrices $\boldsymbol{A}$ at ages a_τ are given by matrices $d\boldsymbol{A}(a_\tau)$. Transition probabilities from age a_1 to a_2 are calculated as (Eqn. 1, de Wreede et al. 2011)

$$\boldsymbol{P}_{a_1,a_2} = \prod_{a_\tau \, in \, (a_1,a_2]} (\boldsymbol{I} + d\boldsymbol{A}(a_\tau)) \qquad \text{(Eqn. 1)}$$

Using the data-based transition probability matrices (Eqn.1), a set of matrices $\boldsymbol{P}_{a,a+1}$ is constructed projecting numbers of embryos in different stages and sharing their age state variable one day forward, for any age $a \in [0, \infty[$. For all ages above the maximum a_{max} observed, it is assumed that $\boldsymbol{P}$ remains equal to $\boldsymbol{P}_{a_{max-1},a_{max}}$. To project numbers of embryos forward in time, we only need to project individuals alive. Therefore the 5 by 5 minorized matrices $\boldsymbol{M}_{66}$ are used, with the sixth row and column of $\boldsymbol{P}$ removed. The life history dynamics in this environmental compartment implies that embryos need, next to their location, their age and stage as state variables. However, as it is only

age as given by the number of days in dry conditions which is assumed to determine developmental progress, we use that number as the individual age variable.

Van Dooren & Varela-Lasheras (2018) found that many embryos don't resume development after a first rewetting. To integrate this in the model, a further 0/1 state variable was added to the individual state denoting whether an embryo would be in a state where development proceeds (1), or they remain in a generally arrested state (0). Each time an embryo experiences wet conditions after the first dry period, it enters this non-advancing state with probability p_d. The probability of survival per day $e^{-\mu_d}$ in the non-advancing state, which was equal in all stages, and the proportion entering the advancing state p_d were estimated based on the data in Van Dooren & Varela-Lasheras (2018) and are given in Table one.

When stage five embryos at a specific location are wetted because the wet pond radius reaches their location, they can hatch on that day only. This occurs with probabilities which differ between the first and the second rewetting and with an age-dependence of the age at first rewetting. The three hatching parameters β_1 , β_2 , β_3 required to model this were estimated and are given in Table one. Upon first rewetting, embryos in stage five hatch with probability $g(\beta_1 + \beta_2 a)$, with g the inverse logit function and a the number of days an embryo has been dry. In subsequent rewettings, embryos hatch with probability $g(\beta_3)$. The eggs which don't hatch remain in the soil at the same location. In wet soil conditions, embryonic development does not proceed, presumably due to lack of oxygen. In a pilot field study on the related species *Acantholebias luteoflammulatus* (van Goor 2009), the probability per day that a stage one egg survives in wet conditions $e^{-\mu_w}$ was estimated in the field and this value was used across all stages (Table one). There are no other stage-specific survival probabilities in wet soil estimated yet.

Fish life histories

Fish are assumed to move freely within the available water body. The fish present share environmental conditions at any point in time. The fish life history is kept simple and based on

Helmstetter & Van Dooren (2019) and Van Dooren & Varela-Lasheras (2018). Individual fish have their age since hatching and sex as state variables. The number of fish and their produced eggs are modelled on a day-to-day basis. Different events which can happen on the same day are treated sequentially and not as competing risks. The sex ratio among the hatchlings on each day is equal. After hatching and before density-dependence, fish have a juvenile survival probability per day $e^{-\mu_J}$ and mature at a fixed age α. The number of fish within a pond is limited by its surface. It is assumed that each square meter of wet surface can contain a maximum of *K* adults. When more than the maximum number of allowed adults is present on a day (*K* times the area of the pond), surplus adults are chosen at random and erased from the population state. When the number of adults is below the maximum which a pond allows, surviving juveniles can coexist with adults in the remaining free surface and with a probability per day of not getting killed by adults equal to the proportion of surface which is free on that day. There is no other competition between juveniles modelled. There is no other source of daily mortality among adults during a wet period. Reproduction occurs each day after density-dependence. Adult females have a fixed individual fecundity per day of eggs *f* which are all fertilized when there are males present in the pond on that day and the sex ratio (ratio numbers of females/males) is within the interval [0.2, 5]. Otherwise, fecundity is set to zero because of presumed sperm limitation in males or male aggression. Eggs are deposited at a random location within the pond. Since the location as an individual embryo state variable is an integer, eggs are distributed across concentric rings at different distances from the center with probabilities proportional to their areas. At the start of a dry period, the remaining adults die.

Simulations of population dynamics

To assess the effects of pond water regimes on the population dynamics of annual fish, the numbers of individuals are projected forward, not their densities. The extent of the wet pond area changes continually. The individuals in the different environmental compartments and their descendants are

essentially projected forward on a per day basis. Across a dry and a wet interval, forward projection was done as follows:

(1) The eggs present at the start of the time interval where the pond contains no water are projected to the first day where the pond contains water. In general, some eggs will die during the interval and there is some development depending on the states of the individuals. The eggs present at the start of an interval where the pond contains water and their descendants are projected to the first day where the pond does not contain water anymore and this involves several steps. (2) Eggs already present from the start of this interval but at different distances from the pond center can become wetted on different days and on those days, stage five embryos can hatch with a probability determined as explained above. Embryos which do not hatch remain in the egg bank. Advancing/non-advancing and hatching states are reset. Eggs at different locations will be dry again on different days. They differ in the amounts of days in wet and dry conditions, in their survival probabilities and development rates. (3) Numbers of hatchlings per day are summed and are the recruits to the fish population, of which the dynamics is iterated per day. Numbers of eggs produced per day and their distribution across location categories are noted. (4) Newly produced eggs all experience wet conditions right after they enter the egg bank. They don't develop in these conditions and the numbers surviving are determined for the first day where they encounter dry conditions. (5) These new eggs are then projected further across the interval where the pond still contains some water, but their locations are already dry. (6) New eggs and the eggs deposited in previous wet periods are the new population state at the start of the new period where the pond is dry.

Stochastic fluctuations in the simulations were kept as small as possible. Only eggs with states that occurred in very low numbers were removed (when the total number of individuals in that location and with that age, hatching and adavancing state variables was below one). In the fish population, fish in (age, sex) states with numbers below one were removed and fecundity was zero for females with states that occurred in numbers below one.

Given an initial population state, an egg bank with a number of embryos can be projected forward over a series of successive years, where each year repeats the pond cycle of the previous year, to find the attractors of the population dynamics. These attractors were found to be stationary. Population states change within a year, but on the same day in different years, the population state is approximately identical. For the purpose of illustration, forward projection was done for example profiles Y0, YA, YD and YS and the stationary dynamics over one year was simulated on a day-to-day basis. The results are shown in figure two.

Mutations and invasion fitness

If we want to assess whether organisms which are prevalent in a given environment are adapted to it, then we should determine in the first place that organisms with genotypes determining different but similar strategies would not be able to increase in frequency when they would appear. This is the idea of adaptation bringing populations to a final stop of phenotypic and genetic evolution (Hammerstein 1996), which became formalized in adaptive dynamics approximations based on invasion fitness (Metz et al. 1992, Metz et al. 1996, Geritz et al. 1998). At such endpoints of evolution, invasion fitnesses of rare (mutant) phenotypic strategies slightly differing from the prevailing or so-called resident strategy should not indicate that these can invade. Therefore, invasion fitness as an approximately linear function of a trait difference from the resident trait should be a flat function. At adapted population states, the invasion fitness as a function of mutational changes is at an optimum and the first condition for that is that the linear approximation of such a function equals zero for small mutational changes, i.e., all partial derivatives (slopes) of invasion fitness with respect to a mutational change in a single trait are zero (Metz et al. 1996).

