**Supplementary Materials**

Supplementary Methods

*Parameter estimation*

Fitting of parameters to data was done in Matlab with the platform BYOM v.6.4 and the package DEBkiss v.2.3a (https://www.debtox.info/byom.html). BYOM uses a Nelder-Mead simplex search to optimize the parameters for a set of ordinary differential equations by minimizing negative log-likelihood (NLL). The DEBkiss package works under BYOM to estimate model parameters based on their effect on the DEBkiss equations and the auxiliary equations. The differential equations predict length, egg production, egg buffer mass, and survival over time with the differences from observations used to calculate NLL. Before estimating any parameters with the optimization described above, we ran simulations with fitting turned off using a set of recommended parameters (Jager, 2018) and parameters we calculated from data on *M. menidia*, as described in the main text (Section 2.3). We visually assessed fit and noted the NLL calculated from each simulation as we adjusted parameters to obtain a reasonable set of initial parameters before estimating any. Testing a range of parameters and obtaining realistic initial parameters helps avoid detecting local minima with the optimization. This also helped us reduce the number of parameters being estimated to avoid overfitting and so that there were not multiple correlated parameters free at once.

*Calculation of volume-specific maintenance costs*

By assuming all structural weight lost during starvation is used for maintenance, we can use data on dry weights during starvation to calculate mass-specific weight change over time. This can then be converted to volume-specific maintenance cost by multiplying it by the dry weight density. Because such data do not exist for *M. menidia*, we used dry weight data from the closely related *M. beryllina* from a study in which larvae were starved for 7 days starting at three ages (7, 14, and 21 days post-hatching) and at three different temperatures (21, 25, and 28°C; Letcher and Bengtson, 1993). We selected the data for the 7 day post-hatching fish at 25°C because it was closest to the temperature at which much of the data were collected, and because it had the greatest sample size (n=22). Based on the calculation used in Stevenson et al. (2023), we fit a function to the dry weights over time. While Stevenson et al. (2023) used an exponential function, we used a linear function because the dataset only reported dry weight at the beginning and end of the starvation period.

The mass-specific rate of change in mass (*kM0*, mg assimilates \* mg dry weight-1 \* d-1, assumed to be the mass-specific maintenance cost) is calculated as:

, (S1)

where *WV0* is the initial dry weight in mg, *WV* is the final dry weight in mg, and *t* is the duration of starvation in days. We then multiplied *kM0* by *dV* to obtain the volume-specific maintenance cost in mg mm-3 d-1. The values involved in the calculation are in Table S1.

We used *JvM* to calculate the volume-specific maturity maintenance costs (*JvJ*) by assuming their values are connected through *κ* as specified by Jager (2018):

(S2)

With a *κ* of 0.8, this gives us *JvJ* = 0.00535 mg mm-3 d-1.

|  |  |
| --- | --- |
| *WV0* | 0.160 mg |
| *WV* | 0.100 mg |
| *t* | 7 d |
| *kM0* | 0.0535 mg assimilates \* mg dry weight-1 \* d-1 |
| *dV* | 0.4 mg dry weight \* mm structural volume-3 |
| *JvM* | 0.0214 mg assimilates-1 \* mm structural volume-3 d-1 |
| *κ* | 0.8 |
| *JvJ* | 0.00535 mg mm-3 d-1 |

**Table S1.** The parameter and variable values used in calculating the volume-specific somatic and maturity maintenance costs.

