Name: Billy Moore
Thesis title: Physiological and Transcriptomic Response of Early-Life Stage Clownfish to Future Ocean Warming and Marine Heatwaves

Research aim:

Increasing ocean temperatures threaten to push coral reef fish beyond their natural thermal limits, with previous studies indicating this will elicit a range of adverse physiological and molecular responses. However, the effects of increasing temperature on early life-stage coral reef fish are currently unclear, as previous studies often focus on later life-stages. This PhD thesis aims to address these knowledge gaps by measuring the physiological response or larval-juvenile clownfish to temperatures associated with future ocean warming. Moreover, it aims to identify the molecular processes underpinning these physiological responses, thus providing a more complete understanding of how coral reef fish will respond to future temperature increase.

Material and method:

Mature *Amphiprion ocellaris* (9 pairs) were collected via SCUBA from multiple sites around Okinawa to serve as breeding stock. Two individuals were subjected to whole-genome sequencing using Illumina short-read, PacBio long read, and Hi-C techniques, with genome assembly and annotation conducted. Next, the response of larval/post-larval (1-20 dph) and juvenile (~60 dph) *A .ocellaris* to temperatures associated with future warming and marine heatwaves (+3 °C) was investigated via a series of aquaria-based studies. These experiments utilized microscopic imaging and weight measurements to determine growth, and respirometry to measure oxygen-consumption based metabolic rates. Following these physiological measurements 6 larval-stages, 4 tissues (brain, liver, muscle, digestive tract) from post-larval stages, and 7 tissues (brain, liver, gill, heart, muscle, intestine, pancreas) from juvenile fish were subjected to RNA extraction and sequencing. Additionally, metatranscriptomic sequencing of 12 post-larval digestive tracts was used to investigate changes in microbiome structure at +3 °C.

Results:

When reared at +3 °C larval clownfish exhibit faster growth and development, reaching key settlement stages earlier. Although variability exists between larval stages and temperature treatments, in general the results indicate that +3 °C causes an increase in clownfish metabolic rates. Nonetheless, increasing developmental exposure to +3 °C reduced the effect of elevated temperatures on metabolic rates at ~60 dph, indicating *A .ocellaris* may display developmental acclimation to future warming. Transcriptome analysis revealed that this developmental acclimation may be driven by molecular changes, characterized by insulin secretion suppression in the pancreas and upregulation of oxidative phosphorylation in the liver. Transcriptome analysis also revealed expression changes to genes involved with heat shock response, epigenetic reprogramming, liver damage, immune response and neurotransmission, indicating +3 °C will affect a range of biological processes. Finally, the microbiome of 20 dph *A. ocellaris* was altered at +3 °C, as potentially pathogenic *Vibrio sp*. replaced *Escherichia coli* as the dominant bacteria.

Conclusion:

Overall, these results indicate that early life-stage clownfish will exhibit a range of physiological and molecular change under future ocean warming, with alterations to growth, metabolism and multiple biological processes observed here, potentially having harmful effects on future fitness.