Low oxygen levels (‘hypoxia’) are a pressing concern for marine and freshwater ecosystems worldwide, and this may deteriorate as ocean temperatures rise. Hypoxia causes stress in organisms, which can cause reproductive impairments that persist across generations — even the offspring that have never been exposed to hypoxia. Previous studies discovered that hypoxia can disrupt sex hormones, resulting in birth defects and affecting reproduction of male fish over several generations. This study shows how hypoxia can also affect female marine medaka (Oryzias melastigma) over multiple generations — and thus may pose a significant threat to the sustainability of natural fish populations worldwide.

Hypoxia is of great concern to aquatic ecosystems and over 400 coastal ‘dead zones’ (zones with less than 2 mg/L of dissolved oxygen) have been identified worldwide. Oxygen-depleted zones in European seas have increased hugely in size in past decades. The Baltic Sea, for example, has the largest oxygen-depleted zone in the world at 60 000km² in size — and this has resulted in the loss of about three million tonnes of large ocean organisms.

Such oxygen loss is largely caused by agricultural fertiliser run-off, but climate change exacerbates the problem. The majority of oceanic dead zones face a temperature increase of 2°C by the end of this century — causing both a further decrease in oxygen solubility, and a rise in demand for oxygen from aquatic organisms. Understanding the effects of climate change and the resultant hypoxia on aquatic ecosystems and fish populations in the future is key: it falls within the European strategy on adaptation to climate change and can inform fisheries management and conservation strategies.

This study explored the transgenerational effects of hypoxia on marine medaka. This fish was selected for the following reasons:

- the researchers have established the fish as the model for marine fish (as zebra fish is the model for freshwater fish);
- it can be kept from generation to generation in the lab;
- the biology, e.g. feeding requirements, growth, reproduction, have all been researched;
- the genome of this species has already been established;
- the life-cycle is relatively short (three to four months), which is good for transgenerational studies.

First-generation fish (F0) were split into two groups and exposed to either normoxia (normal oxygen levels of 5.8 mg/L, F0N) or hypoxia (low oxygen levels of 1.5 mg/L, F0H) for one month. Any embryos produced were collected within one hour of fertilisation; embryos from F0N were immediately transferred to the same normoxic conditions for the development of the F1 and F2 generations (F1N, F2N); whereas the embryos gathered from F0H were split into two groups. Half were reared in hypoxia for two generations (F1H, F2H) and half returned to normoxia and kept for another two generations (F1T, F2T).

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Low oxygen levels affect reproductive function in female fish – across multiple generations (continued)

Each group consisted of five identical net cages, each containing 45 male and 45 female fish. From each treatment group, three cages were chosen at random and 100 embryos sampled 10 days after fertilisation. Hatching success was calculated as a percentage of hatched embryos. Nine female fish from each group and generation were used to create three sets of pooled RNA and DNA for analysis. Fish were anaesthetised in an ice bath before having their ovaries removed. Three ovaries were pooled to produce an RNA sample from a given treatment group, and three more ovaries pooled to create a DNA sample. Each exposure condition and generation (i.e. normoxia control (F0N) and hypoxia (F0H) in the F0 generation, along with F2 normoxia (F2N), hypoxia (F2H), and transgenerational (F2T) groups) had three sets of pooled RNA and DNA. Ingenuity Pathway Analysis software was used to explore the transgenerational effect of hypoxia on the medaka’s ovarian function.

The results show that hypoxia caused two distortions in ovarian development: follicle atresia (the breakdown of follicles due to cell death) and retarded oocyte development (in the form of a greater number of primary oocytes; but fewer vitellogenic oocytes – those containing egg yolk). Hatching success was greatly reduced by hypoxia in F0 fish. Interestingly, similar reproductive impairments were seen in F2 fish of the transgenerational group – although these fish have never been exposed to hypoxia. Hypoxia had no effect on the number of eggs produced, but the quality of the eggs was adversely affected (as seen transgenerationally in the impact on F2 fish). This, coupled with previous findings by the researchers that hypoxia can cause transgenerational reproductive impairment (reduced sperm quality and quantity) in male fish, demonstrated the population-level effects that hypoxia can have on a marine species.

This study highlights that hypoxia poses a significant and lasting threat to the sustainability of natural marine medaka fish populations worldwide and may also impact other fish species. These findings help inform policymakers on the effects of marine hypoxia, and highlight trends and concerns to consider in efforts to mitigate and regulate climate change.