*Calculation of dry weight density*

The dry weight density (*dV*) was calculated using:

(S3)

where *WV* is the dry weight and *L3* is the volume of an individual at a given time. The only volume data available for *M. menidia* is for the embryo stage, so we paired the volume of an embryo immediately before hatching with the dry weight immediately after hatching. An embryo volume of 0.45 mm3, not including the chorion, was measured using microscope images of embryos less than 24 hours before hatching. Dry weight was calculated from a total length at hatching of 5.3 mm (Cross et al., 2019) using two different equations to convert total length to dry weight. The first equation was empirically derived from data on larval to adult stages (Concannon et al., 2021):

(S4)

This gave a dry weight at hatching of 0.18 mg. This conversion may have overestimated dry weight at hatching because it was derived from individuals that were greater than 6 mm total length, suggesting they were a couple of days old and had certainly begun feeding. As this was greater than the initial egg dry weight of 0.15 mg obtained from Klahre (1997), we also attempted the conversion with:

(S5)

This was derived from empirical data on the congeneric *M. peninsulae* immediately after hatching, ranging from 3.7-4.6 mm standard length immediately after hatching (McMullen and Middaugh, 1985). This conversion gave a much lower dry weight of 0.046 mg for a newly hatched larva of 5.3 mm total length, which is equivalent to 5.0 mm standard length according to data from Schwemmer et al. (2020). Equation S4 gave a *dV* of 0.4 mg mm-3, while Equation S5 gave a *dV* of 0.1 mg mm-3. However, only the former allowed a close fit to both the growth and hatching data, while the latter would not allow as close a fit to both, requiring either growth to be underestimated or time to birth to be overestimated. Although the conversion in Equation S4 provided a high value of dry weight at hatching, we decided to use this in our model because it does not require borrowing from a different species, provides a closer fit, and is appropriate for the full life cycle instead of just newly hatched larvae. It is also close to existing *dV* values in DEBkiss models of other fish species, such as the lumpfish with *dV* = 0.28 mg mm-3 (Jager et al., 2022). Importantly, we found that the results of applying the hypoxia-based correction factor to the parameters of interest were the same regardless of which *dV* value we used, with both versions identifying the same parameters (*yVA*, *µemb*, and *µlar*) as the best parameters to which to apply the correction factor based on AICc.

*Relating DEB processes to physiology*

Several genes controlling cell division and protein synthesis are regulated by hypoxia (Ton et al., 2003), such as insulin-like growth factor binding protein 1 (IGFBP-1), a protein controlled by hypoxia-inducible factor 1 (Hif-1) that has been shown to reduce growth and delay development in fish embryos exposed to hypoxia (Kajimura et al., 2005; Kajimura et al., 2006; Sun et al., 2011; Tian et al., 2014). This factor is thought to trade off growth for other oxygen-demanding processes and help fish tolerate hypoxia. By preventing insulin-like growth factors from binding to their receptors, IGFBP-1 inhibits signaling for cell division and differentiation and energy can be diverted to processes necessary for survival (Kajimura et al., 2005). In the DEBkiss model such forms of inhibition to the SU under hypoxia would be represented by reduced assimilation rates, though the link to survival is not represented explicitly.

The conversion efficiency of assimilates to structure determines growth and hatch timing because it represents the fraction of assimilates that are converted into structure rather than burned on overhead costs of growth (Jager, 2018). When oxygen is low enough that anaerobic metabolism must be used, this reduces conversion efficiency so that less growth results from the same amount of yolk or food (Thomas et al., 2019). Damage to the SU may also be responsible for reductions in conversion efficiency through lactate accumulation and consequential declines in internal pH. Even at oxygen levels above the critical level at which oxygen consumption declines, anaerobic glycolysis may increase (Nonnotte et al., 1993; Maxime et al., 2000; Wood et al., 2018). Although capability for anaerobic glycolysis in embryos and yolk sac larvae appears to vary widely across species (Wieser, 1995; Finn, 1995; Rombough, 1988), smaller fishes reach harmful levels of anaerobic end-products much faster than larger fishes due to their higher mass-specific metabolic rates (Nilsson and Östlund-Nilsson, 2008). We hypothesized that this contributed to a smaller hatch size and slower growth post-hatch.

