Project information

Project title
Trophic interactions in pelagic ecosystems
Year
2013/2014
Project leader
Tove M. Gabrielsen, UNIS
Participants
- Tove M. Gabrielsen, UNIS (project leader)

Participants:

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Flagship

Fjord and coast, Theme: Structure, function and change in Arctic and boreal fjord ecosystems

Funding Source
Fram Centre, others (se below)
Summary of Results

Highlights:

- The increased number of sequencing reads produced by NGS (Next Generation Sequencing) is not enough by itself to identify prey in zooplankton.
- Blocking primers or alternative modes of removing predator DNA are necessary to identify prey in zooplankton.
- Group-specific primers work to identify prey in zooplankton guts, but is limited by availability of primers to detect relevant prey.

In 2013, we added more field collections of zooplankton (focusing on copepods) from Adventfjorden, Kongsfjorden, Billefjorden and Rijpfjorden during the polar night and twilight period (with RV Helmer Hanssen in January and with KV Svalbard in February). Our main focus, however, was on the analyses of our experimental tests started in 2012, as well as continuing our experimental setup to optimize the identification of zooplankton food utilizing genetic tools. The results of the experimental tests performed so far are presented in Table 1 and Figs 1-2, and summarized here: In total 7 different experiments have been performed to test the necessity of removing predator DNA prior to the identification of prey in zooplankton guts or whole animals (for meso-sized zooplankton). The first two experiments each utilized single individuals of zooplankton predators (*Thysanoessa inermis* and Mysidae) that were analysed using 454 Roche sequencing based on the 28S nrDNA fragment without removal of predator DNA. About 57000 sequencing reads were identified from each experiment, all of which were identified as predator DNA (Table 1). The next two experiments (3^{rd} and 4^{th}; Table 1 and Fig. 1) tested how well a C3 blocking primer against *Calanus* sp. allowed the amplification of prey sequences from 7 whole individuals of *C. glacialis*. In experiment 4 (no blocking primer), no potential prey sequences were identified whereas in experiment 3 (utilizing the C3 blocking primer), 2.60 % of the sequencing reads were identified as potential prey (Table 1, Fig. 1). The dominant prey identified in 6 of the 7 individuals of *C. glacialis* tested were two ciliates, whereas the dominating prey in the last individual was a diatom. Other potential prey included fungi, ctenophores, cercozoa, chlorophytes and dinoflagellates. Some of the latter may have been accidentally ingested, as they are dominant in sea water.

In experiment 5, we tested if the increased sequencing reads from utilizing Illumina sequencing rather than 454 sequencing may allow identification of zooplankton prey without removing predator DNA. From more than 8 million sequences obtained from the foregut of *T. libellula*, only 0.001% was identified as potential prey (*Calanus* sp.). It should be noted, however, that the quality of the sequences received from this experiment was poor. The last two experiments (6 and 7) performed included another test of Illumina sequencing to identify prey without utilizing blocking primers, this time for *Clione limacina*. In addition, we tested the utility of group-specific primers to amplify prey DNA in *C. limacina* as a cheaper variant to NGS sequencing. The combined approach allowed us to identify *Calanus* sp., *Parasagitta* sp. and Cnidaria as potential prey of *C. limacina* in addition to the only assumed prey *L. helicina*.

The combined results of our experiments suggest that blocking primers (or other ways of removing predator DNA) are necessary to properly identify prey of zooplankton guts/whole animals. The C3 blocking primer utilized in experiment 3 was only partially successful in blocking *Calanus* amplification, and we are now using PNA blocking primers instead. To avoid amplifying potential prey attached to the outside of small zooplankton, we have developed a protocol utilizing diluted chlorine to remove DNA from the outside of the animals while not influencing the gut DNA. We are currently utilizing PNA blocking primers of the 18S nrDNA against whole animals of *Calanus glacialis*, *Pseudocalanus* sp. and guts of *Thyssanoessa inermis* to identify prey of these species focusing on the polar night period. The results from these analyses are not yet ready, and will be reported at a later stage.