To implement this as a check of adaptation, choices need to be made as to what the phenotypic effects of mutations can be. The embryonic annual fish strategy considered here consists of the four transition rates from a developmental stage to the next, five stage-specific mortalities and the three hatching parameters. For the mortalities it is a point of discussion whether these could vary between

genotypes, but let's assume for now that they can. The rates are all age-dependent strategies, which makes them in total nine function valued strategies to assess (Kirkpatrick & Heckman 1989). I opted for a simplification such that a developmental strategy consists of eleven parameters. Nine trait parameters represent multiplicative changes to the cumulative hazards estimated from data and the trait value is one for resident individuals and smaller or larger than that for mutants. For hatching, similar multiplicative changes to hatching parameters β_1 and β_3 of the linear predictor in the logistic regression are the traits. With this choice of traits and the resident trait values all fixed at one, the transition probabilities and hatching probabilities determined from the data represent the assumed common strategy in a resident population of this species.

Some more detail is required to understand what a mutation change in one of the rates will actually change to a transition matrix. If we make the elements of a minorized transition matrix $\boldsymbol{M}_{66}$ from one day to the next explicit, we can write that matrix as consisting of transition probabilities $p_{i\to j}$ from state *i* to state *j*, where each state is a developmental stage (*i*, *j* in 1,..., 5).

$$\boldsymbol{M}_{66} = \begin{matrix} p_{1\to1} & p_{1\to2} & p_{1\to3} & p_{1\to4} & p_{1\to5} \\ 0 & p_{2\to2} & p_{2\to3} & p_{2\to4} & p_{2\to5} \\ 0 & 0 & p_{3\to3} & p_{3\to4} & p_{3\to5} \\ 0 & 0 & 0 & p_{4\to4} & p_{4\to5} \\ 0 & 0 & 0 & 0 & p_{5\to5} \end{matrix} \qquad \text{(Eqn. 2)}$$

Transitions $p_{i\to dead}$ can be inferred from the other transitions as $p_{i\to dead} = 1 - \sum_{j\neq dead} p_{i\to j}$. Diagonal elements in $\boldsymbol{M}_{66}$ can be decomposed as $p_{i\to i} = 1 - \sum_{j\neq i} p_{i\to j} - p_{i\to dead}$. The probabilities of developmental transitions are not conditional on not dying. All transitions are treated on an equal footing. One needs to be careful when changing hazards to keep all elements within the bounds [0, 1] and to preserve the sum constraint on the probabilities out of a state. Therefore, when for a particular day, no events have been observed (due to lack of events or of observations), the matrix for that day is not changed by trait changes. Also, when one of the probabilities on a row is one or the diagonal is zero, trait changes in the involved cumulative hazards are not affecting that row. Only

diagonal elements $p_{i\to i}$ of $\boldsymbol{M}_{66}$ (Eqn. 2) within the interval]0, 1[can be affected by trait changes to the cumulative hazards for death rates. It is assumed that trait changes in stage-specific rates of development, affect all cumulative hazards for changes out of that stage equally. For example, a multiplicative increase *c* in the rate of development out of stage one affects four cumulative hazards, changing all off-diagonal probabilities in]0, 1[in the first row of $\boldsymbol{M}_{66}$ (Eqn.2) with a factor *c* and therefore reduces the diagonal with $(c-1)\sum_{j=2,3,4,5} p_{1\to j}$. Trait changes were limited to the interval [0.9, 1.1] for which it was checked that all constraints on the matrices $\boldsymbol{M}_{66}$ are preserved.

Invasion analysis

It is assumed that all resident individuals are diploid homozygotes of a single allele which determines all traits (Van Dooren 2006). Offspring numbers of residents are determined by female fecundity and the availability of adult males as discussed above. For mutant genotypes which are relatively rare, the number of mutant alleles equals the number of mutant individuals because due to their rareness these are approximately all heterozygotes of a mutant and a resident allele. The mutant phenotype is that of a mutant heterozygote. Mutant adult females contribute the mutant allele to half of their eggs. Male adult mutant individuals contribute their mutant allele to half of the eggs they fertilize each day, which is assumed to equal the total number of eggs produced on that day, *f* times the number of resident adult females present, divided by the resident males present.

To estimate invasion fitness, the population dynamics of the data-based resident strategy was simulated to obtain so-called resident stationary regimes. Then the mutant genotype was introduced and simulated competing against the resident strategy over a low number of years until it achieved a relatively stable distribution across locations and states. Invasion fitness of a mutant was then estimated. The growth of the total mutant population was estimated across a number of days equal to four or eight complete dry-wet period cycles. Respecting this natural periodicity minimizes errors in estimation (Van Dooren & Metz 1998). The final number of mutant individuals was divided by the initial number at the start of this time interval and raised to the power one over the interval length

(in days) and the result log transformed. Invasion fitness is thus the geometric average population growth rate per day of the mutant population given an environmental regime and a resident data-based population strategy.

Fitness sensitivities

To assess the effects of specific trait changes on invasion fitness in a given environmental regime, mutants with trait values between 0.9 and 1.1 were generated for each of the separate traits and their invasion fitnesses were estimated in an established population with the data-based strategy. This was done for each of the pond filling scenarios Y0, YA, YD and YS and allowed an assessment per scenario of strenghts of selection on each trait and a first assessment of which environments the data-based strategy might be adapted to. To compare these strengths of selection more easily between environments, we assigned parameter values to the scenarios YD-Y0 and YA, and to Y0 and YS, such that invasion fitnesses of a specific trait change could be plotted as functions of environmental parameters. To compare effects of accelerating or decelerating pond filling, YD1-3 were given parameter values -1, -2 and -3, Y0 value zero and YA1-3 the values 1, 2 and 3. To compare effects of adding a short wet period where reproduction is impossible, Y0 was again assigned parameter value zero, and YS1-5 the corresponding parameter *m*. Per environmental regime, the invasion fitnesses for mutations in each trait with value 1.1 were used as a measure of invasion fitness sensitivity (Caswell 2019) and plotted to see how environmental properties might affect strengths of selection and change the potential adaptedness of strategies.

Adaptive environments

Here it is assumed that mortality rates are determined by environmental constraints and therefore would remain fixed during adaptation even when a change in mortality would increase invasion fitness. An optimization algorithm using the L-BFGS-B method (Byrd et al. 1995) was programmed using the optim function for R (R Core team 2020), acting on the parametric representation of pond

regimes detailed in Supplementary figure one. Invasion fitnesses were minimized as a function of environmental parameters. The Euclidian norm of the vector of invasion fitnesses of mutants with trait values 1.1 in the four developmental traits and the two hatching probabilities was calculated for each regime and minimized by changing pond regime parameters. In an environment where the data-based strategy is adapted, this norm would become zero. Parallel optimizations were started for a limited number of different initial pond regimes with one or two wet periods. In all cases explored, the algorithm converged to a local minimum of the gradient of invasion fitness. Its final value at these local minima was calculated and compared between different initial conditions. Qualitatively different environmental regimes with the overall smallest gradients found are plotted, their resident population dynamics simulated and shown, as well as the pattern of invasion fitnesses of mutants in these environments.

