



R/V Tiglax, Resurrection Bay, Alaska

# Impact of environmental change on zooplankton physiology and reproduction in the Gulf of Alaska

In the far reaches of the sub-arctic North Pacific, scientists are using genetic profiling of high latitude zooplankton to better understand complex regional oceanography and commercial fish stocks. The Gulf of Alaska supports successful commercial fisheries, but productivity depends on robust fish populations, which in turn depend on a ready supply of zooplankton. Novel genetic sequencing of zooplankton RNA is allowing **Professor Petra Lenz** and her collaborators to investigate controls on adaptive physiology and population structure in the face of a changing climate.

**T**he Gulf of Alaska, a region of the sub-arctic North Pacific, supports numerous large commercial fisheries. Fishery harvests depend on robust fish populations, which in turn depend on a ready supply of nutrition. Many fish species depend on zooplankton (small drifting organisms), either directly, as a primary food source, or indirectly, by

preying on fish that consume zooplankton. However, zooplankton populations fluctuate in response to regional oceanography and large-scale climatic variation such as the Pacific Decadal Oscillation. Years with warmer sea surface temperatures are correlated with lower abundances (numbers) of zooplankton, juvenile pollock and forage fish (i.e., fish preyed on by predators).

Although zooplankton have been resilient to this environmental variability in the past, their long-term success under future climate scenarios is far less certain. Therefore, it is critical to understand how zooplankton may respond to future climate scenarios in order to predict the potential effects on fisheries in the Gulf of Alaska.

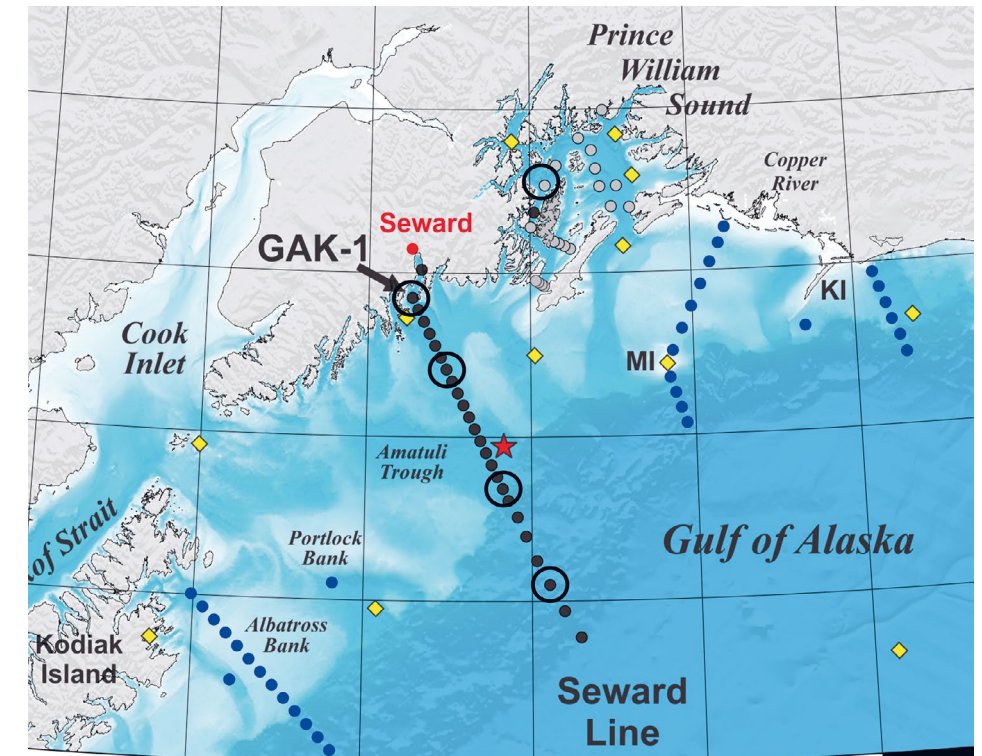
In the Gulf of Alaska, the small crustacean copepod, *Neocalanus flemingeri* (*N. flemingeri*), is an important zooplankton prey species, consumed by larval and juvenile fish, and krill. However, their population exhibits large variations in abundances from one year to the next, which may impact commercial fish stocks. Recent anomalous warming in the Gulf of Alaska has come with notable changes to *N. flemingeri* physiology (i.e., smaller adult females) and abundance (i.e., smaller winter broods).

