Prey DNA as a Biomarker to Assess Feeding in Copepods

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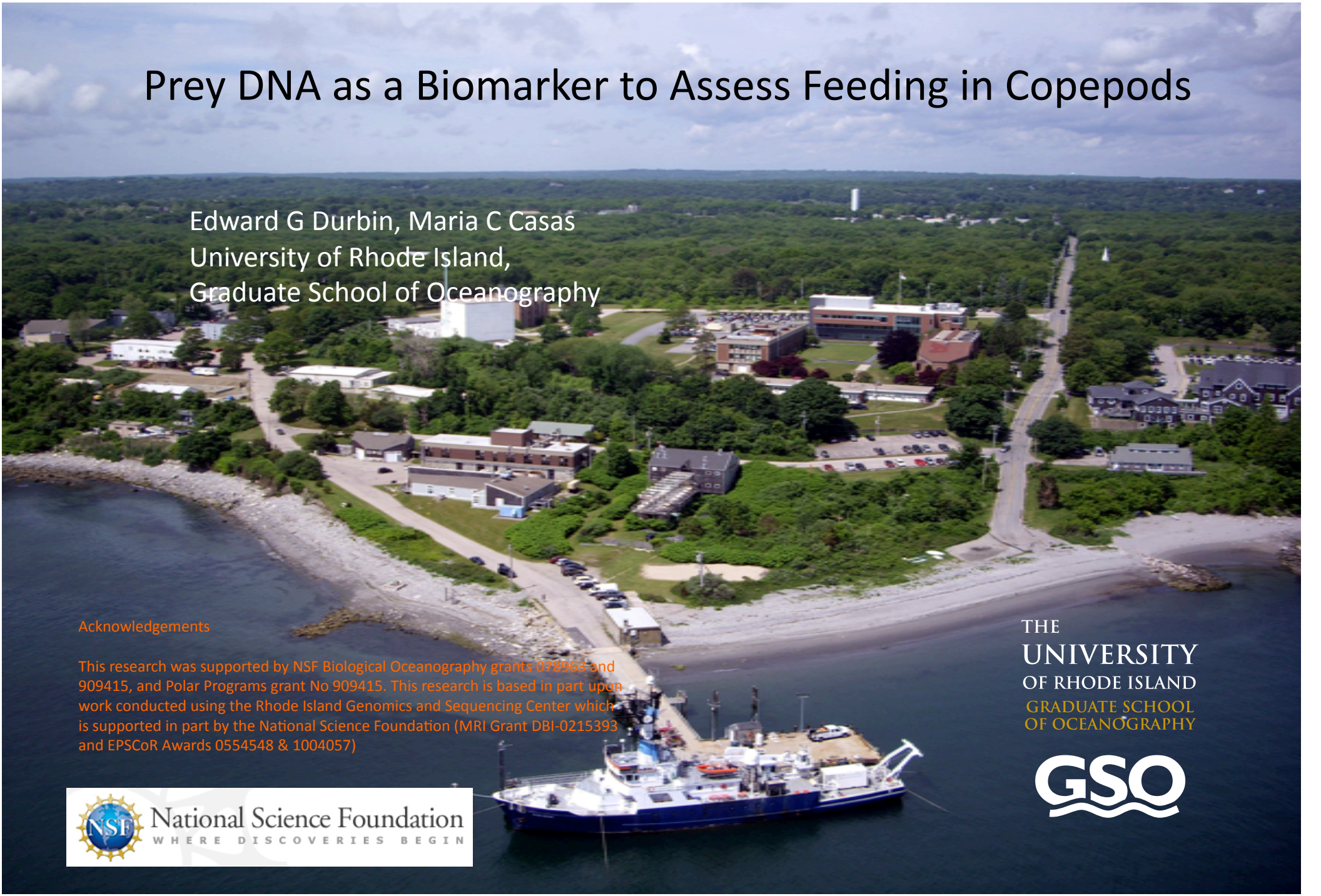

Acknowledgements

This research was supported by NSF Biological Oceanography grants 078963 and 909415, and Polar Programs grant No 909415. This research is based in part upon work conducted using the Rhode Island Genomics and Sequencing Center which is supported in part by the National Science Foundation (MRI Grant DBI-0215393 and EPSCoR Awards 0554548 & 1004057)

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Talk Outline

Motivation

Laboratory Experiments to evaluate the use of prey DNA in copepod guts to assess feeding

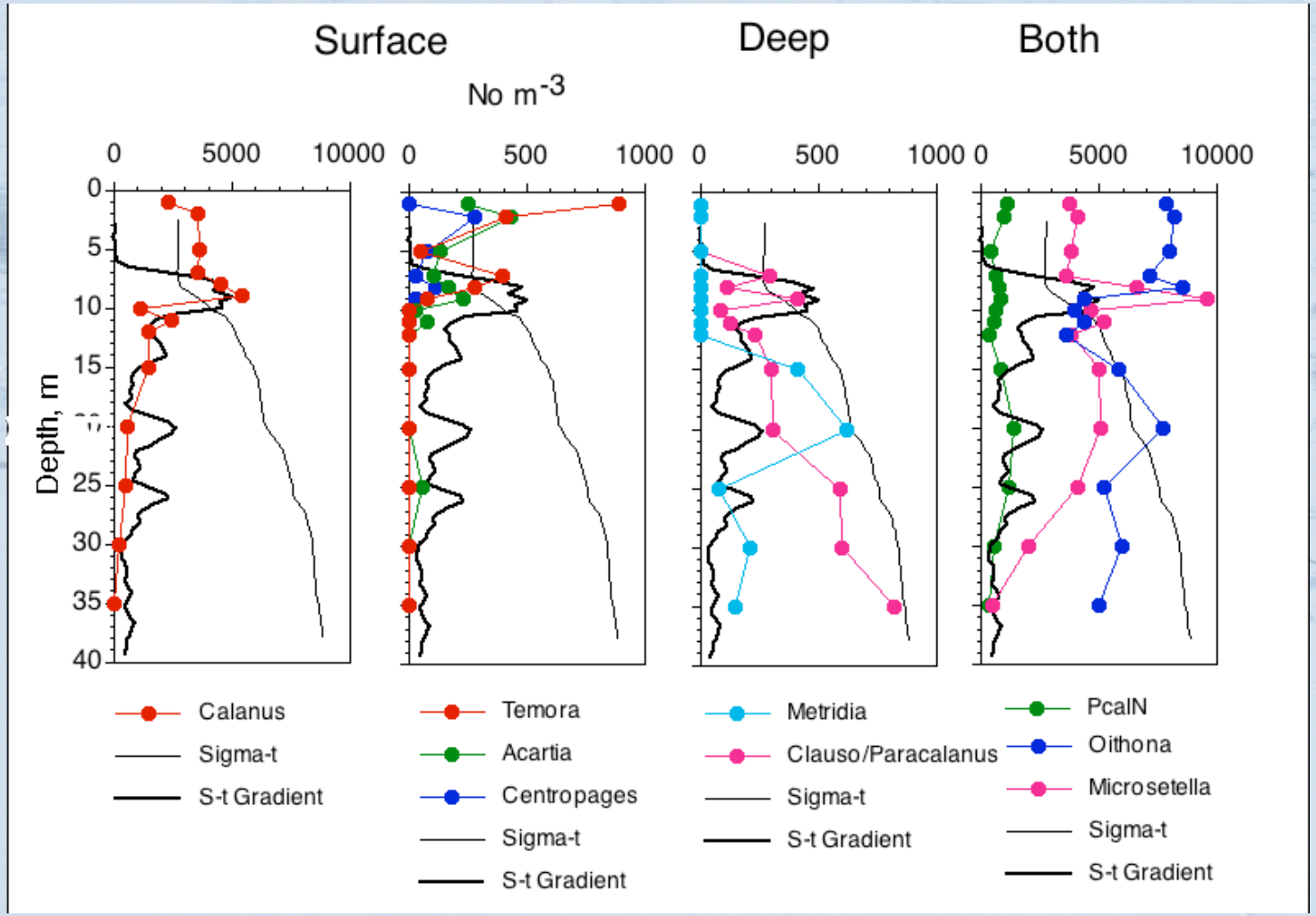
Application to the field for a study of *Calanus glacialis* feeding in the northern Bering Sea in late winter – early spring