Results

Population Dynamics

During dry periods all stages are usually present, but stage five is generally most abundant (Figure two). At different distances from the pond center, the number and distribution over stages of embryos changes predictably, with more advanced stages found sooner at larger distances from the pond center (Supplementary Figure two). At the start of a dry period after reproduction, earlier stages are the most abundant near the pond center and more advanced developmental stages dominate near the pond edge. The largest egg bank changes occur within wet periods, due to hatching, overall lower survival, and addition of new eggs. Patterns of pond filling have clear effects: with more decelerating pond filling, i.e., faster initial and slower later filling when the maximum radius is approached, the overall size of the egg bank increases at all times because more individuals reproduce (Fig. 3A). The pattern of pond filling is mirrored in the speed of increase in the number of first stage embryos during the main wet period (Fig. 3A). With an additional short pre-period of pond

filling covering the pond radius to some extent (Fig. 3B), stage five embryos hatch in that period without any chance of reproducing and the embryos which experienced their first rewetting have their hatching state variable changed. In the main wet periods (Fig. 3B), we can see more intense competition: the fraction of the juvenile population contributing to the pool of reproducing adults is smaller, while overall, there are many more new eggs produced. It seems that the carrying capacity of the pond is more often approached. If we need to guess which among these environments might be the most adapted, based on simple principles such as the optimization of population size (Charlesworth 1994) or the pessimization of the environment (Metz et al. 2008), it would be the pond filling regime where the egg bank is largest and where competition is most intense, so YS1 or YS2. A combination of decelerated pond filling and a short wet period before a long one might be the environment where the data based strategy is the most adapted, if only within year variation would occur.

Invasion fitness patterns

When invasion fitnesses are estimated for changes in each trait in each of the example scenarios, it can be observed that invasion fitness changes are largest for mortality and development in stage one, and for early hatching (Figure three). Note that also for mortalities in all stages except for stage one, the slope of invasion fitness on mutational trait change is small. This can be explained by the already high survival rates in each of these stages. For the development rates, there seems only weak selection to increase developmental speed in most stages (Fig. 3). Invasion fitness slopes changed when environmental parameters changed, in particular for the traits under stronger selection (Figure four). However, for the range of changes explored in (Fig. 4), invasion fitness slopes did not change sign except for hatching at the first rewetting, when short wet periods without reproduction were added to the reference scenario Y0. This suggests that changes in the pond filling regime within a year could make selection weaker still than in the examples investigated, and even make early hatching adapted.

Adaptive environments

It was found that the minimization algorithm could make jumps to environmental regimes where dry periods would disappear and the algorithm would stall. These iterations were restarted with a constraint which imposed that a dry period would exist for of at least 10% of the year. In general, initial conditions with two periods where reproduction could occur were replaced by pond regimes with a single wet period with reproduction. When pond regimes were changed iteratively such that the invasion fitness gradient for the developmental speeds and hatching traits became minimized, the output pond regimes to which the algorithm converged were local minima which had filling regimes with a subset of parameters changed. Figure five shows two representative initial and final pond filling regimes. In general, the length of the invasion fitness gradient vector would become at least halved, but vectors of zero length were not obtained. Therefore, traits would not generally become adapted in any of the scenarios with within-year variation. The only trait which ever became really adapted in one of the environments, was late hatching (Fig. 5C, lower panel). Minimization of the invasion fitness sensitivity in early hatching, a trait which contributed much to the reduction of the gradient, could co-occur with an increase in invasion fitness sensitivities for other developmental speeds (Fig. 5C). The attractors of the population dynamics in each of the regimes at local minima of the invasion fitness gradient, had large maximum numbers of eggs in the egg bank and showed that many juveniles did not become reproducing adults. At the start of the first wet period in the year, the two scenarios differed in number of eggs in the egg bank by a factor of two.

Discussion

Using lab data and knowledge of the field ecology of annual fishes, a model for their population dynamics was constructed. Using invasion fitness analysis and minimization of fitness gradients it was investigated to which patterns of within-year variation the observed embryonic life history strategy might be an adaptation.

Adaptation to pond size variation within years

Searches for an annual pond filling cycle where embryonic life histories are adapted halted at local minima of the invasion fitness gradient, where invasion fitness sensitivities of developmental rates and hatching probabilities were smaller than in the initial environments but not zero. Only for hatching after the first rewetting did it occur that it became adapted in one of the regimes. Selection on hatching at the first rewetting differs in direction between different pond filling regimes. All developmental rates are always selected in any annual environment to increase in speed, and mortality rates are always under selection to decrease. Without considering variation between years, one could thus state that these rates must be constrained, or that trade-offs, likely between developmental and mortality rates keep them at their observed values.

Note that diapause II, claimed to be the hallmark of annualism (Furness et al. 2015b, Furness 2016), was not observed in the data (Van Dooren & Varela-Lasheras 2018). Selection to decrease the rate of development in the involved stage seems unlikely, unless diapause II would be strongly and rather directly coupled to a decrease in a mortality rate. Van Dooren & Lasheras (2018) found that when parental pairs were compared, low mortality in stage one was associated with faster development from stage 4 into the prehatching stage. Trade-offs and constraints on annual fish development might not be just within-stage patterns, but there is thus far no evidence of a trade-off involving rates of stage three, where diapause II could occur.

In some of the simulation steps aiming to minimize the invasion fitness derivative, pond regimes were chosen without a completely dry period. These were avoided as results for now, but might be useful to explore the adaptedness of developmental patterns in non-annual killifish (Varela-Lasheras & Van Dooren 2014) and why killifish clades which evolved annualism never seem to have lost it.

Model validation and falsification

Different types of data can be collected to test and falsify model assumptions and predictions. First of all, snapshot distributions of embryos in the egg bank across developmental stages as in Polacik et al. (2023) can be compared to model predictions. Note from Fig. 2 and Supp. Fig. 2 that the model predicts on many days that a single stage will dominate in the egg bank. Large sample sizes will be required to reject the model on the basis of the relative occurrences of the less abundant stages. Predictions can also be made for transfers of embryos between lab and field (Polacik et al. 2023) and simulations can be used to optimize power of such experiments. Several model assumptions were made based on the lack of more detailed data, such as for the density dependence in the fish population. Data are needed on hatching probabilities after the first rewetting, to assess age and other effects there. Estimates of survival in wet soil conditions in other stages than the first are needed and a longitudinal follow-up of embryos after their first rewetting. It is generally important to replace lab estimates and inspired guesses by estimates in natural environments. Polacik et al. (2021, 2023) renamed developmental stages after the diapauses that mark them but did not collect individual longitudinal data. They could therefore not detect any developmental arrest nor estimate field demographic parameters. More detailed data on pond filling regimes are needed as well, to investigate what the speeds of emptying and filling actually are and how they vary within a year and what the magnitude is of between-year variation. All lacunes in the data call for a field embryology with longitudinal studies in time sequences of natural conditions.

Adaptation to variation between years

Techniques known as evolutionary invasion analysis (Otto & Day 2007), deriving from adaptive dynamics approximations (Metz et al. 1996) were used to check whether a life history structure found in data could be adaptive in specific environments. Patterns of invasion fitness in different pond filling regimes which were each repeated between years were very similar for many traits such that it seems at first sight unlikely that introducing between-year variation would drastically alter the pattern of selection. However, that intuition might be wrong. Between different within-year pond filling patterns, the population size in the egg bank can differ by a factor ten (Fig. 2). Delaying and bet-hedging can be selected against in any constant environment, but become advantageous with population fluctuations (Bulmer 1984, Van Dooren & Metz 1998). Selection for delaying strategies can be understood as selection against fluctuations in population densities and in the underlying demographic processes. The results on the population dynamics in different pond filling regimes suggest that egg bank sizes could vary a lot between years. Extrapolating expectations from the situations modelled here, while faster development rates are favoured with within-year variation, these might be selected against with between-year variability when population sizes fluctuate between years. Selection on early hatching did change in direction already in different annual regimes. This would permit a variable environment where years with positive and negative selection to balance selection overall and make the observed early hatching probability adapted. However, here we need to note again that the attractors of the resident population dynamics differ between pond regimes with different selection on this trait. In sequences of alternating environments, the sign and magnitude of selection on this trait can come to depend on how close the population state is within each year to these respective attractors. This depends on how often the alternative environments switch.

