

From resilient adults to fragile early life stages: salinity tolerance across the life cycle of the green panchax, *Aplocheilichthys blockii*

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Abstract

Freshwater fishes that occupy coastal and estuarine landscapes routinely experience fluctuating salinity, and the capacity to cope with this variation shapes where and how such species persist. The green panchax, *Aplocheilichthys blockii*, is a small indigenous killifish valued both as a larvivorous agent for mosquito control and as an ornamental species, yet its physiological limits and early-life responses to salinity remain poorly quantified. We examined salinity tolerance across ontogeny—in adults, larvae and embryos—and characterised the developmental responses that accompanied it. Under abrupt exposure, adults survived salinities up to 20 ppt without mortality, whereas survival collapsed between 30 and 40 ppt, yielding a probit-estimated median lethal salinity (LC_{50}) of 27.6 ppt. When salinity was raised gradually (5 ppt every 48 h), tolerance increased substantially: no mortality occurred up to 60 ppt, and a proportion of fish survived to 75 ppt. Larvae were consistently more vulnerable than adults, losing roughly one-third of individuals by 20 ppt and dying completely within 3 h at 40 ppt. Embryos hatched across the entire 0–40 ppt range, but hatching success declined and incubation lengthened as salinity increased, and a subset of embryos entered a reversible, diapause-like developmental arrest under the most saline conditions. These results reveal pronounced stage-dependent tolerance—adults being the most robust life stage—and indicate that recruitment in *A. blockii* is likely constrained by the salinity sensitivity of its early stages.

Introduction

Killifishes (Cyprinodontiformes) are a species-rich assemblage of small teleosts that are characteristically tied to shallow, seasonally unstable waters, from temporary pools to coastal marshes (Murphy and Collier 1997; Furness 2016). Within this order, the green panchax *Aplocheilichthys blockii* ranges widely through the freshwater and mildly brackish habitats of India, Sri Lanka and Pakistan (Ilango 1990; Jafari et al. 2019). It is a diminutive fish, seldom exceeding 5 cm in total length (Job 1940), and an efficient predator of the aquatic larvae of *Aedes*, *Anopheles* and *Culex* mosquitoes (Menon and Rajagopalan 1978; Kumar et al. 1998; Chakraborty et al. 2008). This appetite for larvae underlies the long-standing interest in *A. blockii* and other native larvivores as tools for the biological suppression of mosquito-borne disease (WHO 2003). At the same time, the hardiness, small size and attractive colouration of the species have earned it a place in the ornamental fish trade, where indigenous forms increasingly support local livelihoods and small-scale aquaculture.

The habitats in which *A. blockii* is commonly found are rarely stable. Field populations occur in ponds, ditches, paddy margins and backwater fringes where salinity, dissolved oxygen and other water-quality variables can shift markedly over short periods. Persistence under such conditions implies a degree of environmental tolerance that may permit the species to occupy disturbed or human-modified systems. Although the reproductive biology and early development of the green panchax have recently been documented (George et al. 2026), quantitative information on its stress physiology remains sparse. In particular, how the different life stages of *A. blockii* respond to salinity—the dominant environmental gradient in the coastal wetlands it inhabits—has not been systematically examined.

Salinity is a decisive factor governing habitat use and distribution in fishes, and closely related species can differ sharply in the thresholds that separate freshwater from estuarine occupancy (Fuller et al. 2007; DiMaggio et al. 2009). Among killifishes, the ability to withstand osmotic challenge is often coupled to reproductive and developmental strategies. Annual species from Africa and the Americas are the best-studied examples: their embryos can enter diapause, a reversible arrest of development that appears at defined points during embryogenesis and confers resistance to desiccation, osmotic stress and other adverse conditions (Podrabsky and Hand 1999; Furness et al. 2015; Machado and Podrabsky 2007; Podrabsky and Culpepper 2012; Dolfi et al. 2019; Chalar et al. 2021). Whether comparable developmental flexibility exists in non-annual aplocheilids from the Indian subcontinent is essentially unknown.

Tolerance to osmotic and other environmental stress varies widely among fishes and reflects the underlying capacity for ionic and osmotic regulation (Uchida et al. 2000; Fridman 2020; Torres-Rodríguez et al. 2025). The recurrent occurrence of *A. blockii* in salinity-variable waters suggests appreciable euryhalinity, but the actual limits of that tolerance, and the extent to which they differ among life stages, have not been measured. Establishing these limits is a prerequisite for evaluating the ecological performance of the species and its suitability for deployment in the fluctuating, often degraded, aquatic systems where mosquito control is most needed.

The present study addresses this gap by quantifying, in a single experimental framework, (i) the salinity tolerance of adults under both abrupt exposure and gradual acclimation, (ii) the salinity tolerance of early larvae, and (iii) embryonic development and hatching success across a salinity gradient. Together these measurements provide baseline information on the osmoregulatory ecology of *A. blockii* and on the developmental plasticity that may buffer this species against the variable conditions of its native range.

Materials and methods

Fish collection and maintenance

Adult *Aplocheilichthys blockii* were hand-netted from freshwater streams and ponds in and around Panangad, Kochi, Kerala, India, and transported in aerated containers to the laboratory. Fish were held in 40 L glass aquaria maintained at $28 \pm 2^\circ\text{C}$ and pH 7.5–8.0, at a stocking density of 30 individuals per tank. Sprigs of *Hydrilla* sp. were added to provide structural cover. Animals were fed to satiation on live feeds (bloodworms and *Daphnia*) supplemented with a formulated diet (40% crude protein, 8% lipid). Approximately 10% of the tank volume was renewed on alternate days, and temperature, pH, salinity, alkalinity and ammonia were monitored throughout the holding period. Fish were acclimated to laboratory conditions for at least two weeks before use.