Final thoughts

Walrus, AJ Lang

Motivation: How to explain fine-scale vertical partitioning of the water column by nauplii of nine taxa in the Maine Coastal Current

1. Surface mixed layer and pycnocline only: *Calanus, Temora, Centropages, Acartia*
2. Beneath the surface mixed layer only: *Metridia, Clauso/Paracalanus*
3. No preference: *Pseudocalanus, Oithona, Microsetella*



Hypothesis:

Fine scale vertical partitioning of the water column by nauplii reflects predation by older stage *Calanus* finmarchicus and swimming behavior and escape responses of nauplii

How to Measure this?

- Difficult to scale laboratory experiments appropriately.
- Need *in situ* feeding observations
- Prey DNA in the guts of predators!

Use of Prey DNA as a biomarker to assess prey types consumed

Method Overview

Capture copepods and fix DNA

Extract and Purify DNA

Amplify DNA in PCR reaction. We have used both CO1 and 18S as marker genes

Sequence amplification products

Sequence data analysis and identify prey types

Species specific qPCR of common prey types

Issues:

How quickly is prey DNA degraded after ingestion

How to quantitatively extract and purify small amounts of prey DNA

Large amount of predator DNA present compared with prey DNA

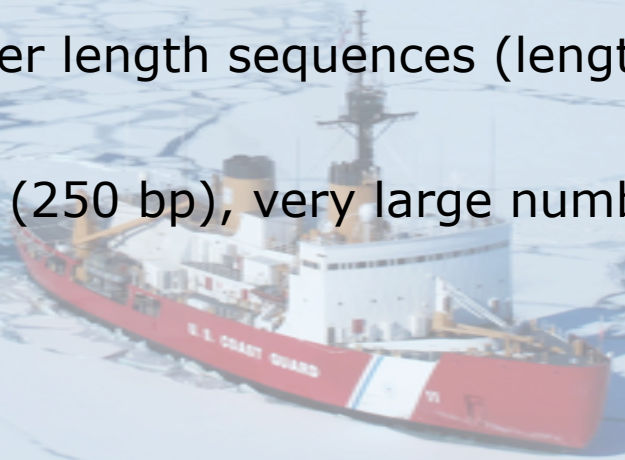
Sequencing of Prey DNA from PCR – Great advances

Methods we have used

Clone Libraries: Can obtain longer full-length 18S sequences, smaller numbers - 10^2

454 Sequencing: Shorter length sequences (length?), larger numbers - 10^5

Illumina: Shorter length (250 bp), very large number of amplicons (10^6 – 10^7)