Unfortunately, with the current trends in environmental change, it is unclear whether we can still expect real populations to be near attractors of their eco-evolutionary dynamics for much of the time. For example, the recently described *Austrolebias wichi* was at that time known from a single pond in a region with large changes in agricultural practise (Alonso et al. 2018), but can be now

found in nearby man-made roadside ditches as well. Similar to the search for environments rendering embryonic life histories adapted as implemented here, simulations of anthropogenic disturbances and rearrangements of the natural habitats could explore which ones would render patterns of development adapted and would minimize adaptational lags (Van Dooren 2022) across a wider range of projected climate change patterns. The novel managed environmental regimes which result could aid in the conservation of the several hundred annual fish species occurring in South-America and Africa, often at risk of extinction and with currently small population sizes. The occurrence of *Austrolebias* annual fish in anthropized environments is already common and made Helmstetter et al. (2018) use road coordinates as a background map for species range modelling.

Potential extensions

.Competition between juvenile and adult fish was modelled according a very simple density-dependence. This ignored size-structured competition to a large extent (e.g., de Roos et al. 2003). However, the size-structure would have to extend to the boundaries of the embryonic stages, to investigate how egg size and hatchling size correlate. Vrtilek et al. (2020) found no correlation between them in *Austrolebias bellottii*, while in a different species hatchling size depended in a hypo-allometric manner on egg size. Developmental stages appear so different, that it seems difficult to define a single size variable which remains valid and useful throughout development. Including more size structure where possible would permit investigating coexistence and co-adaptation of annual fish species (Helmstetter et al. 2020). There is also some urgency to model temperature effects on persistence and adaptiveness. In African *Nothobranchius* annual fish for example, Furness et al. (2015a) demonstrated temperature plasticity in the entrance into diapause. However, these authors defined developmental duration as time to hatching or death, such that separate stage-specific mortalities and speeds of development were not estimated. Polačik et al. (2017) did censor individuals that died in the experiment, which is customary in time-to-event analysis, but did not analyse survival effects on developmental outcomes. It seems that the model

presented here can make predictions for non-*Austrolebias* annual killifish, and that existing data might just need to be reanalysed to collect required parameters.

Eco-evo-devo modelling

Nine of the eleven traits modelled summarize effects of mutations across all ages on function-valued development or mortality rates. This might not correspond to how specific genes would change phenotypes, but at least all possible events in the embryonic life histories are included in the assessment of adaptedness. The multi-state model used for the embryonic life history has cumulative hazards which depends on the number of days in dry conditions, leading to a Markovian model (de Wreede et al. 2010). This might not be the model which integrates developmental mechanisms easily. Van Dooren & Varela-Lasheras (2018) modelled development with age-within stage as timescales, which seems reasonable when a minimum time is required to complete each developmental stage. Deriving multistate predictions is feasible but the model becomes semi-Markov (de Wreede et al. 2010). With such timescales or coexisting other timescales, the only way to arrive at predictions of distributions of individuals over states is forward simulation of all competing processes (de Wreede et al. 2010). This is individual-based modelling. These issues which extensions of this model in such directions raise, resonate ideas of Hogeweg and others in the classical schools of bioinformatics (Hogeweg 2012), dealing with the information exchange of organisms and their environments and the incompressibility of this process. Modelling the dynamics of pools of physiological resources and state variables explicitly is an alternative approach which others have implemented to keep track of processes with different timescales (DEB modelling, Kooijman 2010), and this should be explored as well. Embryos within an egg have well-circumscribed resource pools available to them. In the end, multiple such efforts, even for the same empirical model, seem required to understand evolutionary constraints on development and to be able to project adaptation and evolution as processes fuelled by mutations forward into the future.

Fortunately, for annual fish, rather mechanistic models are available for processes surrounding diapause I (Reig et al. 2017, Montenegro-Rojas et al. 2023).

However, an approach which is customary, even in evo-devo, is to separate the processes generating phenotypic variation from selective processes such as mortality (e.g., Salazar-Ciudad & Cano-Fernández 2023). This should be abandoned. Individual phenotypes are not composed on a conveyor belt moving individuals to a station where their quality is controlled. They can change the speed of the belt, they can fall off, jump off and back on again. At any point in time there are competing risks of advancing their phenotype and of dying which can vary between genotypes.

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Table 1. Model parameters fixed across simulations

Model Parameter	Compartments	Value	Description
r_{max}	All	10	Maximum pond radius
$e^{-\mu_w}$	Egg bank (wet location)	0.95	Probability of survival per day[#]
β_1	Egg bank (wet location)	2.693	Hatching parameter first rewetting*
β_2	Egg bank (wet location)	-0.034	Hatching parameter age effect[*]
β_3	Egg bank (wet location)	-0.080	Hatching parameter later rewettings[*]
p_d	Egg bank (dry location)	0.498	Proportion arresting after rewetting
$e^{-\mu_d}$	Egg bank (dry location)	0.994	Survival per day, those arrested after rewetting
f	Water body	10	Female fecundity per day
$e^{-\mu_J}$	Water body	0.989	Juvenile survival probability per day[$]
a	Water body	60	Age at maturity (days)
K	Water body	10	Maximum density of adults per m^2

#: according Van Goor 2008.

*: according Van Dooren & Varela-Lasheras 2018.

$: according Helmstetter & Van Dooren 2020.