Spawning and egg incubation

For spawning, groups of ten conditioned adults (2.9–3.5 cm, 1:1 sex ratio) were transferred to bare-bottomed 50 L tanks filled with dechlorinated freshwater, which facilitated recovery of the demersal, adhesive eggs, following the captive-breeding and egg-collection protocol established for this species (George et al. 2026). Eggs were collected daily, examined under a stereomicroscope, and only viable, cleavage-stage embryos were retained for experiments. Selected embryos were incubated at salinities of 0, 10, 20, 30 and 40 ppt, with 15 embryos held in 250 mL of test medium per replicate. Each salinity was tested in triplicate at 28°C under a 12 h light : 12 h dark photoperiod. Embryos were inspected daily; hatching success, the proportion of embryos completing normal development, and the mean incubation period were recorded for each treatment.

Larval salinity tolerance (abrupt exposure)

Ten-day-old larvae were transferred directly, without prior acclimation, to salinities of 0 (control), 10, 20, 30 and 40 ppt. Each treatment comprised three replicates of ten larvae in 500 mL of test medium. Survival and behaviour were followed for 240 h, with continuous observation during the first 3 h of exposure and subsequent checks at 24 h intervals. Larvae were fed copepod and *Moina* nauplii three times daily. Dead individuals were counted and removed at each observation.

Adult salinity tolerance (abrupt exposure)

Adult fish (3.1 ± 0.2 cm) were transferred directly to salinities of 0, 10, 20, 30 and 40 ppt without acclimation. Each treatment was run in triplicate with ten fish per 5 L. Survival and behaviour were recorded over 240 h, with frequent observation during the initial exposure phase and subsequent checks at 24 h intervals. Fish were fed *Daphnia* three times daily.

Adult salinity tolerance (gradual acclimation)

To test whether acclimation altered tolerance, a separate batch of adults (3.1 ± 0.2 cm) was exposed to a stepwise increase in salinity. Beginning at 0 ppt, salinity was raised by 5 ppt every 48 h using concentrated seawater (80 ppt) until 75 ppt was reached. Each step was maintained in triplicate with ten fish per 5 L. Survival was scored at every salinity increment, and behaviour and survival were then followed for a further 240 h under the same feeding regime described above.

Water-quality monitoring

Test salinities were prepared by diluting or concentrating natural seawater with dechlorinated freshwater and verified with a calibrated refractometer. Temperature, pH, total alkalinity and ammonia were measured in each experimental series using standard methods, and mean values are summarised in Table 2. Conditions were held within ranges considered non-stressful for small tropical teleosts, so that mortality could be attributed to the imposed salinity treatments rather than to background water quality.

Statistical analysis

Survival was expressed as the percentage of individuals alive at the end of the observation period for each replicate. Because the response variable was a proportion, values were arcsine square-root transformed before parametric analysis. Differences among salinity treatments were tested by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test for pairwise comparisons. The median lethal salinity (LC_{50}) for adults under abrupt exposure was estimated by probit regression of mortality on log-transformed salinity; because the concentration–response was steep, with mortality confined to a narrow salinity band, the LC_{50} is reported as a point estimate. Hatching success, developmental success and incubation period were likewise compared among salinities by one-way ANOVA. All analyses were performed at a significance level of $P < 0.05$, and results are presented as mean $\pm$ standard deviation.

Use of AI-assisted tools

During the preparation of this manuscript, the authors used a generative AI assistant (Claude; Anthropic, San Francisco, USA) to support language editing and document formatting. The AI tool was not used to generate research data or scientific conclusions. All output was reviewed, verified and edited by the authors, who take full responsibility for the integrity and accuracy of the work.

Results

Salinity tolerance of adults under abrupt exposure

Adult *A. blockii* tolerated abrupt transfer to moderate salinities remarkably well. Survival remained complete (100%) throughout the 240 h observation at 0, 10 and 20 ppt (Fig. 1). At 30 ppt, mortality began after roughly 4.5 h; survival fell to 23.3% by 24 h and then stabilised near 16.7% for the remainder of the trial. At 40 ppt the decline was abrupt, with survival dropping to 60% within the first hour, to 36.7% by 2 h, and to zero within 5 h. Fish exposed to the two highest salinities displayed clear signs of osmotic stress before death, including elevated opercular beat rate and bursts of erratic swimming. The effect of salinity on final survival was highly significant (one-way ANOVA, $F_{4,10} = 287.5$, $P < 0.001$), and probit analysis placed the median lethal salinity at 27.6 ppt (Fig. 2).

Salinity tolerance of adults under gradual acclimation

When salinity was raised gradually in 5 ppt steps, the tolerance of adults was far greater than under abrupt exposure (Fig. 3). No mortality occurred at any salinity up to and including 60 ppt. Beyond this point survival declined progressively, averaging 70% at 65 ppt, 53.3% at 70 ppt and 36.6% at 75 ppt; interpolation places the median lethal salinity for gradually acclimated fish at approximately 71 ppt, roughly 2.5-fold higher than under abrupt transfer. Swimming behaviour appeared normal across the

tolerated range, although a reduction in feeding activity was noted at salinities of 45 ppt and above. Salinity had a significant effect on survival under this regime (one-way ANOVA, $F_{15,32} = 12.0$, $P < 0.001$).

Salinity tolerance of larvae under abrupt exposure

Ten-day-old larvae were consistently more sensitive to salinity than adults (Fig. 4). Survival was complete at 0 ppt for the full 240 h. At 10 ppt survival remained high but was no longer perfect, settling at about 83% by the end of the trial, and at 20 ppt it declined more steeply to roughly 70%. Mortality was pronounced at 30 ppt, where survival fell to 40% within 24 h and then held near 30%. At 40 ppt no larva survived beyond 3 h. Whereas adults suffered no losses at or below 20 ppt, larvae experienced measurable mortality from 10 ppt upward, and their complete mortality at 40 ppt occurred faster than in adults (within 3 h versus 5 h). The effect of salinity on larval survival was significant (one-way ANOVA, $F_{4,10} = 24.3$, $P < 0.001$).

