used to conditionally alter properties of the core clock by changing cell-autonomous period, deleting TTFL function, and/ or compromising synaptic signalling. Such studies have revealed a hierarchy of neurochemically defined cell types that contribute to the correct functioning of the SCN as a network and thereby circadian behaviour. Of note are Neuromedin S-positive (NMS) cells, which constitute ca. 40% of SCN neurons. Synaptic signalling in these cells is essential for the SCN to control circadian behaviour, and changes in the properties or competence of the cell-autonomous TTFL in NMS cells are echoed at the level of mouse behavioural rhythms. In contrast, manipulations of AVP- or VIP-expressing cells (which are sub-groups of the NMS population) have a more modest effect. Paradoxically, however, loss of NMS itself has no obvious effect on the clockwork. Furthermore, recent developments of the intersectional approach have shown that, astonishingly, neurons are not the only active cellular participants in SCN timekeeping. Through conditional manipulations of the astrocyte TTFL, it is clear that the astrocytic clock can also control the ensemble SCN molecular clockwork and overt circadian behaviour of the mouse.

## Prospect

The SCN is a unique brain area: two clusters of 10,000 cells exert global, hour-by-hour control over every vital function of the organism. It generates an ensemble circadian signal that has extreme robustness, persistence and precision (varying by ca. ±5 minutes over the 24 h cycle, i.e. 0.35% error). These emergent properties are established by the linkage between cell-autonomous and circuit-level mechanisms, such that outputs of the cellular clock become inputs to it (Figure 4B). Significant questions remain as to how individual SCN neurons generate day-to-day electrophysiological rhythms, what these rhythms mean for the SCN and how they are linked to the molecular clock. At the network level, the neurochemistry that 'glues' the SCN together is poorly understood: we have a fair grasp of the distribution and release of some individual neuropeptides, but no deep understanding of how peptides and other neurotransmitters come together to create a robustly functional network. Finally, we do not know the full hierarchy of signals within the SCN at the level of neurochemicals or cell-type and their topology, nor how these signals are directed outside of the SCN to determine organismal physiology. Time to get to work.

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# Extremely rapid maturation of a wild African annual fish

Milan Vrtílek[1], Jakub Žák[1,2], Martin Pšenička[3], and Martin Reichard[1,*]

Ephemeral habitats can impose challenging conditions for population persistence. Survival strategies in these environments can range from high dispersal capacity to the evolution of dormant stages able to tolerate a harsh environment outside the temporal window of favourable conditions [1]. Annual killifish have evolved to live in seasonal pools on the African savannah and display a range of adaptations to cope with an unpredictable environment [2,3]. For most of the year, killifish populations survive as diapausing embryos buried in dry sediment. When savannah depressions fill with rainwater, the fish hatch, grow rapidly and, after attaining sexual maturity, reproduce daily [2,4]. *Nothobranchius furzeri*, a model species in ageing research [2,3], is distributed in a region where the climate is particularly dry and rains are unpredictable [5]. Here, we demonstrate that the fast juvenile growth and rapid sexual maturation shown by *N. furzeri* in captivity is actually an underestimate of their natural developmental rate. We estimated the age of *N. furzeri* in natural populations by counting daily-deposited increments in the otoliths and performing histological analysis of gonads. We found that *N. furzeri* are capable of reaching sexual maturity within 14 days after hatching, which to our knowledge is the fastest rate of sexual maturation recorded for a vertebrate. We also demonstrate that *N. furzeri* can grow from an initial length of 5 mm up to 54 mm over the course of a two-week period. Such rapid juvenile development is likely to be adaptive since some pools were entirely desiccated 3–5 weeks after filling, but retained a viable killifish population that reproduced before the adults succumbed to the disappearance of their pool.