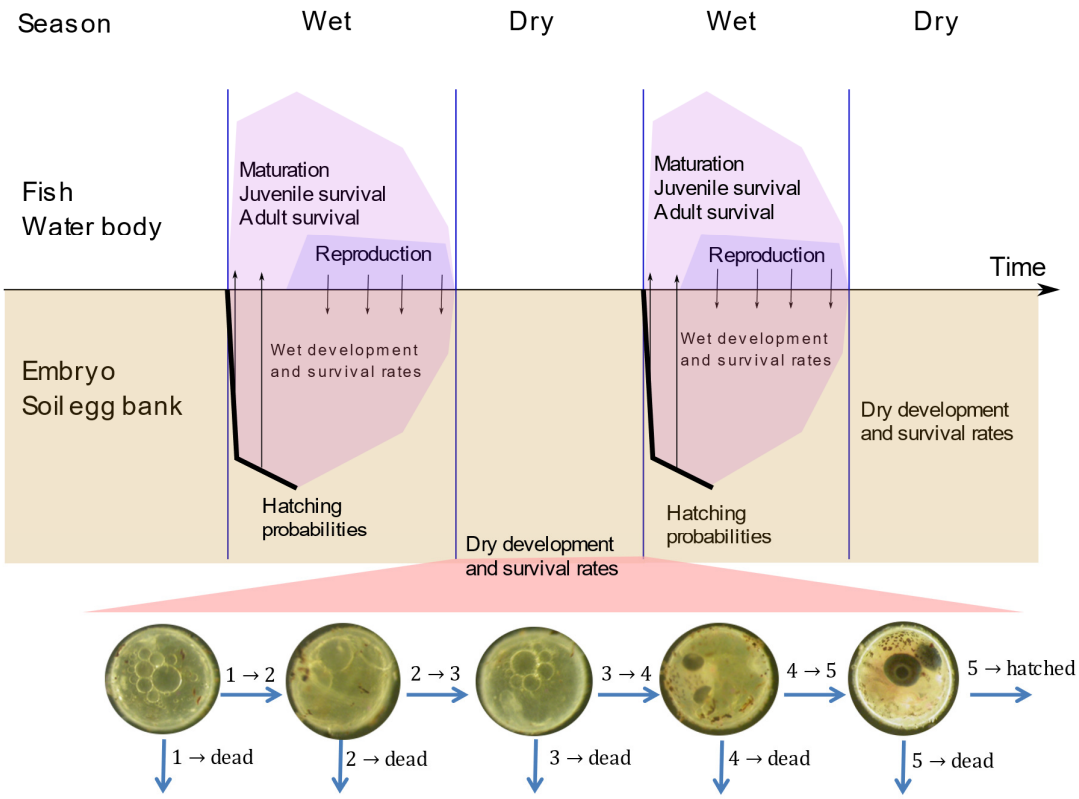


Figure one. Schematic depiction of the annual killifish life history interacting with the pond environment. Egg bank located in the soil and fish in the water when present. Dry and wet periods: during a wet period at least part of the pond is filled. Individuals which hatch from stage five embryos when water reaches the location where they are buried, can survive, mature and reproduce. Reproducing adults contribute eggs to the soil egg bank at locations where water is still covering the soil. Below the diagram, the modelled embryonic life history is shown. Five developmental stages are considered, each with stage-specific mortality and development rates. Stage five embryos can hatch according a sub-model for hatching probabilities.

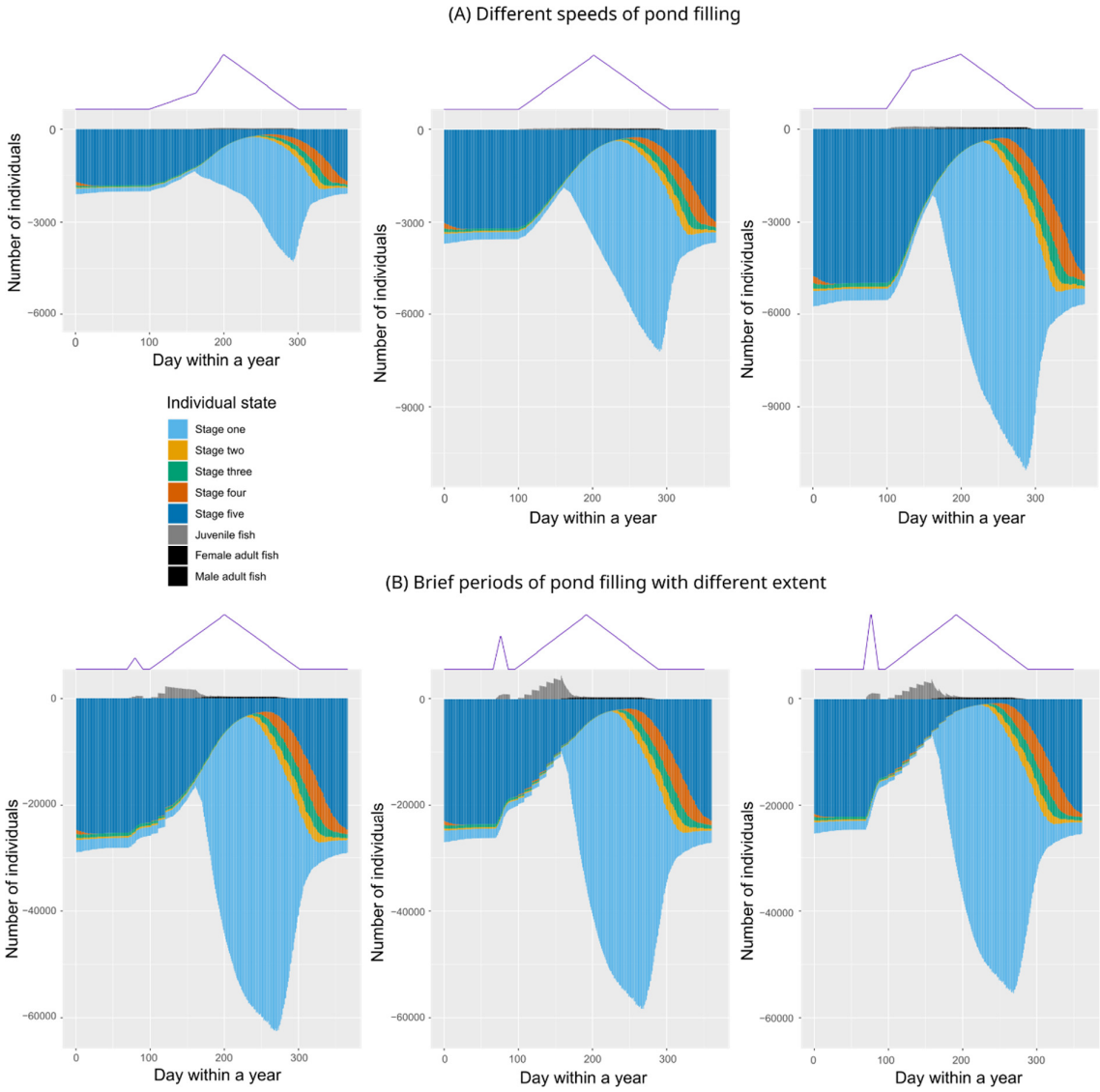


Figure two. Results from simulating different pond filling regimes. These are shown above each panel, with the pond radius varying between zero and r_{max} = 10. The population dynamics was first iterated for twenty years, then it was checked that it had become stationary. The stationary result was iterated from day to day over one year and the numbers of individuals in different states are plotted. The negatives of the numbers of individual embryos in the egg bank are plotted, fish numbers are unchanged. (A) Effects of changes in the speed of pond filling within a wet period. From left to right, the scenario with accelerating filling YA3, the reference scenario Y0 and the scenario with decelerating filling YD3. (B) Effects of the main wet period where reproduction can occur being preceded by a short wet period which does not allow reproduction. From left to right, scenarios YS1, YS3 and YS5. Note the difference in scale between (A) and (B).