Maintenance in DEBkiss is the energy allocated to any processes that support the integrity and functioning of the structural body (Jager, 2018), including homeostasis, damage repair, and activity. Demand for more protein turnover and cell repair can increase the volume-specific maintenance rate following damage (Bouma et al., 1994; Kooijman, 2010a) and indeed maintenance has been shown to increase with damage to structural proteins (Maury et al., 2019). In addition to damage repair, maintenance rate could be elevated by the activity required for some of the behavioral responses fish exhibit under hypoxia (Thomas et al., 2019). *M. menidia* exposed to hypoxia swim to the surface to use aquatic surface respiration, taking advantage of the diffusion of oxygen from the air (Miller et al., 2016). This behavior is impossible in embryos but has been observed in larvae (Cross et al., 2019). Fishes also expend energy on faster ventilation and heartbeat when ambient DO is low to increase oxygen uptake (Kramer, 1987; Maxime et al., 2000) and remove accumulated CO2 and lactate (Perry et al., 2009; Heath and Pritchard, 1965), but these capabilities may be limited until development has progressed further.

The maintenance flux in DEBkiss is represented in units of assimilated biomass required to meet the energy demand from maintenance. It therefore is only indirectly related to respiration rates measured as oxygen consumption. For example, an increase in the abstract maintenance parameter in the model could be caused by an increase in anaerobic processes without impacting oxygen consumption. The *measured* oxygen consumption rates of *M. menidia* early life stages did not significantly increase under experimental chronic hypoxia, but great variability in metabolic rates among individuals combined with the short respirometry periods used (<1 hour) may make small increases related to damage repair or activity difficult to detect (Schwemmer et al., 2020).

Although mortality is not a process directly represented by an SU, it could indirectly be impacted by hypoxia effects on SUs through failure to meet developmental milestones – particularly for hatching – or directly through increase in damage production or inhibition of damage repair rates. In the parameter estimation using data from normoxic conditions (Section 2.3), our estimated survival parameter for embryo mortality was greater than that of larvae (Table 1). If assimilation rate or conversion efficiency of *M. menidia* decreases under hypoxia, the resulting slower egg buffer depletion would delay hatching, extending individuals’ time in the stage with greater mortality and thus accounting for reduced hatch survival under hypoxia. We therefore hypothesized that if either assimilation rate or conversion efficiency is modified by the hypoxia-based correction factor, additionally modifying the embryo mortality parameter would consequently not be necessary to account for hypoxia effects on all four state variables. However, this would not be the case for the post-hatch mortality parameter because none of the processes in the DEBkiss model indirectly affect mortality after hatching, so changing either the assimilation or conversion efficiency parameter in combination with the post-hatch mortality parameter may be necessary to fully replicate the observed changes to growth, hatch timing, and survival under hypoxia.

Reduced food consumption or reduced conversion of food into utilizable compounds, and thus limitation of input of substrate to the SU, is a primary mechanism by which the fish energy budget is thought to be impacted by hypoxia (Chabot and Dutil, 1999; Thomas et al., 2019 with findings reinterpreted for DEBkiss). We did not test food consumption as a mechanism by which hypoxia affects the energy budget because effects on hatch timing and size, before feeding has begun, could not be explained by this mechanism.

*References for Supplementary Methods*

Bouma, T. J., De Visser, R., Janssen, J. H. J. A., De Kock, M. J., Van Leeuwen, P. H., and Lambers, H. 1994. Respiratory energy requirements and rate of protein turnover in vivo determined by the use of an inhibitor of protein synthesis and a probe to assess its effect. *Physiol. Plant*., 92: 585-594. https://doi.org/10.1111/j.1399-3054.1994.tb03027.x

Chabot, D. and Dutil, J.-D. 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions. *J. Fish. Biol.*, 55: 472-491. https://doi.org/10.1111/j.1095-8649.1999.tb00693.x