Embryonic development and hatching under salinity stress

Embryos proved comparatively resilient, hatching across the entire salinity range tested (0–40 ppt), although both the likelihood and the timing of hatching shifted with salinity (Table 1). Hatching success was highest at 10 ppt ($86.6 \pm 13.3\%$) and declined steadily as salinity rose, reaching $64.4 \pm 10.1\%$ at 40 ppt. The proportion of embryos completing normal development followed the same trend, from 100% at 10 ppt to $82.2 \pm 3.8\%$ at 40 ppt. Incubation lengthened with increasing salinity, extending from 8.8 ± 0.3 days at 0 ppt—close to the 8.3-day incubation reported for this species in freshwater (George et al. 2026)—to 14.4 ± 4.2 days at 40 ppt. These trends were consistent across replicates but did not reach statistical significance for hatching success ($F_{4,10} = 2.46$, $P = 0.11$), developmental success ($F_{4,10} = 2.45$, $P = 0.11$) or incubation period ($F_{4,10} = 2.64$, $P = 0.10$), reflecting the modest number of replicates and the variability among clutches.

A distinctive feature of development at higher salinities was the appearance, in a subset of embryos, of a delayed and quiescent phase that persisted beyond day 15 (Fig. 5). Affected embryos showed reduced movement and an extended incubation before eventually hatching, indicating a reversible slowing of developmental progression rather than embryonic death. This diapause-like arrest is discussed below in the context of developmental plasticity in killifishes.

Table 1

Hatching success, developmental success and mean incubation period of *Aplocheilus blockii* eggs at different salinities (mean $\pm$ SD, n = 3)

Salinity (ppt)	Hatching success (%)	Developmental success (%)	Incubation period (days)
0	84.4 $\pm$ 7.6	93.3 $\pm$ 11.5	8.8 $\pm$ 0.3
10	86.6 $\pm$ 13.3	100 $\pm$ 0	9.6 $\pm$ 0.7
20	77.7 $\pm$ 10.1	91.1 $\pm$ 10.1	12.3 $\pm$ 2.9
30	73.3 $\pm$ 6.7	95.5 $\pm$ 3.8	11.7 $\pm$ 0.6
40	64.4 $\pm$ 10.1	82.2 $\pm$ 3.8	14.4 $\pm$ 4.2

Table 2

Mean water-quality parameters (mean $\pm$ SD) recorded during the salinity experiments

Parameter	Adult abrupt	Adult gradual	Larvae abrupt	Egg incubation
Temperature ($^{\circ}$ C)	28.0 $\pm$ 1.4	28.3 $\pm$ 2.2	28.5 $\pm$ 2.5	28.3 $\pm$ 2.0
pH	8.4 $\pm$ 0.4	8.5 $\pm$ 0.4	8.4 $\pm$ 0.3	8.4 $\pm$ 0.4
Alkalinity (mg L ⁻¹)	87.6 $\pm$ 8.4	95.4 $\pm$ 15.4	84.8 $\pm$ 9.2	88.8 $\pm$ 14.3
Ammonia (mg L ⁻¹)	0.002 $\pm$ 0.001	0.004 $\pm$ 0.001	0.003 $\pm$ 0.001	0.001 $\pm$ 0.001

Discussion

This study shows that salinity tolerance in *Aplocheilus blockii* is strongly stage-dependent, with a clear gradient of resilience running from robust adults through more sensitive larvae to embryos whose developmental schedule is itself modulated by salinity. The adult tolerance recorded here—complete survival to 20 ppt under abrupt transfer and to 60 ppt under gradual acclimation—places the green panchax among the more euryhaline of the small freshwater teleosts and is comparable to the tolerances reported for candidate brackish-water killifishes such as *Fundulus seminolis* (DiMaggio et al. 2009).

The contrast between abrupt and gradual exposure is instructive. Direct transfer to salinities above 20 ppt caused steep mortality and yielded a median lethal salinity of 27.6 ppt, whereas stepwise acclimation extended survival to salinities well beyond full-strength seawater. This roughly 2.5-fold widening of the tolerance window indicates that the osmoregulatory machinery of adult *A. blockii* can be substantially up-regulated when osmotic change is slow enough to permit acclimation, a pattern well documented in euryhaline teleosts such as *Oreochromis mossambicus* and *Fundulus heteroclitus* (Uchida et al. 2000; Torres-Rodríguez et al. 2025). The practical implication is that laboratory or field estimates of salinity tolerance in this species depend heavily on the rate of exposure, and that acute-challenge values

considerably understate the range the species can occupy where salinity changes gradually with the tides or the seasons.

Larvae were the most salinity-sensitive of the free-living stages. They sustained losses from 10 ppt upward, dropped to about 30% survival at 30 ppt and died completely, and more rapidly than adults, at 40 ppt. This heightened vulnerability is consistent with the limited osmoregulatory capacity of early life stages in fishes, in which the ionocyte-based machinery required for hypo-osmoregulation is not yet fully developed (Fridman 2020), and echoes the salinity sensitivity reported for the larvae of other killifishes (Araujo et al. 2021). Ecologically, the sharp fall in larval survival between 20 and 30 ppt identifies the larval phase as a likely bottleneck for recruitment: adults may range widely across brackish habitats, but successful establishment of a new cohort should be confined to the lower-salinity portions of that range.