Studying zooplankton residing hundreds of meters below the surface of the North Pacific is no easy task, but in response to the emerging threat from climate change, a group of researchers from Hawai'i and Alaska have accepted the challenge. Professor Petra Lenz (University of Hawai'i at Mānoa), and her colleagues, Professor Russell Hopcroft (University of Alaska Fairbanks) and Professor Andrew Christie (University of Hawai'i at Mānoa) are using a novel approach, transcriptomics (the study of RNA transcripts), to answer critical questions about the life cycle of *N. flemingeri*.

## LIFE CYCLE OF *N. FLEMINGERI*

*N. flemingeri* are key members of sub-arctic marine communities and their life cycle provides ideal features for modelling physiological and population adaptations in response to environmental change. In particular, *N. flemingeri* occur as a single generation per year, and are characterised by non-feeding adults that experience a period of dormancy (known as diapause). In detail (see diagram, next page):

- 1 Pre-adult *N. flemingeri* (copepodite stage C5) migrate to 400 to 1000 m depth during summer and mature to the non-feeding adult stage by molting. After mating, the males die off and the females enter diapause.
- 2 The females depend on stored lipids for energy to survive the period of dormancy, which takes several months. This is followed by the completion of the reproductive programme, which takes approximately six weeks.
- 3 During late winter/early spring, *N. flemingeri* produce a large number of eggs (sometimes more than 1000), which are released as multiple broods over a 4–6 week period.
- 4 Eggs hatch within a few days and nauplii (crustacean larvae) migrate to surface waters, where they exploit the spring phytoplankton bloom (typically in May) for growth and, once they reach the copepodite stages, the accumulation of lipid stores for the winter. Robust populations depend on the ability of nauplii and copepodites to exploit the spring phytoplankton bloom. Without this, low lipid stores translate into fewer eggs and major declines in abundance in the following year. With only a single generation per year,



Map of sampling stations. Map shows the Northern Gulf of Alaska with sampling stations for an NSF-funded Long-term Ecological Research (LTER) Project directed by Prof Russ Hopcroft. A subset of these stations was targeted for physiological studies. *Neocalanus flemingeri* individuals were collected for transcriptome profiling at stations marked with a circle.

population recovery is challenging. Both the timing and magnitude of the phytoplankton bloom are highly variable; therefore, understanding the controls on preparation for and emergence from dormancy and reproduction is critical for predicting variability in the population.

Using transcriptomics, Prof Lenz and her colleagues aim to understand some critical aspects of the *N. flemingeri* life cycle:

- 1 Physiological responses to a poor environment. The main goal here is to identify marker genes for low lipid accumulation (i.e., early warning of low future population).
- 2 Relationship between temperature, food resources, and diapause through analysis of key metabolic and developmental pathways.
- 3 Link between environmental variability and gene profiles and how these relate to the timing and conditions of life cycle completion.

**Professor Petra Lenz and her colleagues are using a novel approach, transcriptomics (the study of RNA transcripts), to answer critical questions about the life cycle of zooplankton**

## TRANSCRIPTOMICS: A NOVEL APPROACH

Transcriptomics relates to the study of all messenger RNA transcripts, which are produced by the genome (the complete set of genetic material) under specific environmental conditions. Critically, while the genome remains largely unchanged, RNA transcripts adapt to environmental conditions. Prof Lenz and her colleagues are using high-throughput transcriptome sequencing (RNA-Seq) to



Prof Russ Hopcroft on the R/V Tiglax getting the Multinet ready to collect diapausing copepods from 400–700 m depth