We surveyed natural populations of *N. furzeri* across its range in southern

Mozambique between January and May 2016 by regular inspection of field sites (Figure 1A) and installation of dataloggers. We collected *N. furzeri* from eight separate pools (A–H, Table S1, see Supplemental Information) within a period of three weeks after the pools first filled with rainwater. Comparison between the timing of pool filling (recorded during our presence at sites and estimated from dataloggers) and fish age (estimated from otolith growth checks, n = 26) indicated that fish hatched within the first three days after rains flooded a pool (Table S1). Histological analysis of male and female gonads of individuals aged 14–15 days (n = 6 fish from 2 populations, and 8 control fish) demonstrated that both sexes were mature. Females possessed ripe ova and males had developed spermatozoa that were present in the sperm ducts (Figure 1C–E). Sexually dimorphic nuptial colouration (Figure 1B, Table S2) suggested that all fish were sexually mature in four populations (A, C–E) estimated to be 14 days old. In a further three populations (B, F and G) fish were estimated to be 12–15 days old and included a combination of juvenile and sub-adult fish. A final population (H) was comprised of 14-day-old fish that were still juveniles. Mean size of fish aged 14–15 days ranged from 18.8 to 42.7 mm in four populations, with an overall range of 17–43 mm in females and 17–54 mm in males. At the age of 17 days one male reached 63 mm (Table S2; n = 260 fish measured overall).

Previous studies demonstrated that under optimal laboratory conditions, *N. furzeri* can reach sexual maturity in as few as 18 days (first mating attempts were observed 1–3 days earlier), followed by rapid senescence in functional traits and high intrinsic mortality [4], resulting in a median lifespan of only 4–6 months [3]. It was questioned, however, whether such a rapid life history was a natural feature of the species, or a consequence of laboratory selection for short lifespan combined with particularly favourable housing conditions with temperature, food supply and population density optimised for rapid growth [6]. Indeed, juvenile growth and sexual maturation are flexible in *N. furzeri* and respond strongly to changes in population density and resource availability.

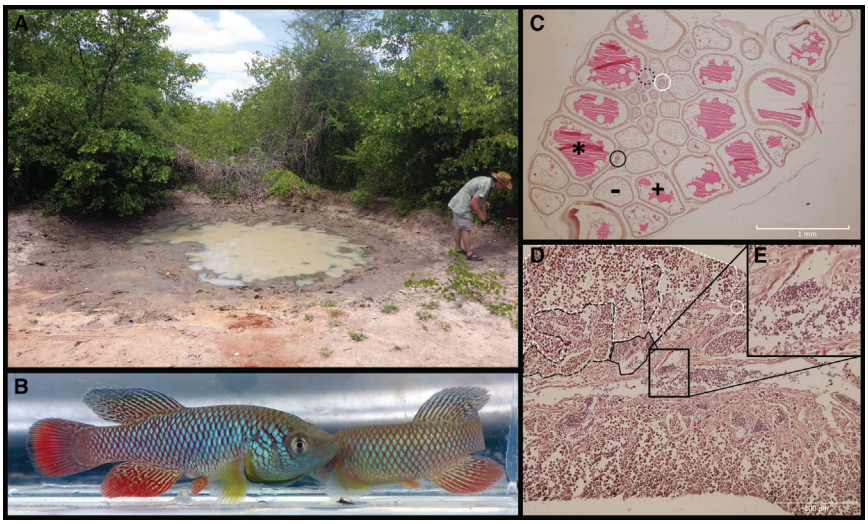


**Figure 1. Adult turquoise killifish (*Nothobranchius furzeri*) and its natural habitat.**
(A) Site 'D' on 27 January 2016 when it contained at least 27 adult fish estimated to be 16–17 days old. (B) Two 14-day-old male *N. furzeri* from site 'A'. (C) Histological section of an ovary from a 14-day-old female from site A that contains oogonia (dotted black circle), primary oocytes at the primary growth phase, chromatin-nucleolus stage (black circle) and perinucleolus stage (white circle), oocytes in the previtellogenic (-) and vitellogenic phase (+) and fully developed oocytes (*). (D) Histological section of the testis from a 14-day-old male from site A that includes spermatogonia (full white circle), spermatocytes (dotted white line), spermatids (broken black line) and spermatozoa (full black line). The boxed area (E) shows a magnified view of mature spermatozoa released into the spermatic duct.