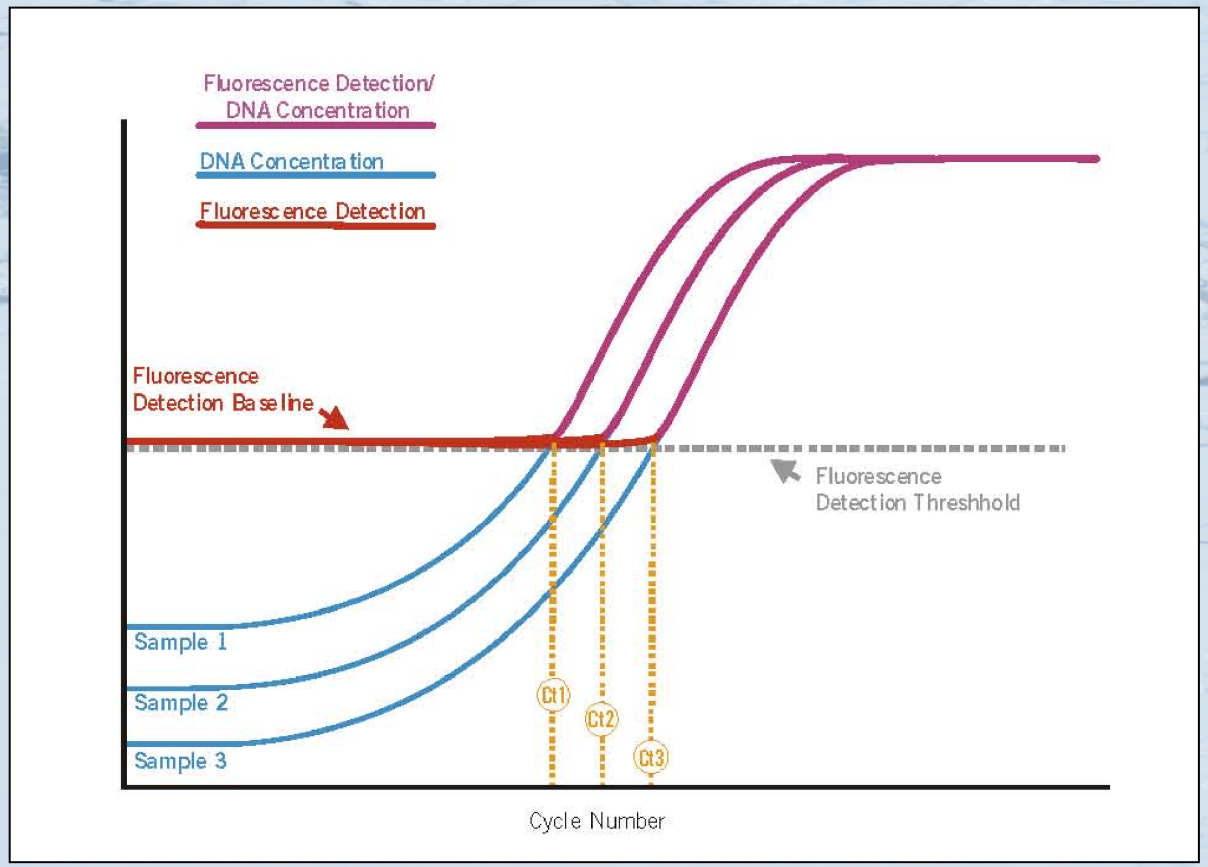
But -

PCR is not quantitative

Method Evaluation - Quantifying DNA Present

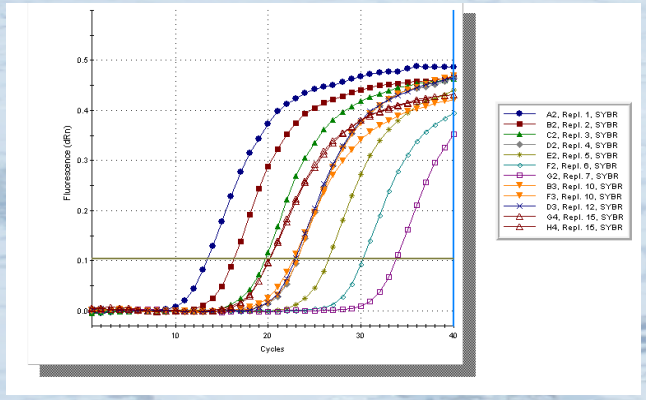
- Need to quantify the amount of DNA present in response to different procedures
- Use real-time quantitative PCR (qPCR)

Illustration of qPCR Amplification Plot

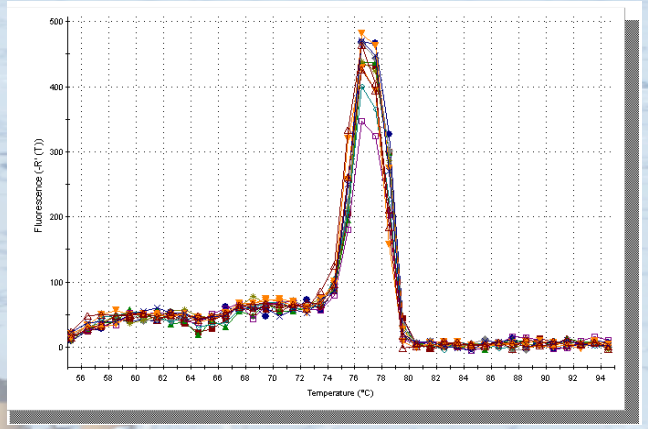


Amplification plots, dissociation plots and standard curve for a qPCR run with three individual *Acartia tonsa* females and 2 groups of 80 *A. tonsa* N2 nauplii. (From Durbin et al. 2007)

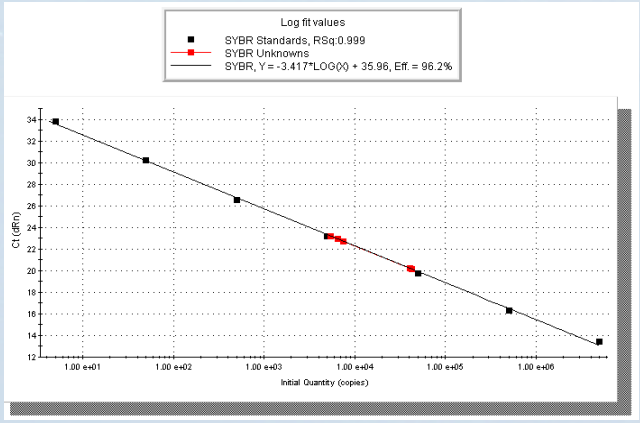
Amplification plots



Dissociation curve



Standard Curve



Method Evaluation- How long does the prey DNA signal remain in copepod guts?

1. DNA is digested very rapidly after ingestion

DNA and chlorophyll disappearance rates in *Acartia tonsa* fed *Heterocapsa triquetra* for 5 min

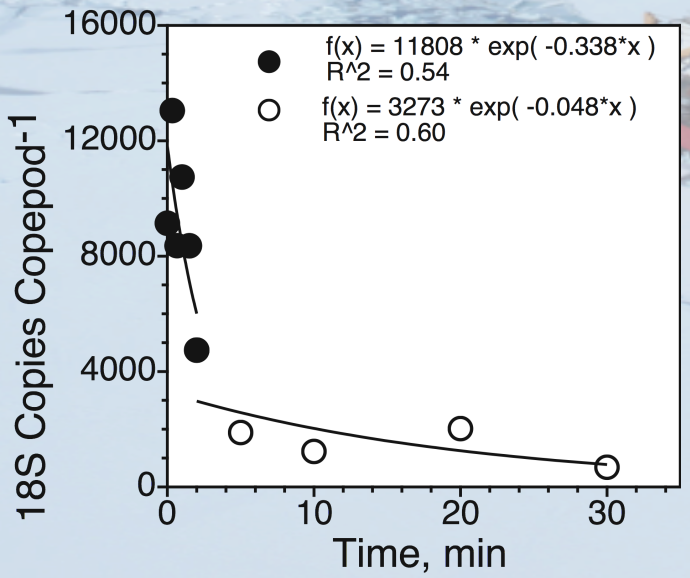
DNA disappearance is bi-phasic. Initial slope similar to enzyme reaction

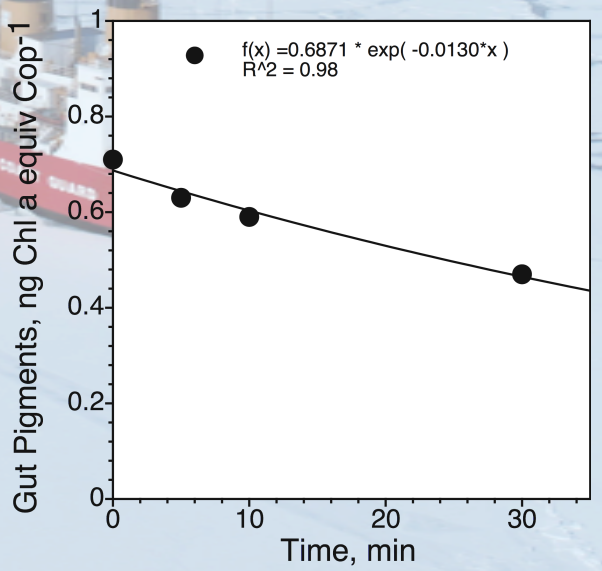
Decline of chlorophyll pigments in the gut was much slower. Reflects fecal pellet evacuation.