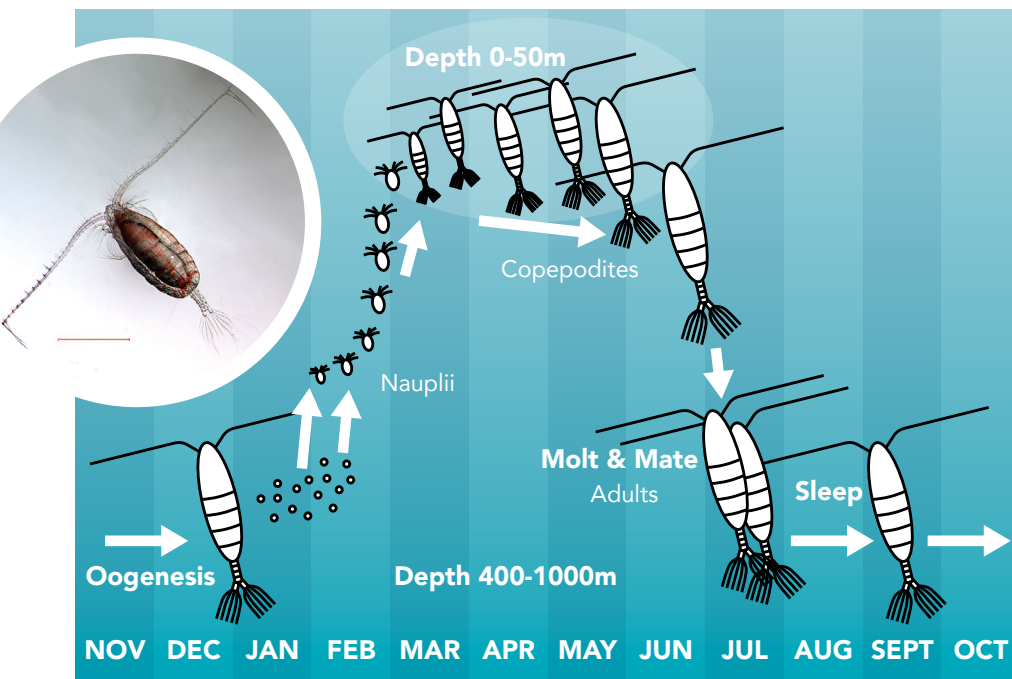


Diagram showing the life cycle of *Neocalanus flemingeri*. Developmental stages feed and grow in the upper 50 m, while the adults are only found at 400–1000 m. Months of the year shown at the bottom. Inset: *N. flemingeri* (copepodite stage C5) preparing to descend. Scale: 2 mm. Photo taken by Prof Russ Hopcroft.

## *N. flemingeri* occur in temperate and polar marine communities and their life cycle provides ideal features for modelling physiological and population adaptations in response to environmental change

characterise the physiological state of pre-adult *N. flemingeri* and how this relates to environmental conditions.

RNA-Seq provides a snapshot of all mRNAs (messenger RNA), which control cellular function. Typically, RNA-Seq is used in laboratory settings on model species, like the mouse or fruit fly. The technique has been used to identify physiological signatures that characterise, among other things, lipid breakdown, diapause, and reproduction. Now, with developments in technology and software, RNA-Seq is being applied to non-model organisms, including zooplankton.

### FIELDWORK IN A UNIQUE LOCATION

Improvements in transcriptomics may have allowed the analysis of *N. flemingeri*, but the technique still relies on a ready supply of genetic material. Mainly resident at > 400 m depth in the sub-arctic North Pacific, *N. flemingeri* females are not easy to come by. Therefore, Prof Lenz and her colleagues

teamed up with research programmes already established in the region.

In particular, Professor Russ Hopcroft leads a 17-year long-term observation programme on zooplankton (the Seward Line). This monitoring programme is now part of the National Science Foundation's Long Term Ecological Research Network. Prof Hopcroft's understanding of the ecology and population biology of zooplankton complements the transcriptomics expertise of the University of Hawai'i team. This interdisciplinary approach is critical as it allows the project to make linkages between the often-distant fields of physiology, ecology, and oceanography.

These linkages are vital to understand zooplankton populations given an uncertain future under climate change. Using existing Seward Line infrastructure, multi-year (May 2015, 2016, 2017, and 2018) and multi-location (inshore and offshore Gulf of Alaska and Prince William Sound) collection of *N.*

## Q&A

### How can we protect zooplankton populations from climate change?

**Petra:** Because oceanic zooplankton populations are dynamic and fluid, the usual concept of protecting a habitat through management cannot be applied. Thus, there is no path to protecting zooplankton using traditional approaches. However, we need to predict how and when the zooplankton community might change.

**Russ:** Indeed, the focus in biological oceanography has been to understand how the community responds to current climate variability, then extrapolate this to future climate scenarios. This has been a challenging task given the multitude of biological and non-biological factors that impact these populations.

### Is commercial fishing in the Gulf of Alaska subject to quotas? If not, do you see this as a way of managing the impacts of climate change on fish stocks?

**Petra:** Gulf of Alaska commercial fisheries are sustainable, and are considered one of the best-managed in the world. The development of an early-warning system that predicts declines in copepod abundances could lead to even better management decisions.

**Russ:** We interact regularly with colleagues at the National Oceanic and Atmospheric Administration and state agencies. We all understand that changes affecting keystone species such as *Neocalanus* need to be documented. Zooplankton biomass is one metric appearing in annual reports used by management agencies to set harvest quotas.