Concannon, C. A., Cross, E. L., Jones, L. F., Murray, C. S., Matassa, C. M., McBride, R. S., and Baumann, H. 2021. Temperature-dependent effects on fecundity in a serial broadcast spawning fish after whole-life high CO2 exposure. *ICES J. Mar. Sci.*, 78(10): 3724-3734. https://doi.org/10.1093/icesjms/fsab217

Cross, E. L., Murray, C. S., and Baumann, H. 2019. Diel and tidal *p*CO2 x O2 fluctuations provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.*, 9: 18146. https://doi.org/10.1038/s41598-019-53930-8

Finn, R. N., Fyhn, H. J., and Evjen, M. S. 1995. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism. *Mar. Biol.*, 124: 355-369. https://doi.org/10.1007/BF00363909

Heath, A. G. and Pritchard, A. W. 1965. Effects of severe hypoxia on carbohydrate energy stores and metabolism in two species of fresh-water fish. *Physiol. Zool.*, 38(4): 325-334. https://doi.org/10.1086/physzool.38.4.30152409

Jager, T. 2018. DEBkiss: A Simple Framework for Animal Energy Budgets. Version 2.0. Leanpub: https://leanpub.com/debkiss\_book.

Jager, T., Malzahn, A. M., Hagemann, A., and Hansen, B. H. 2022. Testing a simple energy-budget model for yolk-feeding stages of cleaner fish. *Ecol. Modell.*, 469: 110005. https://doi.org/10.1016/j.ecolmodel.2022.110005

Kajimura, S., Aida, K., and Duan, C. 2005. Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia-induced embryonic growth and developmental retardation. *Proc. Nat. Acad. Sci.*, 102(4): 1240-1245. https://doi.org/10.1073/pnas.0407443102

Kajimura, S., Aida, K., and Duan, C. 2006. Understanding Hypoxia-Induced Gene Expression in Early Development: In Vitro and In Vivo Analysis of Hypoxia-Inducible Factor 1-Regulated Zebra Fish Insulin-Like Growth Factor Binding Protein 1 Gene Expression. *Mol. Cell. Biol.*, 26(3): 1142-1155. https://doi.org/10.1128/MCB.26.3.1142-1155.2006

Klahre, L. E. 1997. Countergradient Variation in Egg Production Rate of the Atlantic Silverside *Menidia menidia*. [Master’s thesis]. Stony Brook University.

Kooijman, S. A. L. M. 2010. Dynamic Energy Budget Theory for Metabolic Organisation. Cambridge University Press, Cambridge.

Kramer, D. L. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes*, 18: 81-92. https://doi.org/10.1007/BF00002597

Letcher, B. H. and Bengtson, D. A. 1993. Effects of food density and temperature on feeding and growth of young inland silversides (*Menidia beryllina*). *J. Fish Biol.*, 43: 671-686. https://doi.org/10.1111/j.1095-8649.1993.tb01145.x

Maury, O., Poggiale, J.-C., and Aumont, O. 2019. Damage-related protein turnover explains inter-specific patterns of maintenance rate and suggest modifications of the DEB theory. *J. Sea Res.*, 143: 35-47. https://doi.org/10.1016/j.seares.2018.09.021

Maxime, V., Pichavant, K., Boeuf, G., and Nonnotte, G. 2000. Effects of hypoxia on respiratory physiology of turbot, *Scophthalmus maximus*. *Fish Physiology and Biochemistry*, 22: 51-59. https://doi.org/10.1023/A:1007829214826

McMullen, D. M. and Middaugh, D. P. 1985. The Effect of Temperature and Food Density on Survival and Growth of *Menidia peninsulae* Larvae (Pisces: Atherinidae). *Estuaries*, 8(1): 39-47. https://doi.org/10.2307/1352120

Miller, S. H., Breitburg, D. L., Burrell, R. B., Keppel, A. G. 2016. Acidification increases sensitivity to hypoxia in important forage fishes. *Mar. Ecol. Prog. Ser.*, 549: 1-8. https://doi.org/10.3354/meps11695