Embryonic responses reinforce this picture while adding a developmental dimension. Hatching success and developmental success were greatest at low salinity and declined as salinity rose, and incubation lengthened in parallel, indicating that osmotic stress both reduces hatching and desynchronises development. Similar salinity effects on hatching have been reported across cyprinodontiform embryos (Fuller et al. 2007; Mourabit et al. 2011). Most notably, a fraction of embryos at higher salinities entered a prolonged, quiescent phase beyond day 15 before resuming development and hatching. Because embryos of *A. blockii* normally complete development and hatch within about eight days under non-saline conditions (George et al. 2026), this persistence beyond day 15 represents a marked departure from the species' typical developmental schedule rather than ordinary variation in hatching time. The reversibility of this arrest, and its induction under osmotic stress, are hallmarks of embryonic diapause as described in annual killifishes, where diapause states are associated with pronounced metabolic depression, altered cell-cycle regulation and specialised control of water and ion permeability (Podrabsky and Hand 1999; Machado and Podrabsky 2007; Podrabsky and Culpepper 2012; Gao et al. 2022). We therefore interpret the delayed embryos as displaying a diapause-like developmental arrest rather than confirmed diapause in the strict sense; *A. blockii* is a non-annual aplocheilid, and rigorous demonstration of a discrete diapause stage would require staging against the criteria established for annual taxa together with metabolic or molecular corroboration (Podrabsky et al. 2017). Even so, the observation is significant, because facultative developmental delay under osmotic stress would provide a mechanism by which non-annual killifishes could buffer their embryos against transient salinity extremes, and would point to a broader distribution of diapause-like plasticity within the Cyprinodontiformes than is usually assumed (Furness et al. 2015, 2018).

Taken together, the results underscore the central role of ontogenetic stage in shaping salinity tolerance and developmental trajectory in *A. blockii*. Adults combine intrinsic euryhalinity with a capacity for acclimation that allows them to persist across a very wide salinity range, whereas embryos and larvae are more constrained. The broad adult tolerance revealed under gradual acclimation is consistent with the observed field distribution of the species in estuarine and backwater fringes subject to tidal salinity fluctuation, and parallels the buffering role of gradual acclimation reported in other euryhaline teleosts (Ahokas and Duerr 1975). For applications such as larvivorous mosquito control in coastal wetlands,

these findings suggest that adult *A. blockii* could be deployed across a considerable salinity range, but that self-recruiting populations will establish only where lower-salinity refugia are available for embryonic and larval development.

Conclusion

Salinity tolerance in *Aplocheilichthys blockii* is strongly stage-dependent. Adults tolerated abrupt exposure only to 20 ppt but survived gradual acclimation to 75 ppt, revealing a pronounced capacity for osmoregulatory acclimation. Larvae were markedly less tolerant, with survival falling sharply above 20 ppt, while embryos hatched across the full range tested but showed reduced and desynchronised development at higher salinities, including a reversible, diapause-like arrest under the most saline conditions. These patterns indicate that although adults can occupy a very wide salinity range, recruitment depends on the availability of low- to moderate-salinity conditions suited to embryonic and larval development. The study provides baseline information on the osmoregulatory ecology and developmental plasticity of this widely distributed larvivorous killifish and identifies its early life stages as the key constraint on its distribution in salinity-variable habitats.

Declarations

Competing interests

The authors declare that they have no competing interests.

Ethics approval

All procedures involving fish complied with the institutional and national guidelines for the care and use of experimental animals.

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Author Contribution

G.G and B.V conceived and designed the study. G.G. conducted the experiments and collected the data. G.G., S.T. and B.V. analysed and interpreted the results. G.G. drafted the manuscript, and S.T. and B.V. revised it critically. All authors read and approved the final manuscript.

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Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

References

1. Ahokas RA, Duerr FG (1975) Salinity tolerance and extracellular osmoregulation in two species of euryhaline teleosts, *Culaea inconstans* and *Fundulus diaphanus*. *Comp Biochem Physiol A* 52:445–448. [https://doi.org/10.1016/S0300-9629\(75\)80063-6](https://doi.org/10.1016/S0300-9629(75)80063-6)
2. Araujo LMD, Gonçalves LP Jr, Silva WDS, Luz RK (2021) Salinity and prey concentration on larviculture of killifish *Hypsolebias radiseriatus* (Cyprinodontiformes: Rivulidae). *Acta Sci Anim Sci* 43:e52075. <https://doi.org/10.4025/actascianimsci.v43i1.52075>
3. Chakraborty S, Bhattacharya S, Bhattacharya S (2008) Control of mosquitoes by the use of fish in Asia with special reference to India: retrospects and prospects. *J Manusia Lingkungan* 15:147–156. <https://doi.org/10.22146/jml.18687>
4. Chalar C, Clivio G, Montagne J, Costabile A, Lima A, Papa NG, Berois N, Arezo MJ (2021) Embryonic developmental arrest in the annual killifish *Austrolebias charrua*: a proteomic approach to diapause III. *PLoS One* 16:e0251820. <https://doi.org/10.1371/journal.pone.0251820>
5. DiMaggio MA, Ohs CL, Petty BD (2009) Salinity tolerance of the Seminole killifish, *Fundulus seminolis*, a candidate species for marine baitfish aquaculture. *Aquaculture* 293:74–80. <https://doi.org/10.1016/j.aquaculture.2009.04.009>
6. Dolfi L, Ripa R, Antebi A, Valenzano DR, Cellerino A (2019) Cell cycle dynamics during diapause entry and exit in an annual killifish revealed by FUCCI technology. *EvoDevo* 10:29. <https://doi.org/10.1186/s13227-019-0142-5>
7. Fridman S (2020) Ontogeny of the osmoregulatory capacity of teleosts and the role of ionocytes. *Front Mar Sci* 7:709. <https://doi.org/10.3389/fmars.2020.00709>
8. Fuller RC, McGhee KE, Schrader M (2007) Speciation in killifish and the role of salt tolerance. *J Evol Biol* 20:1962–1975. <https://doi.org/10.1111/j.1420-9101.2007.01368.x>
9. Furness AI (2016) The evolution of an annual life cycle in killifish: adaptation to ephemeral aquatic environments through embryonic diapause. *Biol Rev* 91:796–812. <https://doi.org/10.1111/brv.12194>