Implications:

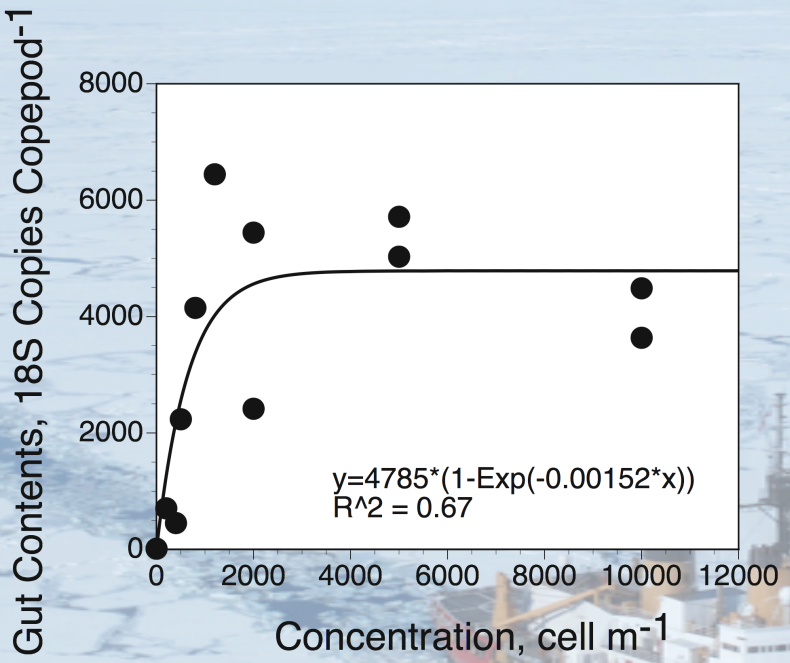
Copepods must be fixed very rapidly. We found ethanol most effective. Freezing resulted in much lower DNA copy numbers

DNA in gut only represents short-term feeding





2. Do we see similar gut content vs food concentration relationships when comparing DNA and traditional bottle incubation methods?



I_{crit} (90% of I_{max}):

DNA Method:

I_{crit} = 1,590 cell ml $^{-1}$, I_{max}= 4,310 18S copies copepod^{-1}

Bottle incubation method:

I_{crit} = 940 cell ml $^{-1}$, I_{max}= 1,920 cell copepod^{-1} hr $^{-1}$ (from Thompson et al. 1994).

Application to the field

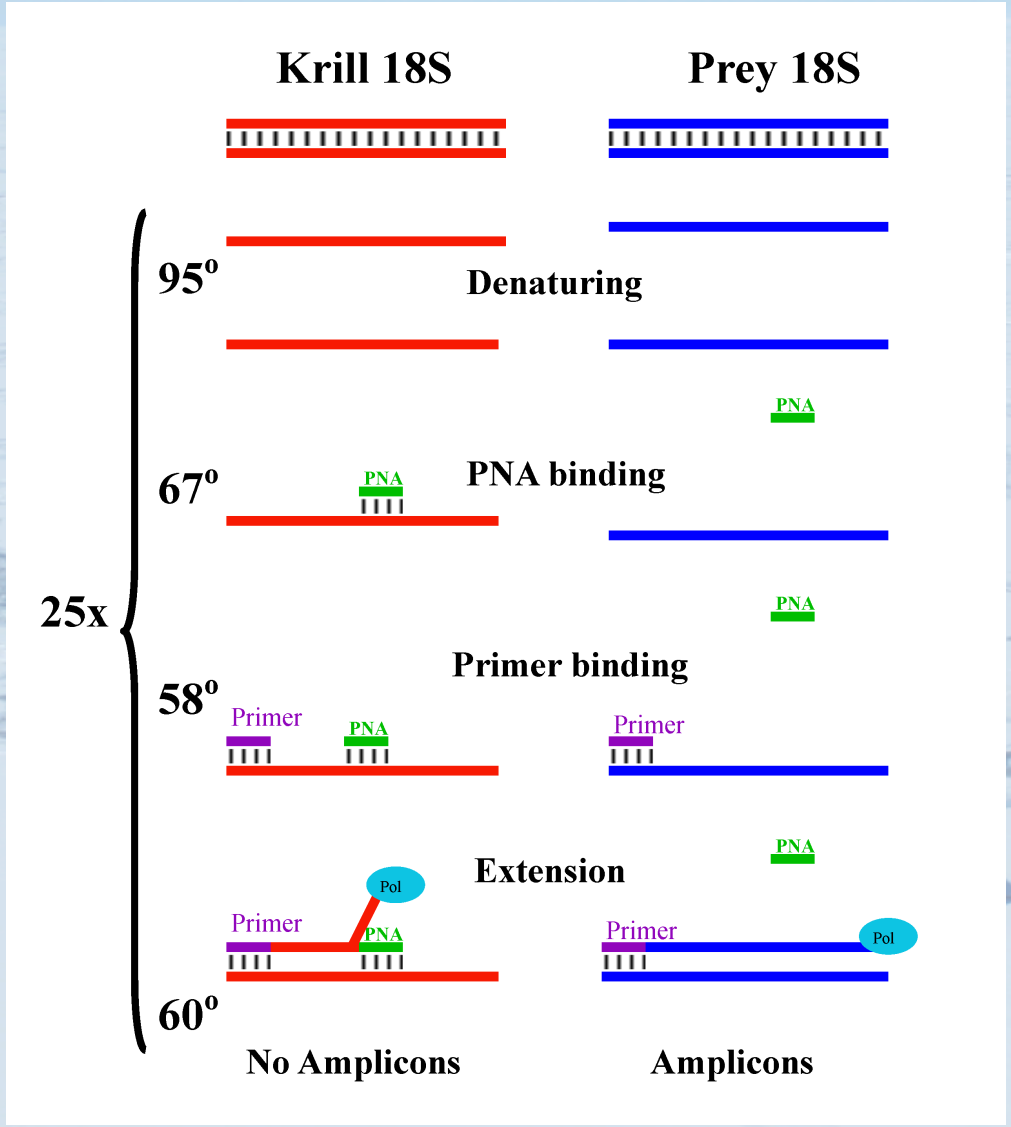
Copepods caught in the field, preserved in alcohol