Surveys of natural *N. furzeri* populations in 2016 revealed that pools typically desiccated 1–4 months after filling [7]. The shortest period for which a pool contained water and supported a killifish population that reached sexual maturity was 20 days. In that population we sampled fish at the age of 7 days (all juveniles, n = 12), 14 days (adult colouration, size not measured, n = 5) and 16–17 days old (all fish adult, 32–45 mm long, n = 16) (Table S2). Two other pools held water (and fish) for just 33 days and the median pool duration was 65 days [7].

This finding demonstrates that fast juvenile development is adaptive in killifish despite an apparent tradeoff with early-onset ageing; even a pool that desiccated in three weeks permitted successful reproduction of *N. furzeri* and, thereby, supported a viable population that year. *Nothobranchius* species display two contrasting life-cycle phases. The first is a long-lasting embryonic period with high resistance to ageing and a bet-hedging strategy of three facultative diapause stages that spreads out the developmental time of individual embryos [2,3]; this strategy serves as an adaptation to promote survival during extended periods of drought. The second is an exceptionally short post-embryonic period characterised by rapid juvenile growth and sexual maturity — an adaptation to unpredictable pool longevity that comes with a cost of short post-embryonic lifespan [2,3].

Pronounced seasonality often gives rise to life cycles combining resting and active stages. In non-vertebrate animals, early developmental stages are usually encased in protective envelopes that shield individuals from a harsh environment. In vertebrates, it is typically the adult stage that is adapted to cope with periods of extreme conditions at seasonally dormant stages, varying from annual winter hibernation to adaptation to periodic desiccation [1]. Nevertheless, several clades of annual killifish in Africa and the Neotropics [8], along with a semelparous chameleon in Madagascar [9], have evolved annual life cycles featuring a brief post-embryonic lifespan. Among these taxa, the rapid post-hatching development and maturation shown by *N. furzeri* represent the most extreme life history, combining a vertebrate body plan with a characteristically invertebrate solution to survival in unpredictable conditions.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes two tables, experimental procedures, and references and can be found with this article online at https://doi.org/10.1016/j.cub.2018.06.031.

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## AUTHOR CONTRIBUTIONS

M.R. conceived and designed the study, M.V. and J.Ž. collected field data, M.P. performed histological analysis, and M.V. and M.R. drafted the manuscript.

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# The cephalo-thoracic apparatus of *Caputoraptor elegans* may have been used to squeeze prey

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Alienoptera is an insect order recently described from mid-Cretaceous amber [1] and is phylogenetically nested in the Dictyoptera lineage. Alienoptera currently comprises three species: *Alienopterus brachyelytrus* [1], *Alienopterella stigmatica* [2] and *Caputoraptor elegans* [3]. The most interesting is *Caputoraptor elegans*, which was recently described in *Current Biology* by Bai and colleagues [3] and which has an unusual cephalo-thoracic device formed by wing-like extensions of the genae and the corresponding edges of the pronotum. Bai and colleagues [3] suggested that the cephalo-thoracic apparatus may have been used to hold the female and male together during copulation. According to this possible function, the cephalo-thoracic apparatus of the female would fit together with the spread forewings of the male while the female was on the back of the male during copulation. This function was proposed based on examination of females and nymphs, and the authors stated that it could be falsified if a male with a similar apparatus were discovered. After examining a male nymph of this species (Figure 1), I here suggest that the cephalo-thoracic apparatus was not used for copulation but was instead used for predation and feeding.