Nilsson, G. E. and Östlund-Nilsson, S. 2008. Does size matter for hypoxia tolerance in fish? *Biol. Rev.*, 83: 173-189. https://doi.org/10.1111/j.1469-185X.2008.00038.x

Nonnotte, G., Maxime, V., Truchot, J. P., Williot, P., and Peyraud, C. 1993. Respiratory responses to progressive ambient hypoxia in the sturgeon, *Acipenser baeri*. *Respir. Physiol.*, 91: 71-82. https://doi.org/10.1016/0034-5687(93)90090-W

Perry, S. F., Jonz, M. G., and Gilmour, K. M. 2009. Oxygen Sensing and the Hypoxic Ventilatory Response. In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 193-253. San Diego: Academic Press.

Rombough, P. J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In: *Fish Physiology, Vol. 11: The Physiology of Developing Fish, Part A: Eggs and Larvae*. (ed. W. S. Hoar and D. J. Randall), pp. 59-162. San Diego: Academic Press.

Schwemmer, T. G., Baumann, H., Murray, C. S., Molina, A. I., and Nye, J. A. 2020. Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of the coastal forage fish *Menidia menidia*. *J. Exp. Biol.*, 223: jeb228015. doi: 10.1242/jeb.228015

Stevenson, L. M., Muller, E. B., Nacci, D., Clark, B. W., Whitehead, A., and Nisbet, R. M. 2023. Connecting Suborganismal Data to Bioenergetic Processes: Killifish Embryos Exposed to a Dioxin-Like Compound. *Environ. Toxicol. Chem.*, 42(9): 2040-2053. doi: 10.1002/etc.5680

Thomas, Y., Flye-Sainte-Marie, J., Chabot, D., Aguirre-Velarde, A., Marques, G. M., and Pecquerie, Laure. 2019. Effects of hypoxia on metabolic functions in marine organisms: Observed patterns and modelling assumptions within the context of Dynamic Energy Budget (DEB) theory. *J. Sea Res.*, 143: 231-242. https://doi.org/10.1016/j.seares.2018.05.001

Tian, Y.-M., Chen, J., Tao, Y., Jiang, X.-Y., and Zou, S.-M. 2014. Molecular cloning and function analysis of insulin-like growth factor binding protein 1a in blunt snout bream (*Megalobrama amblycephala*). *Dongwuxue Yanjiu*, 35(4): 300-306. 10.13918/j.issn.2095-8137.2014.4.300

Wieser, W. 1995. Energetics of fish larvae, the smallest vertebrates. *Acta Physiol. Scand.*, 154: 279-290. https://doi.org/10.1111/j.1748-1716.1995.tb09912.x

Wood, C. M. 2018. The fallacy of the *P*crit – are there more useful alternatives? *J. Exp. Biol.*, 221: jeb163717. doi: 10.1242/jeb.163717

Sun, C.-F., Tao, Y., Jiang, X.-Y., and Zou, S.-M. 2011. IGF binding protein 1 is correlated with hypoxia-induced growth reduce and developmental defects in grass carp (*Ctenopharyngodon idellus*) embryos. *Gen. Comp. Endocrinol.*, 172(3): 409-415. https://doi.org/10.1016/j.ygcen.2011.04.005

Ton, C., Stamatiou, D., and Liew, C.-C. 2003. Gene expression profile of zebrafish exposed to hypoxia during development. *Physiol. Genomics*, 13(2): 97-106. https://doi.org/10.1152/physiolgenomics.00128.2002

**Supplementary Figure**

A diagram of a curve

Description automatically generated with medium confidence

**Figure S1. Best fit of DEBkiss model to all experimental data from four DO levels.** The model was fitted to early life data (embryos, larvae, and juveniles) and the best fitting model was selected based on lowest AICc. (A) is total length (mm) over time (days), (B) is egg buffer mass (mg) over time (days), and (C) is survival over time (days).