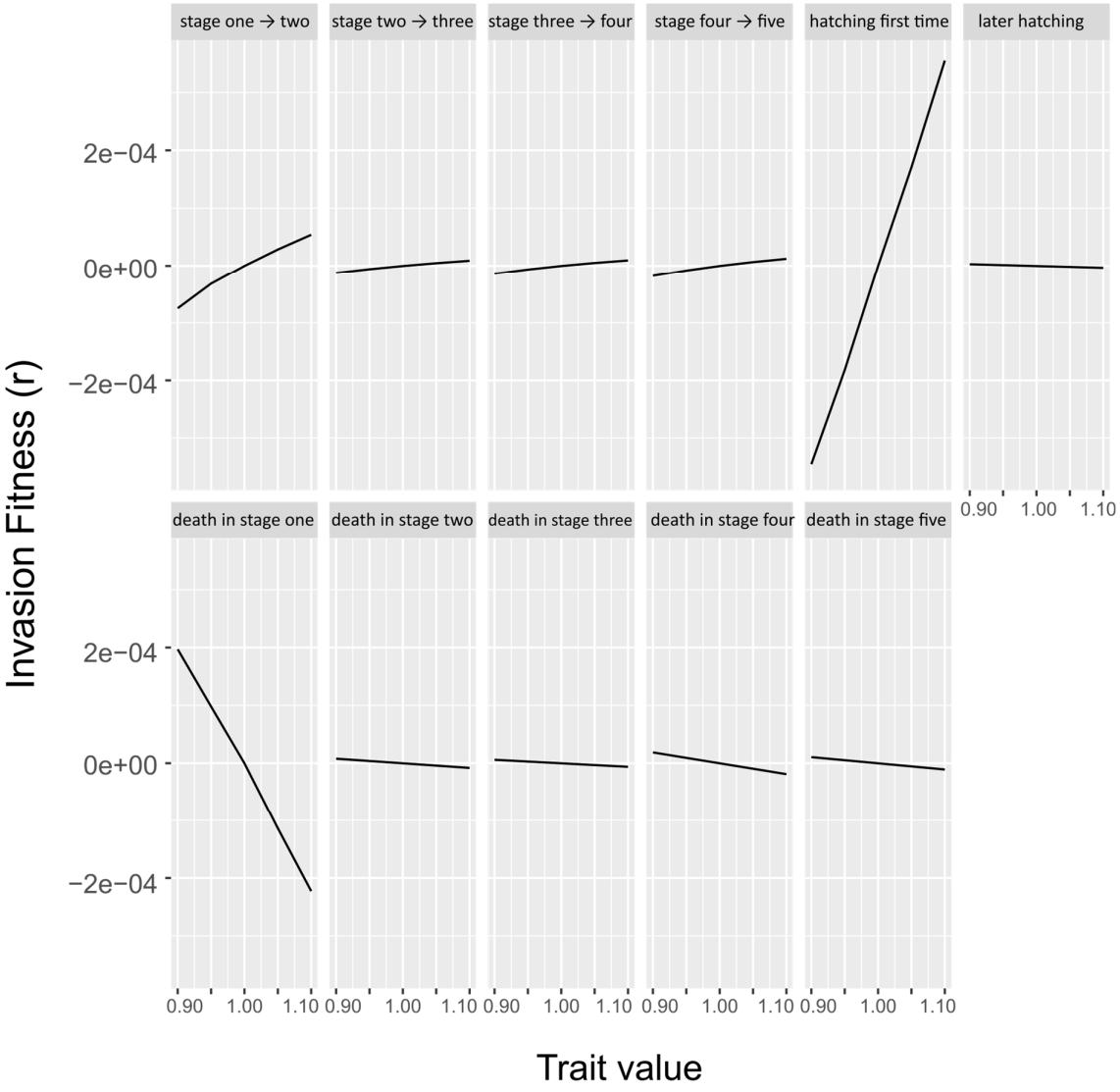


Figure three. Invasion fitnesses of phenotypic mutational changes in each of the embryonic life history traits. Invasion fitnesses are shown for invasions in a resident population which has become stationary in scenario Y0. The resident trait value for each trait is at value one. Invasion fitnesses are estimated at five equidistant points and connected by a line.

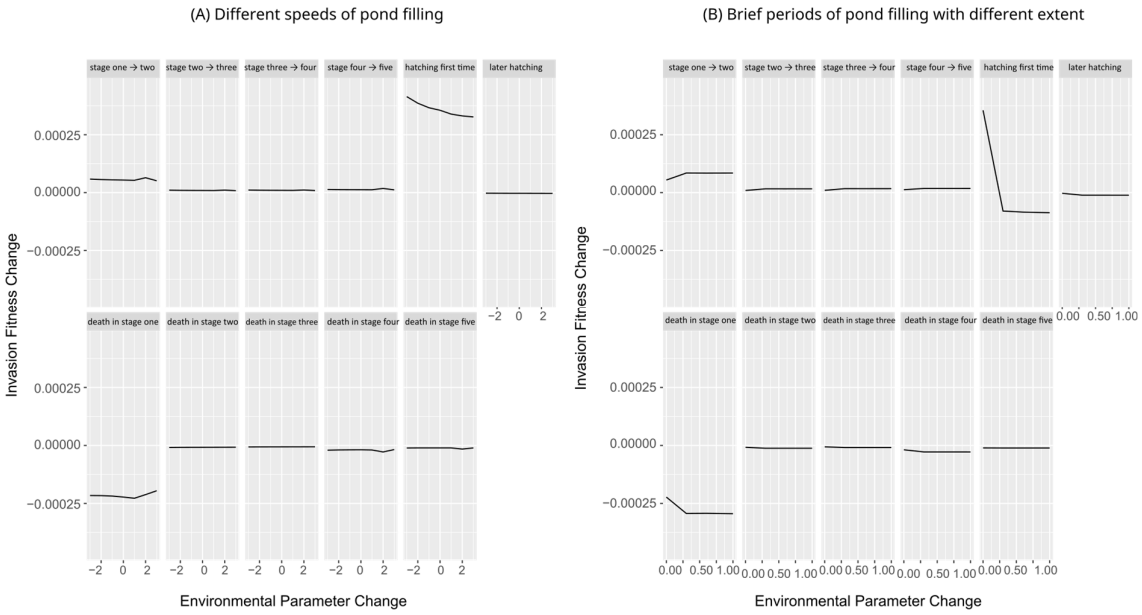


Figure four. Patterns of invasion fitness for a fixed difference between mutant phenotype and resident phenotype. Invasion fitness of mutants increasing cumulative hazards and logistic regression parameters with a multiplication factor of 1.1 plotted as a function of an environmental parameter. This gives a qualitative impression of strengths of selection in different environmental regimes. (A) Decelerating and accelerating pond filling. At zero parameter change, the pond filling regime is the one of the middle panel in row A of Figure two. (B) Short wet periods with increasing fractions of the egg bank covered. At zero parameter change, the pond filling regime is the leftmost one in row (B) of Figure two.

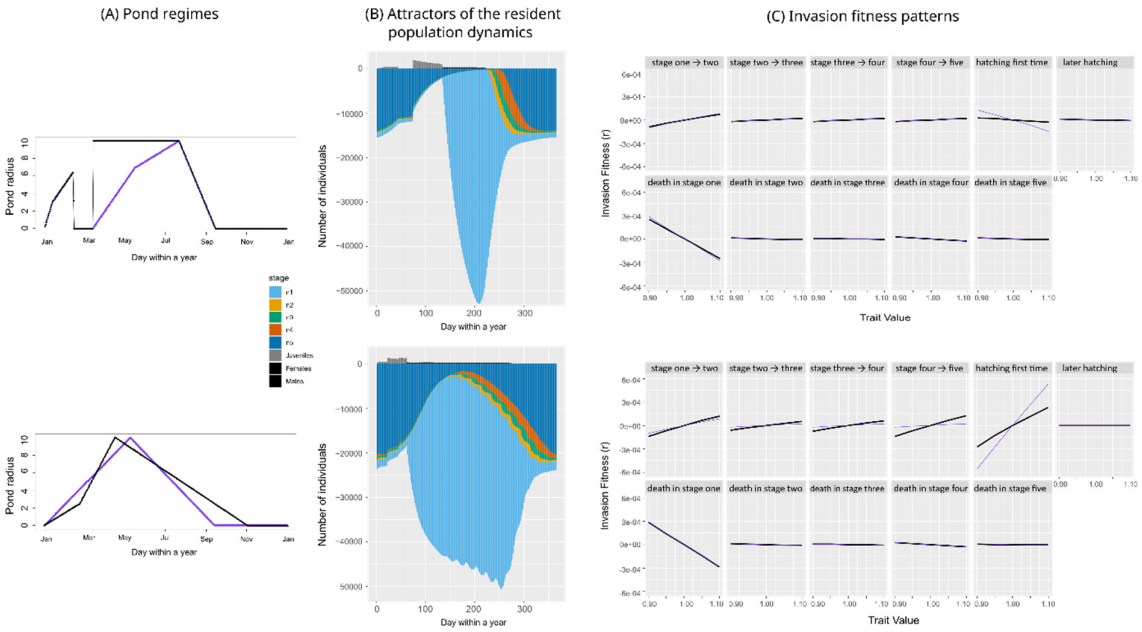


Figure five. Results of optimizing environmental regimes such that the data-based strategy becomes as adapted as possible. (A) Two initial pond filling regimes from which optimization started (blue), the upper one with two wet periods, the lower profile with a single one. Results after minimization of the invasion fitness gradient in black. (B) Attractors of the resident population dynamics in each of the two pond regimes to which the algorithm converged (with lowest length of the invasion fitness vector for development and hatching) (C) The patterns of invasion fitnesses per trait in the regimes obtained after minimization. The invasion fitnesses in the initial pond regimes are shown in blue for comparison.