Table 1. Summary of zooplankton samples analysed so far

Experiment	Species	Blocking primer	Gene	No of inds	Type of tissue	Sequencing method	Total no of sequencing reads	No and potentia reads
1	*Thysanoessa inermis*	No	28S nrDNA	1	Gut	454	57175	
2	Mysidae	No	28S nrDNA	1	Gut	454	56649	
3	*Calanus glacialis*	Yes (C3)	28S nrDNA	7	Whole animal	454	96915	25
4	*Calanus glacialis*	No	28S nrDNA	7	Whole animal	454	118537	
5	*Themisto libellula*	No	18S nrDNA	1	Gut	Illumina	8376402	9
6	*Clione limacine*	No	18S nrDNA	15	Gut	Illumina	1634728	21
7	*Clione limacine*	n/a	Several	141	Gut	Sanger	n/a	

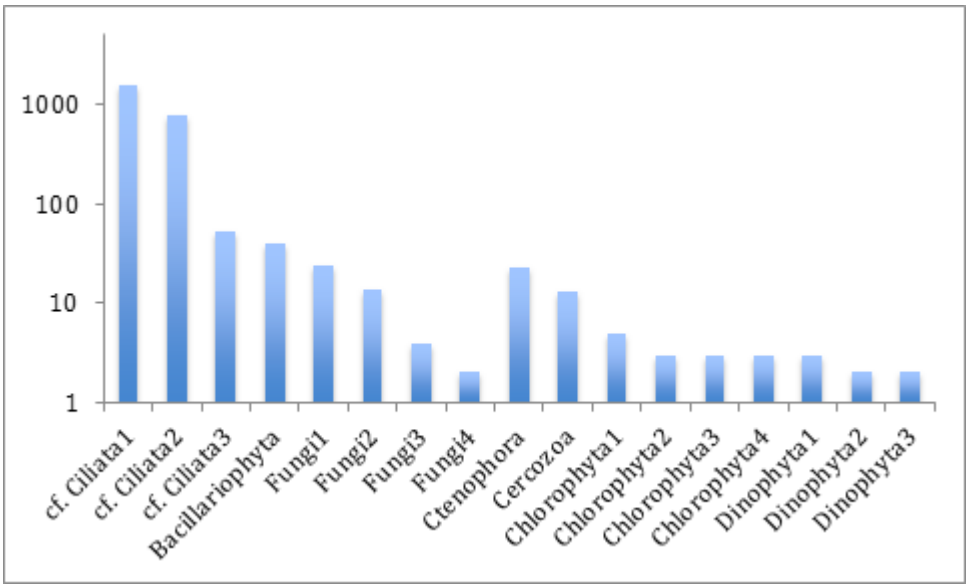

Figure 1. Sequencing reads of potential prey detected in the 7 analysed individuals of *Calanus glacialis* that were analysed utilizing the nrDNA 28S gene including a blocking primer. Note the logarithmic scale of the Y axis. The X axis shows the BLAST hit of the 17 potential prey OTUs that were detected.