10. Furness AI, Reznick DN, Springer MS, Meredith RW (2015) Convergent evolution of alternative developmental trajectories associated with diapause in African and South American killifish. *Proc R Soc B* 282:20142189. <https://doi.org/10.1098/rspb.2014.2189>
11. Furness AI, Reznick DN, Tatarenkov A, Avise JC (2018) The evolution of diapause in *Rivulus* (Laimosemion). *Zool J Linn Soc* 184:773–790. <https://doi.org/10.1093/zoolinnean/zly021>
12. Gao X, Cai T, Lin Y, Zhu R, Hao W, Guo S, Hu G (2022) The function of glucose metabolism in embryonic diapause of annual killifish. *Comp Biochem Physiol D Genomics Proteomics* 42:100965. <https://doi.org/10.1016/j.cbd.2022.100965>
13. George G, Thiruvarasu S, Varghese B (2026) Embryonic and larval development of green panchax (*Aplocheilus blockii*): insights into the reproductive strategy of native killifish with biocontrol potential. *Curr Sci* 130:239–248. <https://doi.org/10.18520/cs/v130/i3/239-248>
14. Ilango K (1990) Larvivorous fishes of Madras and adjoining areas. *Rec Zool Surv India* 86:243–252
15. Jafari A, Enayati A, Jafari F, Haghi FM, Hosseini-Vasoukolaei N, Sadeghnezhad R, Azarnoosh M, Fazeli-Dinan M (2019) A narrative review of the control of mosquitoes by larvivorous fish in Iran and the world. *Iran J Health Sci* 7:49–60. <https://doi.org/10.18502/jhs.v7i2.1064>
16. Job TJ (1940) On the breeding and development of Indian ‘mosquito-fish’ of the genera *Aplocheilus* McClelland and *Oryzias* Jordan & Snyder. *Rec Indian Mus* 42:51–79
17. Kumar A, Sharma VP, Sumodan PK, Thavaselvam D (1998) Field trials of biolarvicide *Bacillus thuringiensis* var. *israelensis* strain 164 and the larvivorous fish *Aplocheilus blockii* against *Anopheles stephensi* for malaria control in Goa, India. *J Am Mosq Control Assoc* 14:457–462
18. Machado BE, Podrabsky JE (2007) Salinity tolerance in diapausing embryos of the annual killifish *Austrofundulus limnaeus* is supported by exceptionally low water and ion permeability. *J Comp Physiol B* 177:809–820. <https://doi.org/10.1007/s00360-007-0177-0>
19. Menon PKB, Rajagopalan PK (1978) Control of mosquito breeding in wells by using *Gambusia affinis* and *Aplocheilus blockii* in Pondicherry town. *Indian J Med Res* 68:927–933
20. Mourabit S, Edenbrow M, Croft DP, Kudoh T (2011) Embryonic development of the self-fertilizing mangrove killifish *Kryptolebias marmoratus*. *Dev Dyn* 240:1694–1704. <https://doi.org/10.1002/dvdy.22668>
21. Murphy WJ, Collier GE (1997) A molecular phylogeny for aplocheiloid fishes (Atherinomorpha, Cyprinodontiformes): the role of vicariance and the origins of annualism. *Mol Biol Evol* 14:790–799. <https://doi.org/10.1093/oxfordjournals.molbev.a025819>
22. Podrabsky JE, Culpepper KM (2012) Cell cycle regulation during development and dormancy in embryos of the annual killifish *Austrofundulus limnaeus*. *Cell Cycle* 11:1697–1704. <https://doi.org/10.4161/cc.19881>
23. Podrabsky JE, Hand SC (1999) The bioenergetics of embryonic diapause in an annual killifish, *Austrofundulus limnaeus*. *J Exp Biol* 202:2567–2580. <https://doi.org/10.1242/jeb.202.19.2567>
24. Podrabsky JE, Riggs CL, Romney AL, Woll SC, Wagner JT, Culpepper KM, Cleaver TG (2017) Embryonic development of the annual killifish *Austrofundulus limnaeus*: an emerging model for

25. Torres-Rodríguez M, Martínez-Rodríguez G, Rodríguez-Viera L, Mancera JM, Martos-Sitcha JA (2025) Physiological effects of water salinity on metabolism and fatty acid biosynthesis in the model fish *Fundulus heteroclitus*. *Animals* 15:2549. <https://doi.org/10.3390/ani15172549>

26. Uchida K, Kaneko T, Miyazaki H, Hasegawa S, Hirano T (2000) Excellent salinity tolerance of Mozambique tilapia (*Oreochromis mossambicus*): elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. *Zool Sci* 17:149–160. <https://doi.org/10.2108/zsj.17.149>

27. World Health Organization (2003) Use of fish for mosquito control. WHO Regional Office for the Eastern Mediterranean, Cairo