Supplementary Material

The equations are given for the population dynamics of a single mutant genotype and phenotype across wet and dry periods. The equations include the feedback from the resident population. It is explained how to obtain the population dynamics of individuals with the resident genotype and phenotype using similar equations. In principle, development and mortality are processes occurring or modelled on a continuous timescale, but the periodicity in environmental conditions within a day is strong (Jacobs et al. 2008) and a motivation to use days as the discrete time step to carry out the modelling.

Embryos have different individual state variables. Numbers of embryos *N* are indexed *i* for stage (*i* = 1...5), *j* for their location, i.e., their distance from the pond center in meter (*j* ≥ 0), *k* for the hatching state first rewetting variable (*k* = 0, 1) and *l* for the advancing (*l* = 1) and non-advancing states (*l* = 0). Embryos are not differentiated according their sex.

A location *j* is dry when $r_{pond}(t) < j$ or $r_{pond}(t) = 0$. In dry conditions at their location on day *t*, embryos are projected forward into the next day according (except for stage one on the first day)

$$\boldsymbol{N}_{j,k,1}(a+1,t+1) = \boldsymbol{M}_{66}^{T}(a)\boldsymbol{N}_{j,k,1}(a,t) \qquad \text{(Eqn. A1.1)}$$

$$N_{i,j,k,0}(a+1,t+1) = e^{-\mu_d}N_{i,j,k,0}(a,t) \qquad \text{(Eqn. A1.2)}$$

where ***N*** is the vector of numbers N_i of embryos in the five developmental stages. A location *j* is wet when $r_{pond}(t) \geq j$. On the first day of wet conditions at a location *j*, embryos in the pre-hatching stage can hatch. To determine the number of individuals hatching $H_{j,s}(0,t)$ at time *t*, with sex *s* and with their age as fish zero, from embryos of all ages and per location *j* receiving water, we need to use the hatching probability functions and account for the fact that half of the individuals are

assumed to be females, half males. Index s is *f* for females and *m* for males. When a location is already wet for at least one day $H_{j,s}(0,t)$ is zero.

$$H_{j,s}(0,t) = \frac{1}{2}\sum_{a,l} g(\beta_1 + \beta_2 a) N_{5,j,1,l}(a,t) + g(\beta_3)\, N_{5,j,0,l}(a,t) \qquad \text{(Eqn. A2)}$$

The individuals which did not hatch are

$$N_{5,j,0,l}(0,t)' = \sum_a (1 - g(\beta_1 + \beta_2 a)) N_{5,j,1,l}(a,t) + (1 - g(\beta_3)) N_{5,j,0,l}(a,t) \qquad \text{(Eqn. A3)}$$

Since these individuals all experienced a rewetting now, the index *k* is zero for all of them and $N_{5,j,1,l}(0,t)'$ equals zero. Then on the same day, the advancing and non-advancing states are reset and the age state variable, the number of days in dry conditions, is reset at zero. This occurs in all embryos. For stages one to four

$$N_{i,j,k,0}(0,t)'' = p_d \ \sum_{a,l} N_{i,j,k,l}(a,t) \qquad \text{(Eqn. A4.1)}$$

$$N_{i,j,k,1}(0,t)'' = (1 - p_d) \ \sum_a N_{i,j,k,l}(a,t) \qquad \text{(Eqn. A4.2)}$$

For stage five, this occurs in the individuals remaining in the egg bank after they decided not to hatch

$$N_{5,j,0,0}(0,t)'' = p_d\left(N_{5,j,0}(0,t)' + N_{5,j,1}(0,t)'\right) \qquad \text{(Eqn. A5.1)}$$

$$N_{5,j,0,1}(0,t)'' = (1-p_d)\left(N_{5,j,0}(0,t)' + N_{5,j,1}(0,t)'\right) \quad \text{(Eqn. A5.2)}$$

The reset individuals *N''* are then advancing to the next day according the survival probability in wet conditions

$$N_{i,j,k,l}(0,t+1) = e^{-\mu_w} N_{i,j,k,l}(0,t)'' \quad \text{(Eqn. A6)}$$

and on subsequent wet days at location *j*, individuals are updated according

$$N_{i,j,k,l}(0,t+1) = e^{-\mu_w} N_{i,j,k,l}(0,t) \quad \text{(Eqn. A7)}$$

Fish can be present when $r_{pond}(t) \geq 0$. The fish population is initiated from hatchlings.

The number of juvenile fish of age one are the summed individuals that decided to hatch on the previous day. For older ages (a below α), they are the juveniles which survived from the previous day, including the effects density dependence from the resident population

$$N_{J,s}(1,t) = \sum_j H_{j,s}(0,t-1) \quad \text{(Eqn. A8.1)}$$

$$N_{J,s}(a,t) = e^{-\mu_J} \left[\frac{KS_{pond}(t-1) - N_A^R(t-1)}{KS_{pond}(t-1)}\right]_{\geq 0} N_{J,s}(a-1,t-1) \quad \text{(Eqn. A8.2)}$$

where $N_A^R(t-1)$ is the number of adult resident individuals present at the start of day $t-1$ and $S_{pond}(t-1)$ the pond wet surface area on that day. Factor $\left[\frac{KS_{pond}(t-1) - N_A^R(t-1)}{KS_{pond}(t-1)}\right]_{\geq 0}$ is non-negative.

Juveniles become adults at age α. Adults only die because of density dependence, or when the pond radius has become zero.

$$N_{A,s}(a,t) = \left[\frac{KS_{pond}(t-1)}{N_A^R(t-1)}\right]_{\leq 1} N_{A,s}(a-1,t-1) \qquad \text{(Eqn. A9)}$$

The density dependent factor $\left[\frac{KS_{pond}(t-1)}{N_A^R(t-1)}\right]_{\leq 1}$ cannot be larger than one. Adult females which will survive until the next day *t* produce new eggs every day throughout their lives, which are fertilized by males, which will also survive until the next day. Reproduction requires that resident individuals both sexes are present and it is also set at zero for very biased resident sex ratios (ratio resident females to males outside of the interval [0.2, 5]). If all conditions are fulfilled, the number of new eggs with a mutant genotype appearing on day t equals

$$M(0,t) = \frac{1}{2} f \sum_a N_{A,f}(a,t) + \frac{1}{2} \frac{\sum_a N_{A,m}(a,t)}{\sum_a N_{A,m}^R(a,t)} f \sum_a N_{A,f}^R(a,t) \qquad \text{(Eqn. A10)}$$