Can't dissect stomachs

Extract all the DNA – Prey + Predators

- **Problem:** Too much predator DNA compared with prey
- **Solution:** PNA (peptide nucleic acid) - PCR blocking probe to block predator DNA amplification

Illustration of how PNA works during PCR



Issues to be aware of:

1.Need to preserve field samples very rapidly

We found that ethanol preservation gave much **higher** prey 18S copy numbers than for copepods that had been frozen

RNAlater may also be used but we have not evaluated it

2. Possible presence of contaminant DNA

We found large numbers of *Parasagitta* sequences in *Calanus glacialis* gut content samples in the Bering Sea

Contaminant DNA

Parasagitta elegans 61% of gut content clone libraries???

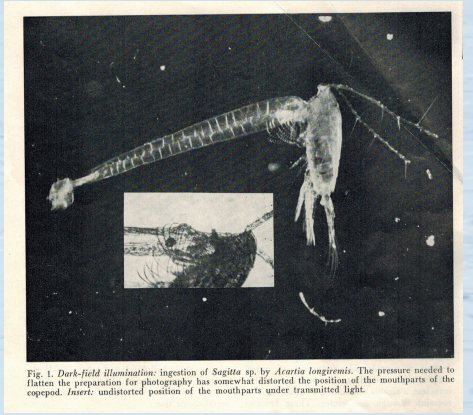


Fig. 1. *Dark-field illumination:* ingestion of *Sagitta* sp. by *Acartia longiremis*. The pressure needed to flatten the preparation for photography has somewhat distorted the position of the mouthparts of the copepod. *Insert:* undistorted position of the mouthparts under transmitted light.

We suggest two possible origins.

1. They were ingested by *Calanus*.
   Acartia longiremis eating *Sagitta*
   Davis (1977) Astarte 10:1-3.

2. They represent material regurgitated by *Parasagitta* during capture and adhered to the *Calanus* exoskeleton. *Parasagitta* regurgitates as much as 80% of gut contents during capture (Baier and Purcell 1997).

   Parasagitta DNA was present on *Calanus* antennae. Using a weak bleach solution we were able to remove this DNA.

   More testing of removal of contaminant DNA needed

- Take Home Message:
  DNA in PNA-PCR amplification products are not always from from ingested organisms!!

Use of Prey DNA as a biomarker to assess prey types consumed

Method Overview

Capture copepods and fix DNA

Extract and Purify DNA

Amplify DNA in PCR reaction. We have used both CO1 and 18S as marker genes

Sequence amplification products

Sequence data analysis and identify prey types

There have been several studies using this approach

Gives a **qualitative** measure of the different prey types that have been consumed

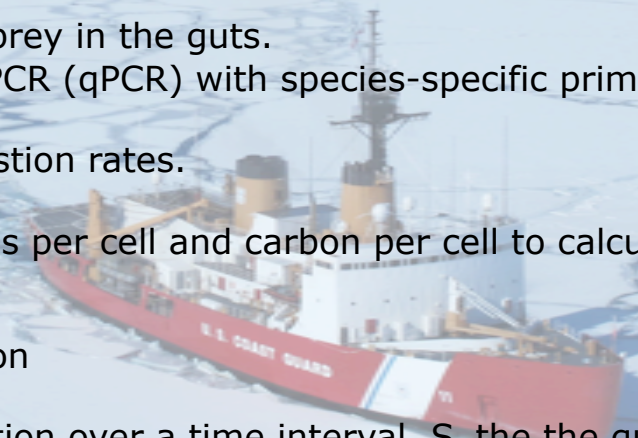
The Holy Grail:

Determining **Feeding Rates** from prey DNA in guts of predators –

Application to the field for a study of *Calanus glacialis* feeding in the northern Bering Sea in late winter – early spring

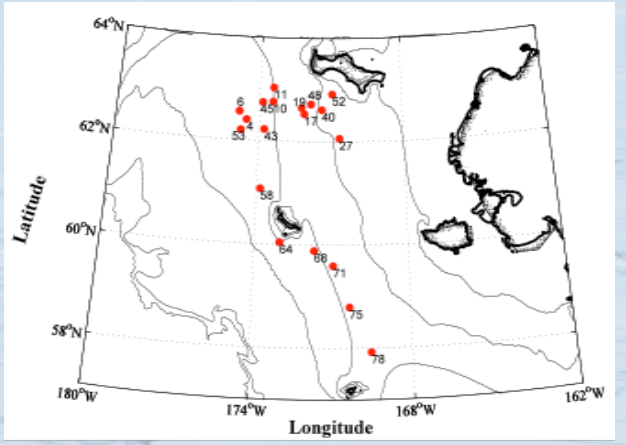
Our Approach:

1. Identify prey species from DNA
2. Quantify amount of prey in the guts.
   Quantitative PCR (qPCR) with species-specific primers
3. Determine DNA digestion rates.
4. Determine 18S copies per cell and carbon per cell to calculate consumption in terms of cells
5. Calculate consumption
   $I_t = S_t \times R$
   where I_t is the ingestion over a time interval, S_t the the gut content and R the exponential DNA disappearance rate



Durbin, E.G., Casas, M.C. Early reproduction by *Calanus glacialis* in the northern Bering Sea: the role of ice algae as revealed by molecular analysis. Journal of Plankton Research. 2014

Application: Coast Guard ice breaker Polar Sea patrol to the northern Bering Sea (PSEA1001), March, 2010



Spectacled Eiders in the Bering Sea

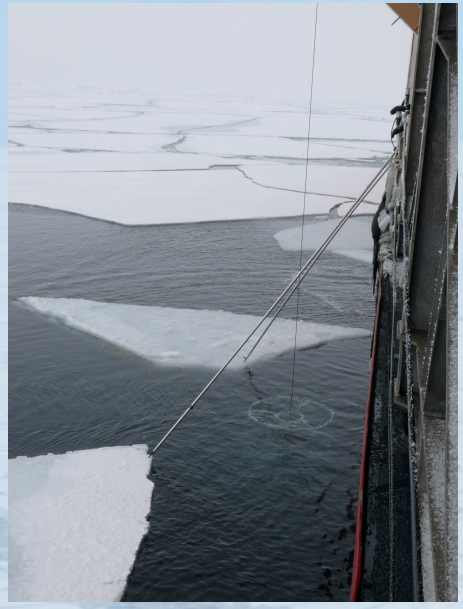
Observation:

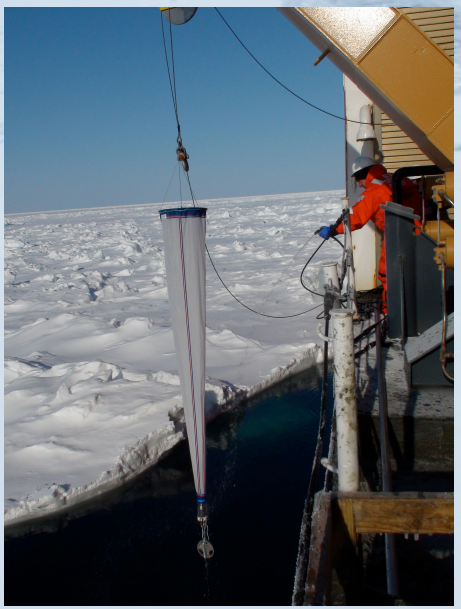
- *Calanus glacialis* abundance in the Bering Sea varies inversely with temperature and ice cover (Smith and Vidal, 1986, Baier and Napp, 2003).

- Onset of reproduction appears to occur well before the phytoplankton bloom (Baier and Napp, 2003).

Why?

Ice Algae – A dense, potential food source?





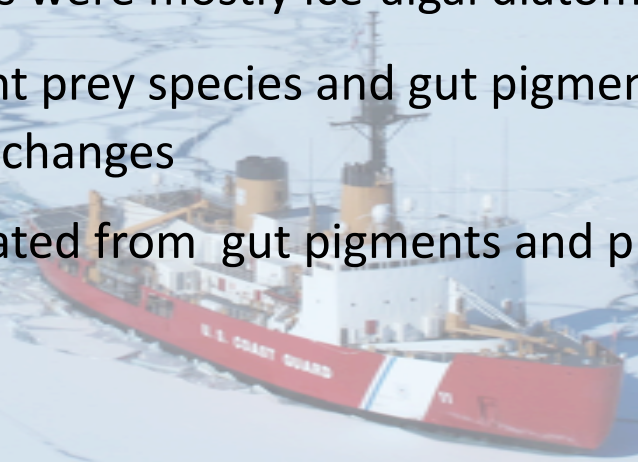
What we did:

Calanus glacialis

- Egg abundance
- Gut pigments
- PNA-PCR amplification of DNA in guts for prey species identification
- qPCR quantification of DNA of more abundant prey
- Characterized water column and ice algae protists
- Estimated ingestion rate from gut pigments and prey DNA

What we found:

- Very low phytoplankton abundance in the water column
- Despite this eggs began to appear in the water column
- At the same time we observed large amounts of phytoplankton pigments in the guts of *C. glacialis*
- DNA revealed prey species were mostly ice-algal diatoms
- DNA of the more abundant prey species and gut pigments showed the same temporal changes
- Consumption rates calculated from gut pigments and prey DNA were similar



Clone Libraries of water column and sea ice samples. 18S full length sequences. Very different communities were present.

Only 3 OTUs of a total of 51 were common to both!

	Water Column		Ice Algae	
Group	No. OTUs	% Total	No. OTUs	% Total
Pennate	2	1.6	28	76.7
Centric	5	79.4	4	6.6
Flagellate	0	0	1	0.6
Cercozoa	0	0	5	9
Ciliate	5	5.4	4	16.6
Dinoflagellate	2	3.2	2	1.2

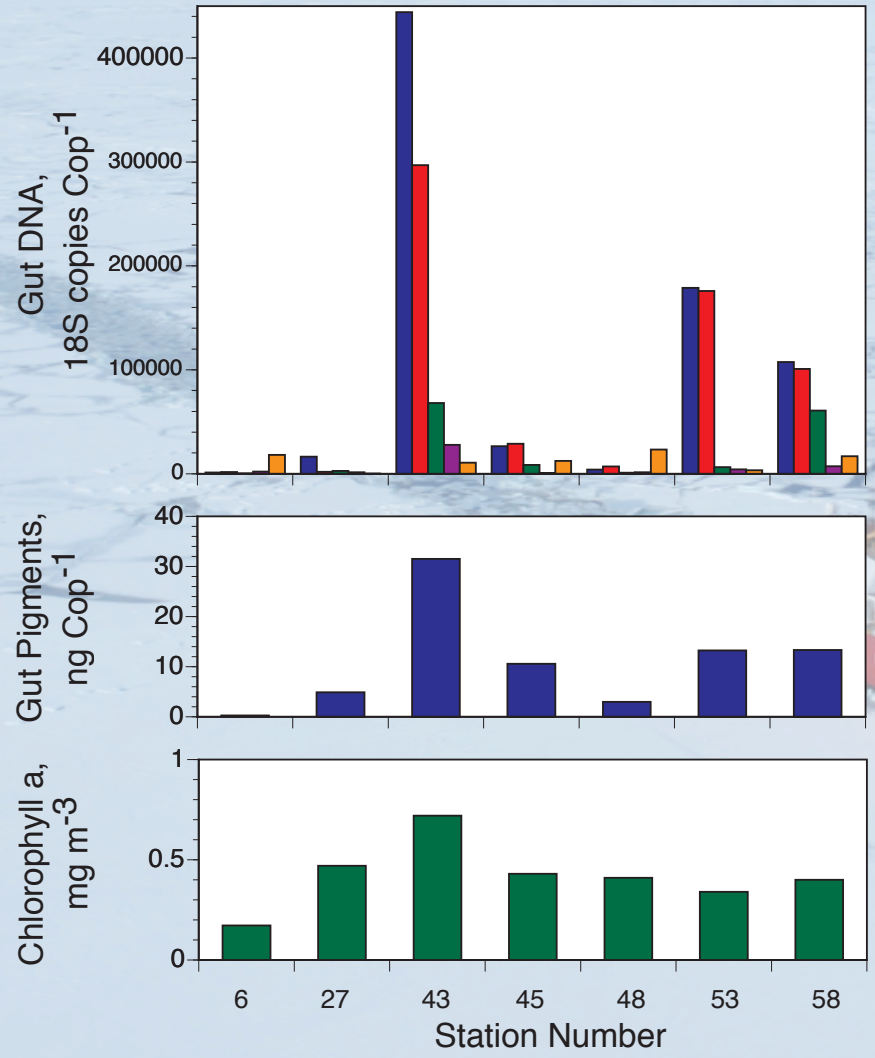
Gut Content Clone Libraries

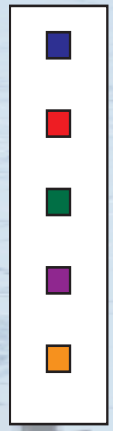
- 403 sequences of 34 OTUs.
- 11% clustered with *Calanus.*
- 61% clustered with *Parasagitta elegans*???
- 11% Clustered with gelatinous zooplankton.
- Two other OTUs were copepod parasites.
- 11% Ice algal diatoms – 8 OTUs.
- The number phytoplankton sequences were positively related to gut pigment levels.