**Andy:** Having grown up in Maine, I have seen the importance of suitable management strategies. Maine has a very healthy lobster population; the young are allowed to grow up, large breeding adults are allowed to

reproduce. This has been achieved through minimum and maximum size limits on lobsters, and limits on types of gear. This said, each fishery is unique and requires tailored management strategies.

### Given your success, will you now apply this technique to other zooplankton species?

**Petra:** Given funding, we plan to start investigations of three other Gulf of Alaska copepod species that depend on the phytoplankton bloom and undergo seasonal diapause.

**Russ:** Expansion to other species is a top priority. Many copepod species have similar life histories to *Neocalanus flemingeri*, but differ in preferred habitat, diet and/or timing of migration and diapause. Enlarging the scope will give a more detailed picture of how climate change will affect the whole ecosystem.

**Andy:** Enlarging the scope will also give a better idea of which physiological/behavioural control systems are ubiquitously impacted by environmental variation and which are species-specific.

### The human genome contains approximately three billion base pairs (source: Human Genome Project). How long is the zooplankton genome?

**Petra:** Copepod genome sizes vary, but if *Neocalanus flemingeri* is similar to its cousin *Calanus finmarchicus*, its genome is double the human size (~6 billion base pairs).

**Russ:** Since the number of operational genes in copepods and humans is expected to be similar, this suggests a surprising amount of "junk" DNA in copepods.

**Petra:** In fruit flies and other model species, much "junk" DNA has a regulatory function. It is a mystery why a small copepod has or needs such a large genome.

**Andy:** A larger genome also comes at a

cost. For example, plants species with larger genomes tend to be at greater risk of extinction, as they tend to be less tolerant to environmental change. One possible explanation is that it takes more time to copy and divide a large amount of DNA. If an organism needs to grow extremely rapidly, let's say when scarce resources become available, having a small genome may be advantageous; this may explain the extremely small genomes of many desert plants. If the same holds true for copepods, those with larger genomes may be more sensitive to environmental variability.

### Apes and humans share 98% of their DNA, while humans and fruit flies share 36% of their genes (Source: Stanford University Museum of Innovation). How similar are humans and zooplankton?

**Petra:** Fruit flies and copepods are both arthropods, which would suggest that humans and copepods would also share ~ 35% of genes. However, the genome of the fruit fly is 10x smaller than the copepod, which means that we may be surprised once we have a genome for *N. flemingeri*.

**Russ:** However, the term zooplankton includes over a dozen different phyla ranging from jellyfish to larval fish and these will show different levels of similarity to humans.

**Andy:** What is important here is not the percentage of shared genes, but rather which genes are transcribed (used), and what is the temporal and spatial sequence of gene expression. Distinct patterning of expression allows for different gene/protein networks to evolve, and hence generate a diversity of physiological/behavioural control systems. This type of evolution allows organisms with similar genetic makeup to be very different.

## Detail

### RESEARCH OBJECTIVES

Prof Lenz investigates the impact of environmental change on zooplankton physiology and population abundance through genetic sequencing of RNA from sub-arctic, North Pacific *Neocalanus flemingeri*, a planktonic crustacean and a food source for commercial fish stock.

### FUNDING

- National Science Foundation (NSF)
- North Pacific Research Board (NPRB)

### COLLABORATORS

- Prof Andrew E. Christie (University of Hawai'i at Mānoa)
- Prof Russell R. Hopcroft (University of Alaska Fairbanks)

Co-investigators on project: Prof Daniel K Hartline and Dr Vittoria Roncalli from the University of Hawai'i at Mānoa.

### BIO

A graduate of both the University of California, San Diego and University of California, Santa Barbara, Prof Lenz has been a member of the faculty of the Pacific Biosciences Research Center at University of Hawai'i at Mānoa since 1990.

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*flemingeri* has been facilitated, resulting in a unique dataset on abundances and physiological condition.

### RESULTS AND FUTURE WORK

Data analysis is on-going and final results are yet to be known; however, the project is already a triumph, having successfully implemented challenging fieldwork and a

complex novel technique. To date, the team has successfully used females collected from the Pacific and incubated in the laboratory to generate gene expression profiles over a ten-week period, from dormancy to reproduction. Collections during the diapause preparation period include several unusual warm years that provide key baseline physiological data. Work is continuing, with field collections in

the Gulf of Alaska, experimental incubations being performed at the University of Alaska Fairbanks, and further molecular and tissue analysis being completed at the University of Hawai'i at Mānoa. The eventual outcomes will provide a breakthrough in our understanding of North Pacific zooplankton cycles, and the potentially devastating impacts of climate change.