Figures

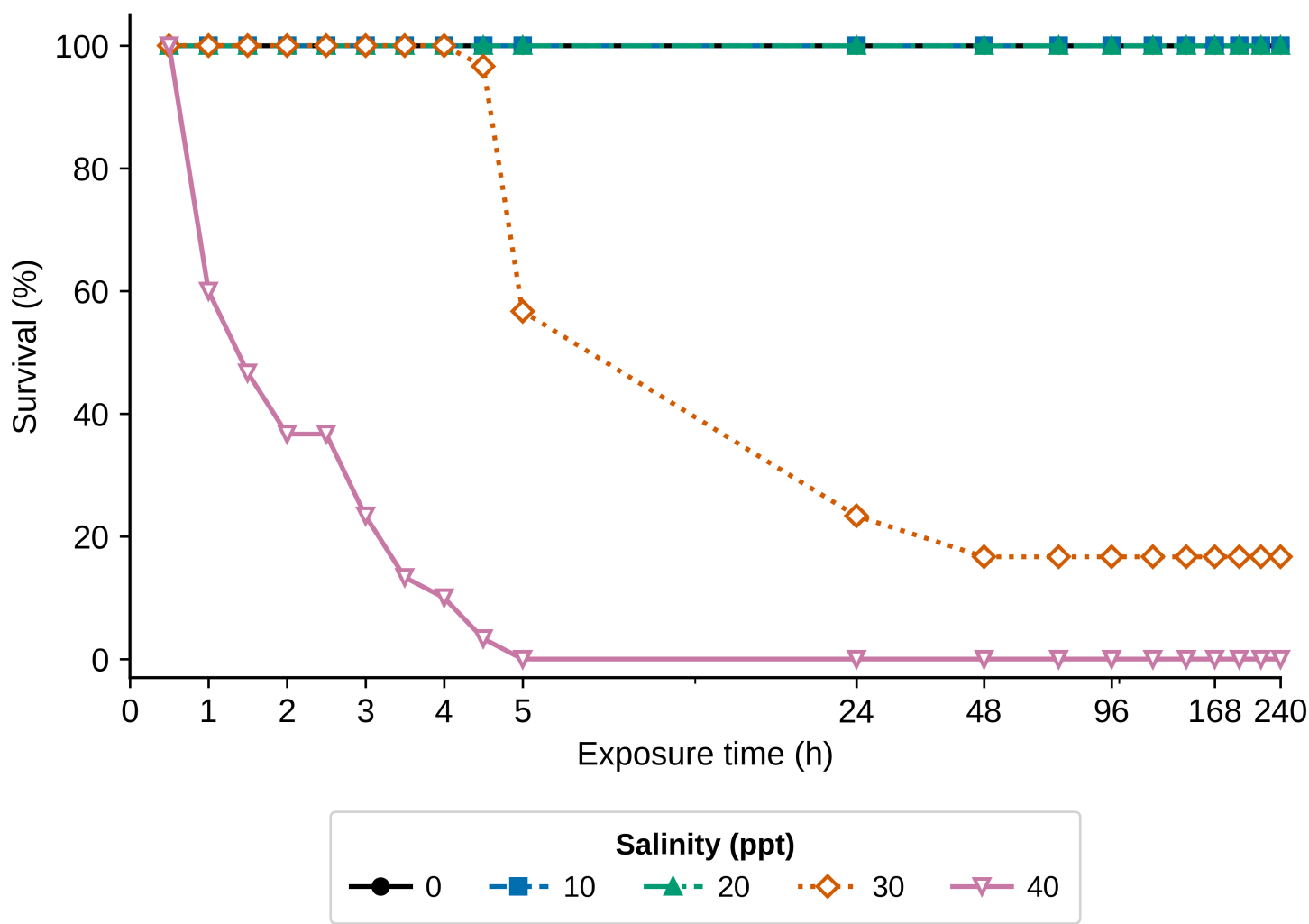


Figure 1

Survival of adult *Aplocheilus blockii* under abrupt (direct) exposure to salinities of 0–40 ppt over 240 h. Survival was complete at 0–20 ppt; mortality was rapid at 40 ppt (complete within 5 h) and delayed but severe at 30 ppt. The time axis is shown on a split (symlog) scale to resolve both the early and later observation intervals.

