**1. Publishable summary**

The research part of this Marie Curie ERG project aimed to

1. quantify the effect of temperature and oxygen on metabolism, growth and survival of aquatic ectotherms.
2. investigate if responses on the short term are related to those on the long term
3. identify the drivers behind differences in these responses of aquatic ectotherms (e.g. are such differences are rooted in their mode of respiration and ecology?), which would allow prediction of the vulnerability of aquatic ectotherms to chronic effects of global warming from traits related to their mode of respiration and ecology.

In warmer waters, ectotherms grow faster, but reach maturity at a smaller size. This life-history puzzle is called the temperature-size rule (TSR) and may be related to animals having insufficient oxygen in warmer waters to support large body sizes. We tested this by growing animals under different temperature and oxygen conditions and found that a classic TSR was only observed under conditions where oxygen was limiting, supporting our hypothesis (Hoefnagel & Verberk, 2015). In a follow-up experiment we found that variation in growth performance can be traced back to individual differences in rates of food and oxygen consumption. Growth performance was strongly affected by oxygen and temperature conditions during rearing, even in the pulmonate snail *L. stagnalis* that can compensate for hypoxia to some extent by aerial respiration (Hoefnagel & Verberk in press).

In contrast to considerable effects of rearing conditions to which animals were exposed for periods of months, oxygen conditions had only minor effects on survival of *L. stagnalis* when exposed to acute heat (hours), suggesting that low oxygen conditions have greater consequences on the long term. As a direct test of this idea, we compared acute and chronic effects by assessing lethal impacts of warming under three oxygen conditions in the laboratory and assessing nonlethal impacts of warming in the field using data on mayfly occurrence with coupled measurements of water temperature and biochemical oxygen demand from 2,632 sites in the UK, which were repeatedly sampled (42,293 samples in total) (Verberk et al., 2016a). Hypoxia lowered lethal limits of the mayflies by 5.5-8.2 °C. Field data confirmed the importance of oxygen limitation in warmer waters; poor oxygenation drastically reduced site occupancy, and reductions were especially pronounced under warm water conditions. Consequently, poor oxygenation lowered optimal stream temperatures for both species. The broad concordance between laboratory results and extensive field data supports the idea that oxygen limitation not only impairs survival at thermal extremes but also restricts species abundance in the field at temperatures well below upper lethal limits (Figure 1). Improving water oxygenation and reducing pollution therefore provide key facets of climate change adaptation for running waters, which provides relevant approaches for policy makers.



Figure 1. Projected model responses of site occupancy for the mayfly Serratella ignita (proportion of samples where species was present at a given site) along a gradient of deteriorating oxygenation (increasing BOD-values). Model responses are calculated for ambient temperatures and warm temperatures (relative temperature +2). Note that at a BOD of 2 (the average water quality in the UK, roughly corresponding to levels of dissolved oxygen of 9 mg O2 l-1), there is already a 30% reduction in site occupancy.

At the same time a dataset was generated on thermal tolerance limits in response to water oxygenation that included data on 58 different species, differing in ecological habitat use (Figure 2A) and mode of respiration (Figure 2B). Low oxygen conditions significantly (P<0.001) reduced heat tolerance (CTmax), especially in species from running waters and those that perform gas exchange across their integument and gills. In contrast, species from standing waters and those with aerial respiration were much less affected by low oxygen conditions and had the highest CTmax (Figure 2). Together, respiratory mode, habitat use and oxygen conditions during the heating trials explained 53% of the variation in CTmax, and differences among species (nested within orders) explained a further 30% (in total 83% of the variation was explained by our model). These results show that there are consistent differences among species in their sensitivity to the interacting effects of hypoxia and heat that are related to how they breathe and their habitat use. This allows an extrapolation to the vulnerability of other taxa not included in this study.



Figure 2. Projected model responses of CTmax as a function of oxygen conditions (normoxia is at 20 kPa). Model responses are calculated for 58 species differing in habitat use (A) and mode of respiration (B).

The above studies have led to several scientific publications and are being continued. Furthermore, they contributed to a good integration of the fellow at the host institute: the fellow is currently (co-)supervising 4 PhD students within the institute (see <http://www.ru.nl/animal/>). Furthermore, he has already successfully supervised 2 PhD students, one in collaboration with the Department of Environmental Science of the institute and the other in collaboration with the NIOO in Wageningen, the Netherlands. The fellow has also passed his probationary period and secured a permanent position. Furthermore, the scientific achievements of the fellow in the field of thermal biology and respiratory physiology has enabled him to obtain a prestigious NWO-VIDI research grant, a personal grant for 5 years to continue his studies on temperature, growth and ectotherm life-histories.

The work on oxygen-limitation and thermal responses is generating much scientific debate (e.g. Verberk et al., 2016b; Lefevre 2016) and unravelling the mechanistic basis will have far reaching consequences for how best to respond to the problem of global warming.

References:

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