Table 7. OTUs in *Calanus glacialis* gut contents during late March-early April, 2010, on the Bering Sea shelf, together with nearest taxonomic identity. All identities were within 98% unless noted. The number of each OTU sequence and % of each is given. Stations sampled were: 6, 27, 43, 45, 49, 53, 58. Station 6 was considered a "low food" station while the others were considered "high food".

Group	Species Name	Low Food		High food		Total	
		No.	%	No.	%	No.	%
Chaetognath	*Parasagitta elegans*	46	53.5	172	54.3	218	54.1
	Parasagitta sp. <98%	5	5.8	24	7.6	29	7.20
Copepod	*Calanus* sp.	6	7.0	14	4.4	20	4.96
	Calanus sp. <98.0%	13	15.1	12	3.8	25	6.20
Diatom	*Fragilaria* sp.	0	0.0	8	2.5	8	1.99
	Fragilaria sp.<98%	1	1.2	3	0.9	4	0.99
	Fragilariopsis cylindrus	0	0.0	9	2.8	9	2.23
	Fragilariopsis sp. <98%	0	0.0	0	0.0	0	0.00
	Pseudonitzschia sp.	0	0.0	19	6.0	19	4.71
	Pseudonitzschia sp. <98%	0	0.0	1	0.3	1	0.25
	Thalassiosira sp.	0	0.0	3	0.9	3	0.74
	Thalassiosira sp. < 98%	0	0.0	1	0.3	1	0.25
Dinoflagellate	*Heterocapsa triquetra*	1	1.2	0	0.0	1	0.25
	Gymnodinoides pitelkae	0	0.0	2	0.6	2	0.50
	Gymnodinium sp. <98%	0	0.0	1	0.3	1	0.25
Ciliate	*Vampyrophyra* sp. <98%	0	0.0	2	0.6	2	0.50
Gelatinous	*Velamen parallelum*	0	0.0	1	0.3	1	0.25
	Velamen sp. <98%	0	0.0	1	0.3	1	0.25
	Bolinopsis infundibulum	0	0.0	1	0.3	1	0.25
	Lizzia sp. <98%	0	0.0	1	0.3	1	0.25
	Ocyropsis sp. <98%	0	0.0	1	0.3	1	0.25
	Mertensia ovum	0	0.0	35	11.0	35	8.68
	Mertensia sp. <98%	0	0.0	5	1.6	5	1.24
Gregarine	*Thiriotia* sp. <98%	0	0.0	1	0.3	1	0.25
Fungi	*Cladosporium* sp.	3	3.5	0	0.0	3	0.74
	Mycosphaerella sp.	1	1.2	0	0.0	1	0.25
	Sirococcus conigenus	1	1.2	0	0.0	1	0.25
	Sporopachydermia lactativora	2	2.3	0	0.0	2	0.50
	Ceriporia purpurea	1	1.2	0	0.0	1	0.25
Terrestrial plants	*Acanthophysium cerassatum*	1	1.2	0	0.0	1	0.25
	Chlorophytum tetraphyllum	1	1.2	0	0.0	1	0.25
	Betula pendula	1	1.2	0	0.0	1	0.25
	Prunus dulcis	1	1.2	0	0.0	1	0.25
	Picea smithiana	2	2.3	0	0.0	2	0.50

Calanus glacialis ingested ice algal diatoms, **not** *Thalassiosira antarctica*, the water column dominant





Fragilariopsis cylindrus

Pseudo-nitzschia sp.

Fragilaria sp.

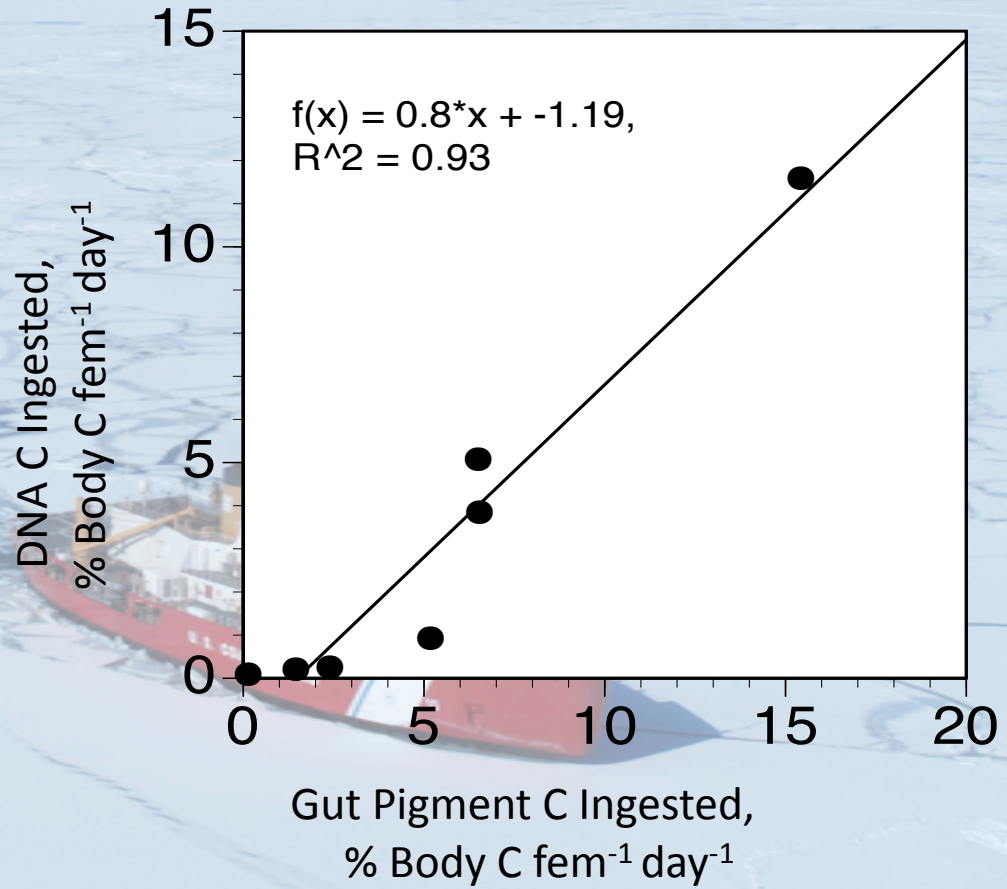
Thalassiosira antarctica

Laboea sp.