The hypothesis that females use the cephalo-thoracic apparatus to hold onto males during copulation is improbable for two reasons. First, if the apparatus was used only for coupling, it would not be developed (or fully developed) in nymphs where it would not fulfil its purpose. Second, a character that is related to coupling and that occurs in only one of the sexes is almost always subject to sexual selection. Such selection, however, usually leads to sex-specific features in the sex with less investment in offspring, *i.e.*, in the sex that produces microgametes, and these are the males [4,5]. Because males are exposed to more intense sexual selection than females, conspicuous secondary sexual characters usually evolve in males rather than in females [6]. Although exceptions exist in insects [7], they are rare and related to cases in which male investment in offspring involves more than sperm donation, as is the case in Heteroptera: Belostomatidae [8]. Such a case, however, is unknown in dictyopteran insects [9,10].

As indicated above, the hypothesis about the use of the cephalo-thoracic device by the female seems unlikely due to the finding of a male nymph (Supplemental Information) with the fully developed apparatus. The studied nymph has nine visible abdominal coxosternites (Supplemental Information), and it is therefore an immature male. Bai and colleagues [3] also suggested that the cephalo-thoracic device may have been used for capturing prey or for defence.

A detailed study of the morphology of the cephalo-thoracic apparatus in the studied nymph (Figure 1; Supplemental Information) has revealed new findings that provides insight into its probable function. The area between the posterior part of the head (gula) and the anterior part of the pro- and mesothorax forms a cavity (Figure 1A). A sharp ridge surrounds the cavity, except in the area of the mouth and of the gap for the forelegs. The thoracic part of the ridge consists of projections of the pro- and mesonotum. The pronotal projections have a straight serrate distal part and a non-serrate proximal part, and these parts curve towards each other. A narrow, sharp extension of the mesonotum abuts the pronotal part and borders the gap for the forelegs.

If the cephalo-thoracic device is closed, the edge of the pronotal ridge is inserted beneath the edge of the wing-like extensions of the genae, and the non-serrate proximal part of the pronotal ridge fits into the narrow groove in the gula under a part of the gena and subgena. The short mesonotal ridge probably attaches to the distal part of the maxillae (stipes) in the closed stage. The closed cephalo-thoracic device remains opened only in the space formed partly

# Supplemental Information: Extremely rapid maturation of a wild African annual fish

Milan Vrtílek, Jakub Žák, Martin Pšenička & Martin Reichard

## Supplemental Results

**Table S1. Geographic and phylogeographic information on study populations and details on hatching date estimates, related to the main text and Figure 1.**

Data on population membership to one of the three *N. furzeri* phylogeographic lineages (Clade) and male colour morph (morph), exact site location (GPS coordinates) and site code. Sex and size (Total Length, TL in mm) of individual fish sampled for otolith analysis, sampling date, age estimate on the basis of otolith readings (Age), and back-calculated hatching date (Hatching) are given.