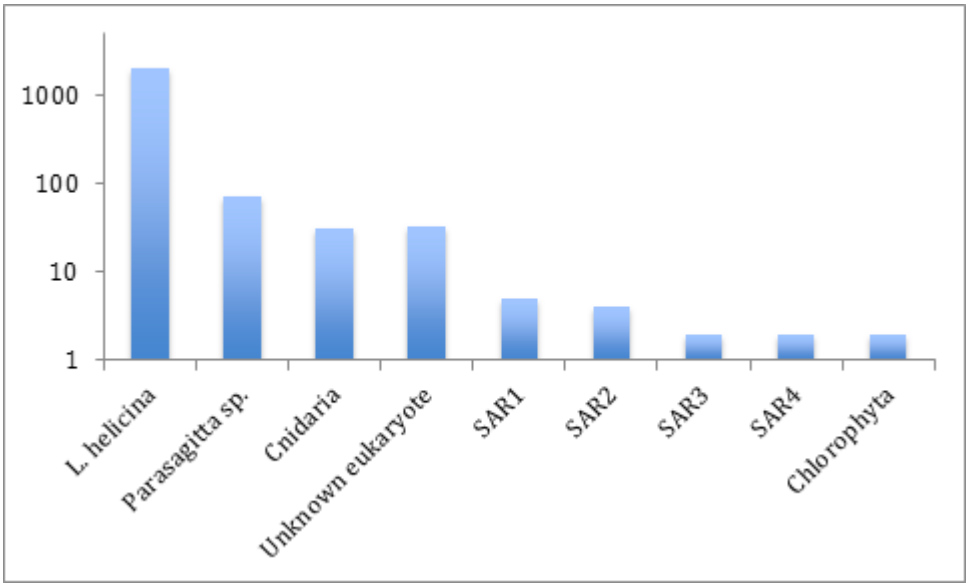

Figure 2. Sequencing reads of potential prey detected in the 15 analysed individuals of *Clione limacina* that were analysed utilizing the V9 region of the nrDNA 18S gene without blocking primer. Note the logistic scale of the Y axis. The X axis shows the BLAST hit of the 9 potential prey OTUs that were detected.

For the Management

A thorough understanding of the lower levels of the arctic marine food web is of high importance for understanding the responses in the arctic ecosystem to changes in the marine food web. In particular, our findings that the traditionally considered monophagous species *Clione limacina* has a broader diet consuming at least copepods and amphipods in addition to the previously considered only prey *Limacina helicina* is highly relevant for understanding how zooplankton species will cope with a changing marine food web due to increased sea water temperature and increased ocean acidification. The broader diet of *C. limacina* may allow the species to survive and thrive even if *L. helicina* becomes less abundant due to ocean acidification influencing its aragonite carbonite shell.

Published Results/Planned Publications

Master thesis entitled “Alternative prey choice in the pteropod *Clione limacina* (Gastropoda) studied by DNA-based methods” by Ida Helene Funderud Kallevik submitted Nov 15th 2013 to the University of Tromsø.

Planned activities and publications:

Big Black Box (NOR-USA POLPROG project) workshop in Fairbanks February 17th-19th 2014.

Visit to the Durbin Lab at URI, USA and research stay by PhD student Julie Grenvald at URI in March-April 2014.

We expect a number of publications in high-ranked journals from this project, but we need to analyse the results from the current NGS sequencing experiments utilizing PNA primers to identify relevant journals/tentative titles.

Communicated Results

Not yet, except for the master thesis submitted by Kallevik (reported above).

Interdisciplinary Cooperation

This project benefits from inter-disciplinarity in terms of utilizing molecular biology to answer questions in arctic ecology. The combination of traditional ecology and molecular biology allow us to answer questions regarding food web interactions during the polar night period without having to e.g. microscopically identify prey.

If Yes

The developed technology can be adapted to a number of different utilizations, including bioprospecting.

Conclusions

a) The results from the project (the master thesis of Kallevik in particular) show that the diet of *Clione limacina*, considered a monophagous species, is more varied than previously considered. The analyses of *Calanus glacialis* (Fig. 1; from samples collected in August) suggest that the diet of *C. glacialis* (at least in the fall) is less dependent on diatoms than previously considered. Our ongoing analyses will allow us to conclude more firmly, but suggest a more complex food web for arctic zooplankton than is usually considered.

b) The development of the pipeline for NGS (Illumina) library preparation and sequencing of zooplankton guts/whole animals to identify prey allow identification of prey items that are not possible to identify by microscopy, either because the prey organisms are too small, or because the stomach content is just a visceral mass. This same pipeline can be utilized in general to investigate predator-prey interactions.