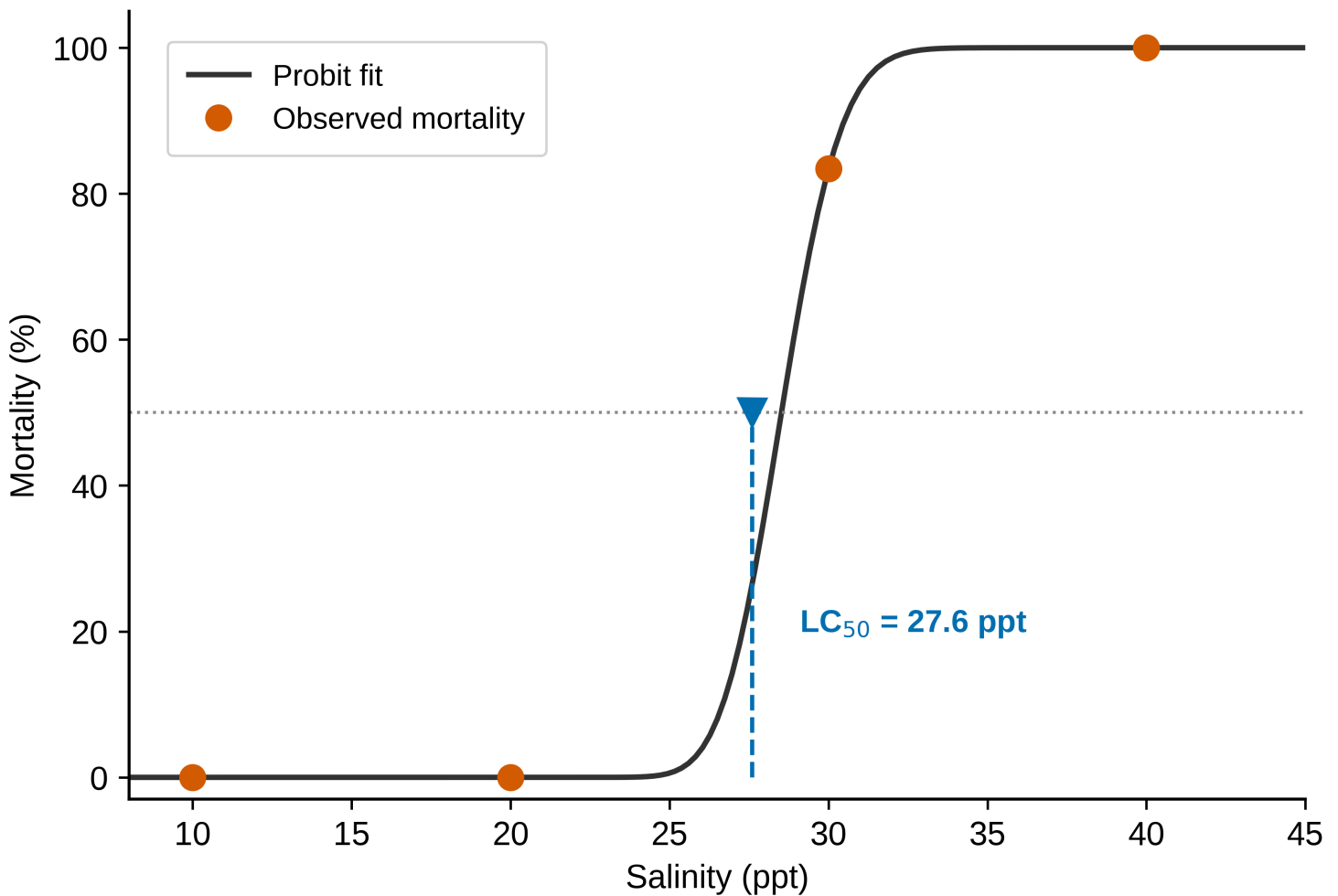


Figure 2

Probit concentration–response relationship for adult *Aplocheilus blockii* under abrupt salinity exposure. The fitted curve yields a median lethal salinity (LC_{50}) of 27.6 ppt. Filled symbols are observed mortality; the dashed line marks the LC_{50} .

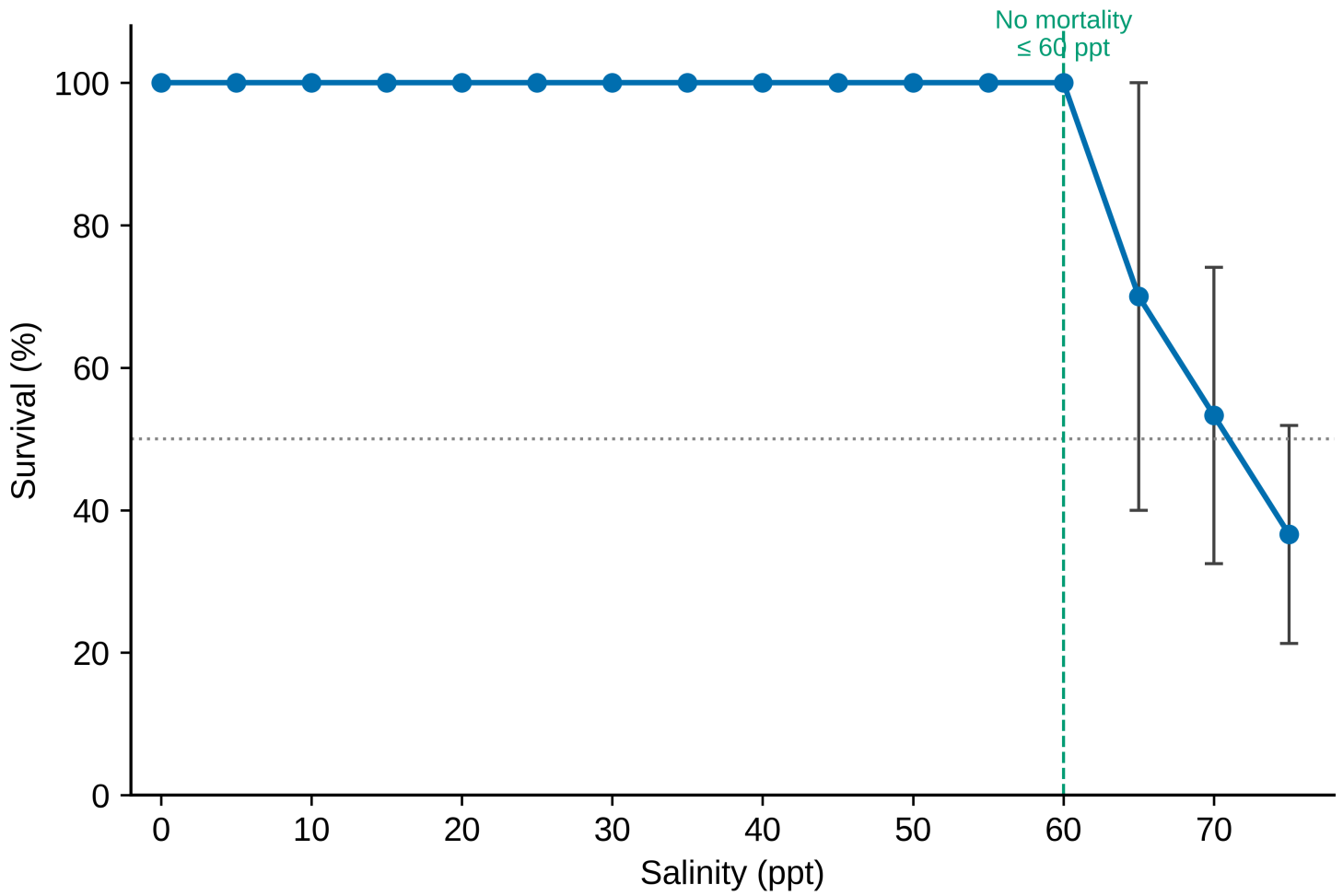


Figure 3

Survival of adult *Aplocheilus blockii* under gradual salinity acclimation (5 ppt every 48 h). No mortality occurred up to 60 ppt; survival then declined to 36.6% at 75 ppt. Points are means $\pm$ SD (n = 3).