| Site | Clade (morph) | GPS coordinates | Sex | TL | Sampling | Age | Hatching |
|---|---|---|---|---|---|---|---|
| A | Limpopo | E 32°36'35" | Male | 28.97 | 24 Mar | 14 | 10 Mar |
| | North | S 23°41'37" | Male | 32.48 | 24 Mar | 14 | 10 Mar |
| | (red) | (MZCS 007) | Female | 32.01 | 24 Mar | 14 | 10 Mar |
| | | | Female | 31.38 | 24 Mar | 14 | 10 Mar |
| B | Limpopo | E 32°58'27" | Male | 33.78 | 27 Mar | 15 | 12 Mar |
| | North | S 24°21'28" | Juvenile | 25.77 | 27 Mar | 15 | 12 Mar |
| | (red, yellow) | (MZCS 121) | Juvenile | 25.23 | 27 Mar | 15 | 12 Mar |
| C | Limpopo | E 32°46'44 | Male | 57.11 | 27 Jan | 17 | 10 Jan |
| | South | S 24°25'08" | Male | 50.21 | 27 Jan | 17 | 10 Jan |
| | (red, yellow) | (MZCS 119) | Female | 39.96 | 27 Jan | 16 | 9 Jan |
| D | Limpopo | E 32°45'58" | Male | 40.28 | 27 Jan | 17 | 10 Jan |
| | South | S 24°24'11" | Male | 44.18 | 27 Jan | 16 | 9 Jan |
| | (red, yellow) | (MZCS 405) | Male | 40.04 | 27 Jan | 17 | 10 Jan |
| E | Limpopo | E 32°36'30" | Female | 38.56 | 24 Mar | 14 | 10 Mar |
| | North | S 23°41'26" | Female | 42.84 | 24 Mar | 13 | 9 Mar |
| | (red, yellow) | (MZCS B04) | Male | 47.6 | 24 Mar | 13 | 9 Mar |
| F | Chefu | E 33°16'22" | Juvenile | 12.23 | 21 Mar | 12 | 9 Mar |
| | (red, yellow) | S 22°10'42" | Juvenile | 23.11 | 21 Mar | 12 | 9 Mar |
| | | (MZCS 421) | Male | 26.08 | 21 Mar | 12 | 9 Mar |
| G | Chefu | E 32°34'54" | Male | 27.61 | 10 Feb | 14 | 27 Jan |
| | (red, yellow) | S 22°30'28" | Male | 31.11 | 10 Feb | 14 | 27 Jan |
| | | (MZCS A29) | Female | 20.6 | 10 Feb | 14 | 27 Jan |
| H | Chefu | E 32°53'54" | Juvenile | 27.05 | 9 Feb | 14 | 26 Jan |
| | (red) | S 22°16'33" | Juvenile | 26.35 | 9 Feb | 14 | 26 Jan |
| | | (MZCS A41) | Juvenile | 19.53 | 9 Feb | 14 | 26 Jan |
| | | | Juvenile | 17.37 | 9 Feb | 15 | 27 Jan |

**Table S2. Fish development and habitat conditions in study pools, related to the main text and Figure 1.**
On-site body size measurements, state of male colouration and key information on pool inundation and desiccation. Fish body size is given as the range of Total Length (TL) with the number of individuals measured in parentheses.

| Site | Inundation* | Hatching | Date | Age | Female size | Male size | Additional information on key events |
|---|---|---|---|---|---|---|---|
| A | 10 Mar[1] | 10 Mar | 24 Mar | 14 | 30-38 mm (10) | 28-40 mm (8) | Nuptial colouration developed in males (Fig. 1a), 8 fish for gonad histology |
| | | | 11 Apr | 32 | 34-39 mm (8) | 37-47 mm (2) | Very low water level (<5 cm water depth) |
| B | 9 Mar[1,2] | 12 Mar | 9 Mar | - | - | - | A similar pool, located 1.5 km away filled with water (datalogger) |
| | | | 27 Mar | 15 | 37 mm (1) | 33-41 mm (2) | Subadults, male nuptial colouration starts to appear, 2 fish for gonad histology |
| | | | 7 April | 26 | 30-44 mm (7) | 32-39 mm (5) | Nuptial colouration developed in males |
| | | | 28 April | 47 | - | - | Wet mud, no fish present |
| C | 10 Jan[2] | 10 Jan | 17 Jan | 7 | - | - | Juveniles only |
| | | | 24 Jan | 14 | 37-43 mm (5) | 38-54 mm (9) | Nuptial colouration developed in males |
| | | | 27 Jan | 17 | 41-53 mm(13) | 45-63 mm (14) | |
| | | | 8 Feb | 29 | 57-66 mm (6) | 57-73 mm (6) | |
| | | | 6 Apr | 87 | - | - | Dry (regular visit) |
| D | 10 Jan[2] | 10 Jan | 17 Jan | 7 | - | - | Juveniles only (14-18 mm, n =12) |
| | | | 24 Jan | 14 | - | - | Nuptial colouration developed in males |
| | | | 27 Jan | 17 | 32-38 mm (18) | 35-45 mm (8) | |
| | | | 30 Jan | 20 | - | - | Dry (regular visit) |
| E | 10 Mar[3] | 10 Mar | 24 Mar | 14 | 32-43 mm (15) | 37-50 mm (10) | Nuptial colouration developed in males |
| F | 8 Mar[1] | 9 Mar | 21 Mar | 12 | - | - | Subadults, male nuptial colouration starts to appear (22-26 mm, n = 6) |
| | | | 8 April | 30 | 33-42 mm (15) | 36-43 mm (11) | Nuptial colouration developed in males |
| G | 25 Jan[3] | 27 Jan | 10 Feb | 14 | - | - | Subadults, male nuptial colouration starts to appear |
| | | | 23 Feb | 27 | 30-34 mm (15) | 31-43 mm (15) | Nuptial colouration developed in males |
| | | | 5 Mar | 38 | 34-48 mm (11) | 39-48 mm (11) | |
| | | | 17 April | 81 | - | - | Dry (regular visit) |
| H | 25 Jan[3] | 26 Jan | 9 Feb | 14 | - | - | Juveniles only |
| | | | 24 Feb | 29 | 32-43 mm (9) | 38-57 mm (8) | Nuptial colouration developed in males |