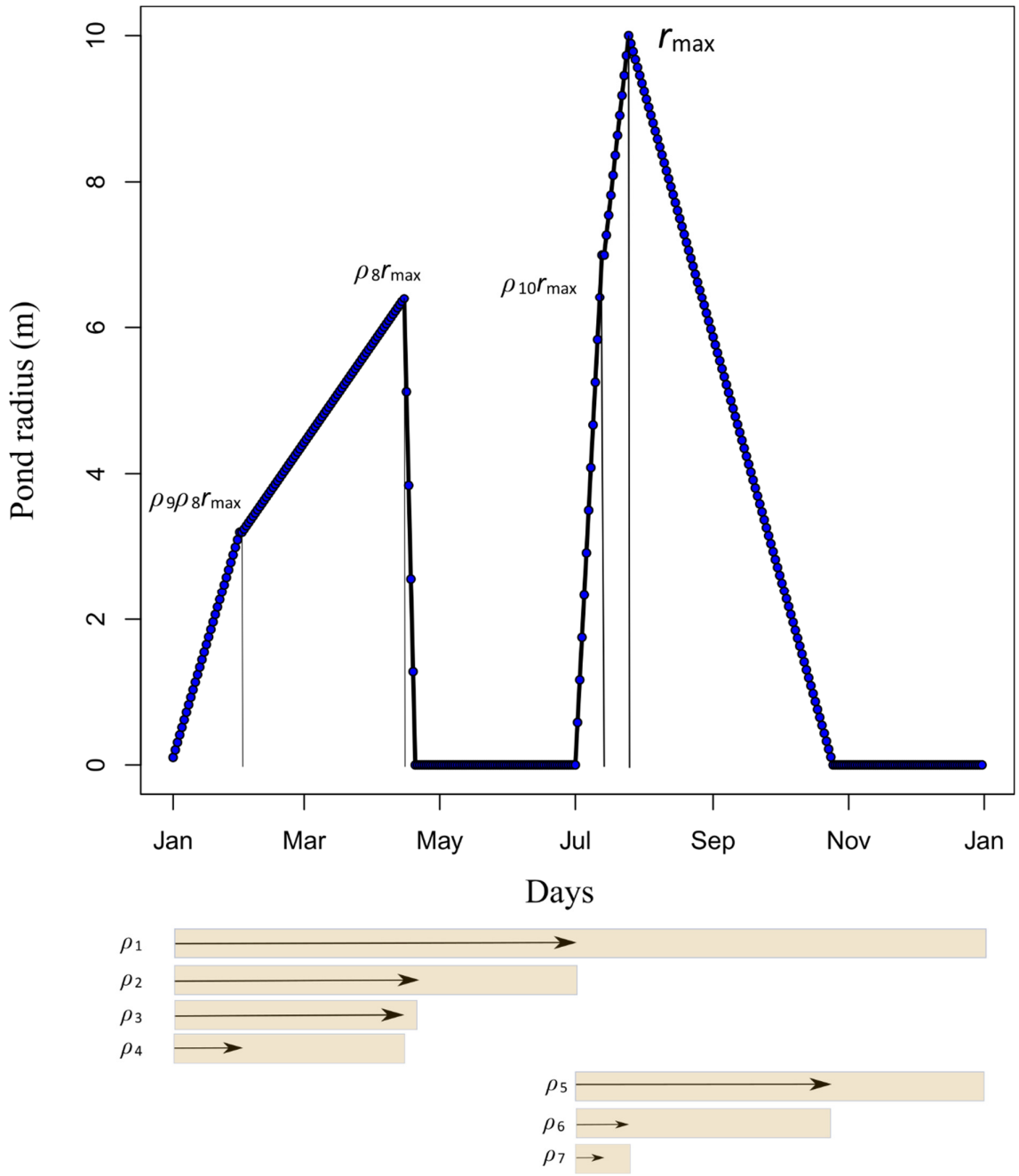
where the factors $\frac{1}{2}$ account for the fact that mutant individuals are heterozygotes and only transfer the mutant allele to half of their offspring. $N_{A,m}^R(a,t)$ is the resident number of adult males and $N_{A,f}^R(a,t)$ the number of resident adult females of a specific age present at time *t*. New eggs and their embryos have experienced zero dry days, they are put in wet conditions in the soil. These eggs are distributed at random over the pond bottom which adults can reach. This implies that location category *j* receives eggs proportional to its surface (which is $\pi(2j+1)$ giving the $M_j(0,t)$ per location category.

On the first day with dry conditions at location *j*, all new eggs deposited in that location in the preceding wet period $M_j(0,t)$ are added to the stage one embryos from older wet periods. The buffer with new eggs is emptied.

$$N_{1,j,1,1}(0,t) = e^{-\mu_w} N_{i,j,1,1}(0,t-1) + \sum_a e^{-\mu_w a} M_j(0,t-a) \qquad \text{(Eqn. A11)}$$

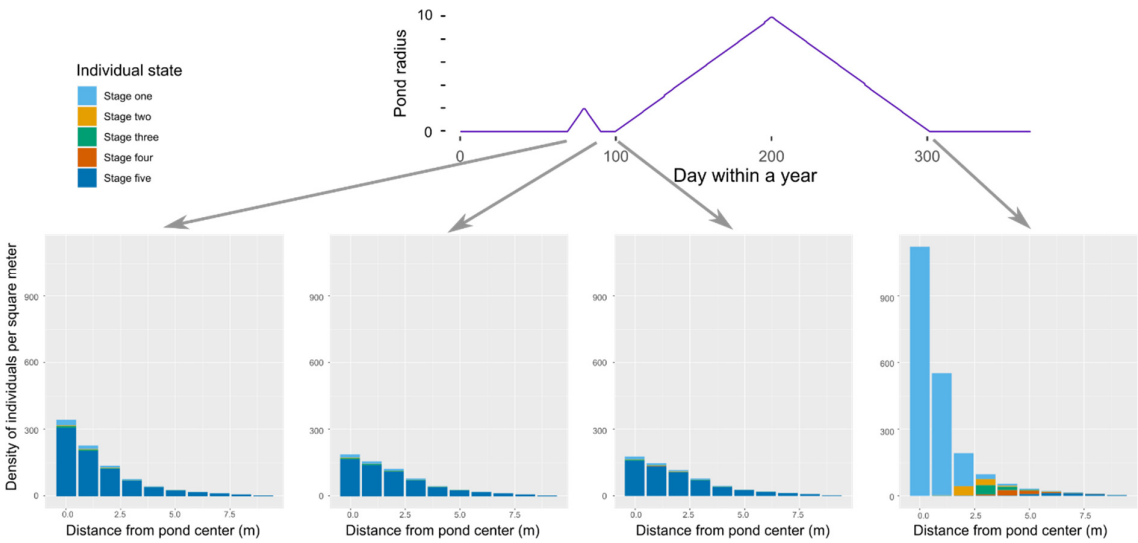
Resident individuals have the homozygous genotype and the phenotype of a homozygote. To determine their numbers we can use mostly the same equations as above where resident individuals are now counted. Only in the number of new eggs produced (Eqn. A10) a modification can be made to make it represent the number of new eggs with resident genotype. Only the route via females needs to be accounted for and resident genotypes are transmitted with probability one, not one half as all males contribute the resident allele.

As further supplementary material, two figures are provided.



Supplementary figure one. Parameterization of pond filling time profiles. Ten parameters, all constrained between zero and one, are involved in the specification of the pond filling regime, for a fixed parameter r_{max}, the maximum radius the pond can have within a year. Parameter ρ_1 is the proportion of the entire year taken up by a first pond filling and drying period. Parameter ρ_2 is the proportion of that period which is wet. Parameter ρ_3 if the proportion of that wet period at which maximum radius is reached. Parameter ρ_4 is the proportion of the first wet period where the radius increases, at which the speed of filling changes. Parameters ρ_5 to ρ_7 are the corresponding

parameters for the second pond filling and drying period. Parameter ρ_8 is the proportion of r_{max} which is the maximum reached within the first period. Parameter ρ_9 determines the radius at which the speed of filling changes in the first wet period and parameter ρ_{10} determines this radius for the second wet period.



Supplementary figure two. Distributions of embryos in different stages at the end of each period in a pond filling regime drawn above the stacked barcharts. The pond filling regime has a short period where reproductions cannot occur and a main longer one where new eggs are added to the egg bank. By the end of the wet period, on day 300, all eggs at nine to ten meter from the pond center have been dry since day 212.