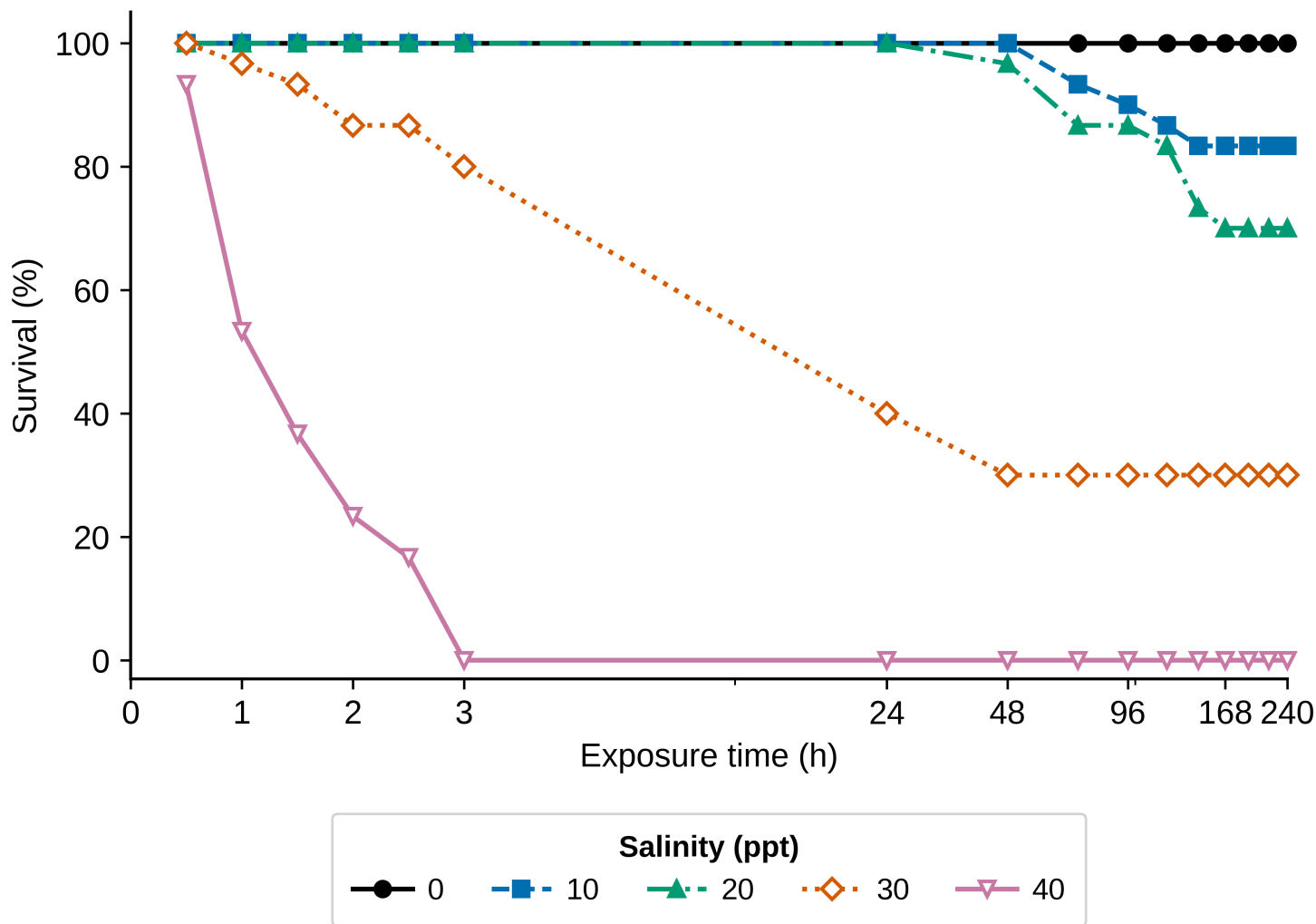


Figure 4

Survival of 10-day-old *Aplocheilus blockii* larvae under abrupt exposure to salinities of 0–40 ppt over 240 h. Larvae were more sensitive than adults, with measurable mortality from 10 ppt and complete mortality within 3 h at 40 ppt.

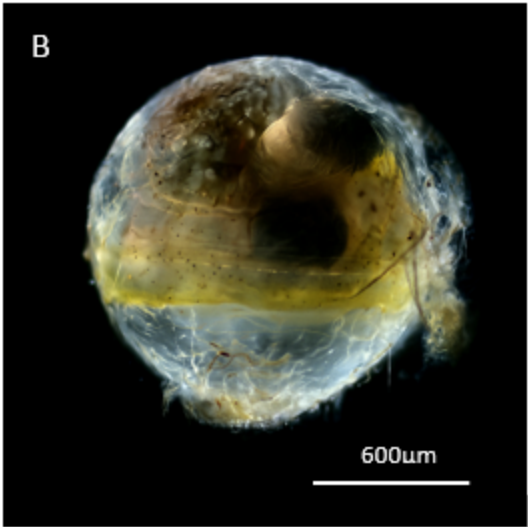
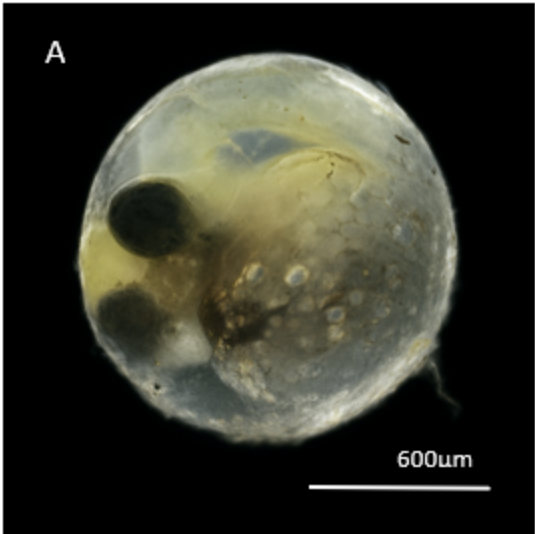


Figure 5

Embryos of *Aplocheilus blockii* under salinity treatment. (A) normally developing embryo at the hatching stage at 0 ppt ($\approx$ day 8); (B) embryo showing delayed, diapause-like developmental arrest with extended incubation at 40 ppt ($\geq$ day 15).