*Superscripts denote information on pool inundation: [1]Site filled with rainwater during a regular site visit; [2]Datalogger from a nearby pool indicated inundation; [3]Precipitation forecasted for the local area (http://www.yr.no).

## Supplemental Experimental Procedures

We monitored populations of *Nothobranchius furzeri* between 17 January and 26 May 2016 across the species' range in southern Mozambique. Study sites contained populations from all three phylogeographic clades of the species and both red and yellow colour morphs (Table S1). During a visit to a field site, we collected fish using standardized hauls (5 m long) of a Seine net (2.7 m long, 0.7 m deep, 4 mm mesh size), covering all habitats in the pool. In the case of a recently filled pool (when small juveniles were expected), the sampling net had the same dimensions but a 2 mm-mesh size to retain the smaller fish. A subsample of fish was measured for their total length (TL, from tip of the snout to the end of caudal fin) and a record of the development of their colouration was made (juvenile colouration, start of nuptial colouration in males, full sexual dichromatism). For a subset of collections (Table S1), fish were stored in Baker's solution or 96% ethanol and returned to the laboratory for analysis. Removal of fish for sample collection was quantitatively limited, as we tracked demographic parameters of each population over their lifespan [S1]. Water temperature was measured every 3 hours using data loggers (HOBO UA-001-08, Onset Computer Corp., Bourne, MA, USA) in a subset of pools and ranged from 18.7 to 37.4° C, with strong fluctuation over each 24-hour cycle that exceeds differences among pools [S2]. Details on field sampling are available in [S1].

The dates of pool inundation were recorded from our presence at the sites, estimated from the dates of precipitation and from data loggers that were installed in the pools before inundation (Table S2). Dataloggers were installed in a subset of pools that were selected for long-term environmental monitoring across the entire region. Pools inundate synchronously after major precipitation events [S2] and the dates of inundation were cross-validated by our presence in the region and from otolith readings. The age of the fish was estimated from otoliths removed from fish stored in 96% ethanol. Three individuals (mixed sex) per species per site (total of 26 individuals) were collected and analysed (Table S1). The number of daily increments in otoliths was read by a commercial facility (Barcelona Otolith Reading Services, Spain). The full description of the methods used is available at [S2].

Histological examination of gonad maturation was performed on samples preserved in Baker's solution. Three males and three females from site A (at age 14 days) and site B (age 15 days) were used (Table S2), along with eight control fish of age > 4 weeks. Gonads were dissected, dehydrated using an ethanol series and xylene, embedded in Paraplast, sectioned (5 μm) and stained in H&E.

All work was carried out in accordance with relevant guidelines and regulations. Sample collection complied with legal regulations in Mozambique (collection licence: ADNAP-170/7.10/16).

## Supplemental References


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