Comparable ingestion rates from gut pigments and prey DNA!

Ingestion rates from DNA and gut pigments linearly related

DNA rates 80% of gut pigment rates



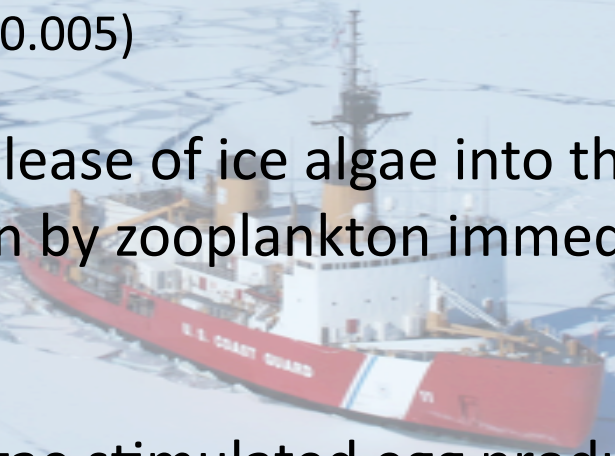
Conclusions

- Feeding rates observed could not have been sustained by the low level of phytoplankton in the water column

- Higher feeding rates associated with warmer air temperatures
  Surface chlorophyll significantly related to air temperature lagged by 2-3 days ($p<0.005$)

- Warming allowed the release of ice algae into the water column where it was fed on by zooplankton immediately under the ice

- The availability of ice algae stimulated egg production



Observation

Calanus can lay eggs at high rates for several months given suitable food

Conclusions

- Cold years when ice cover is more extensive and duration longer, results in an extended period of higher food availability for *Calanus glacialis*

- This results in a longer period of reproduction and a greater abundance later during the spring

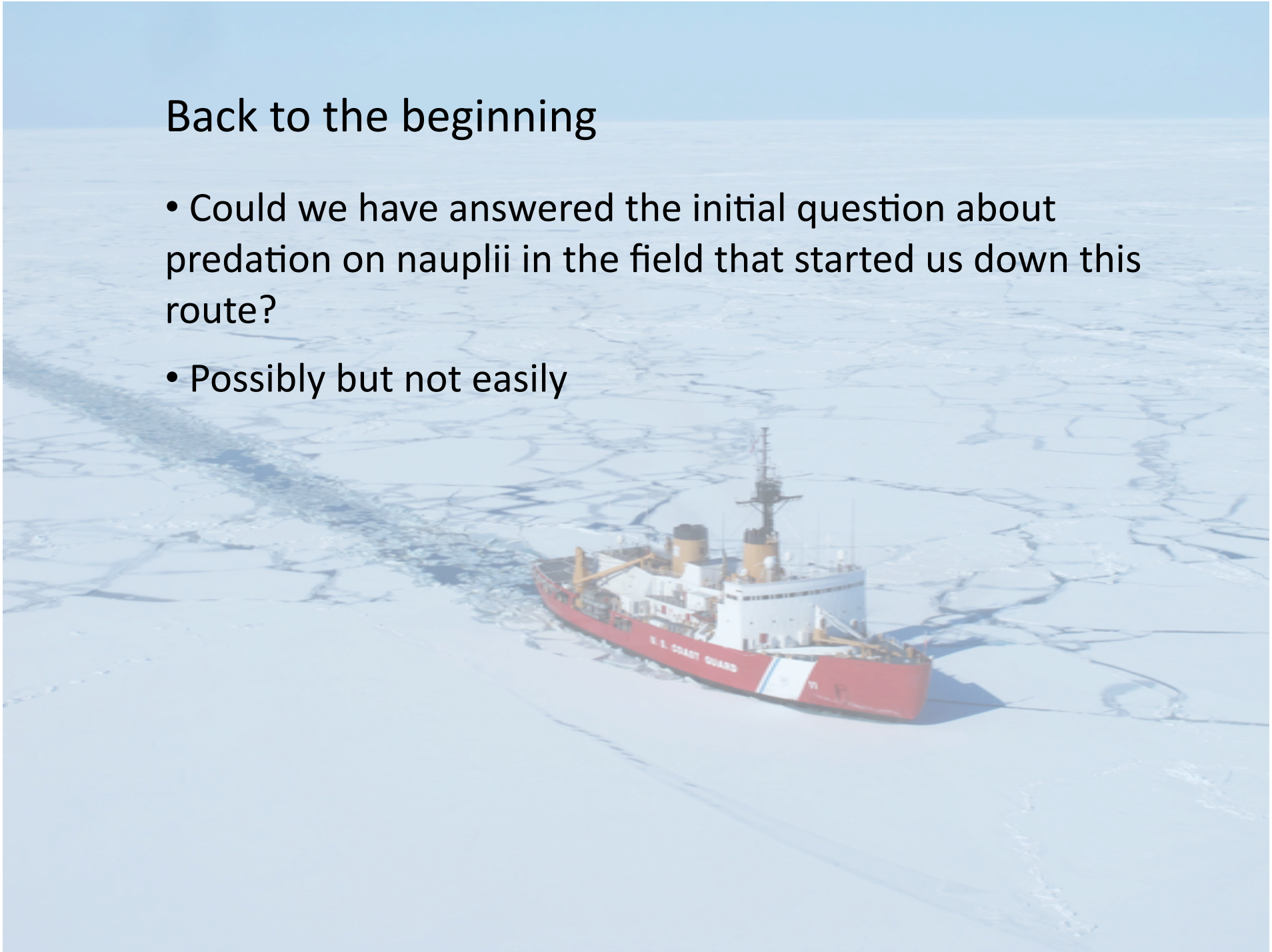
- By comparison the ice-edge and water column phytoplankton blooms are quite brief resulting in a shorter period of reproduction

Final Thoughts:

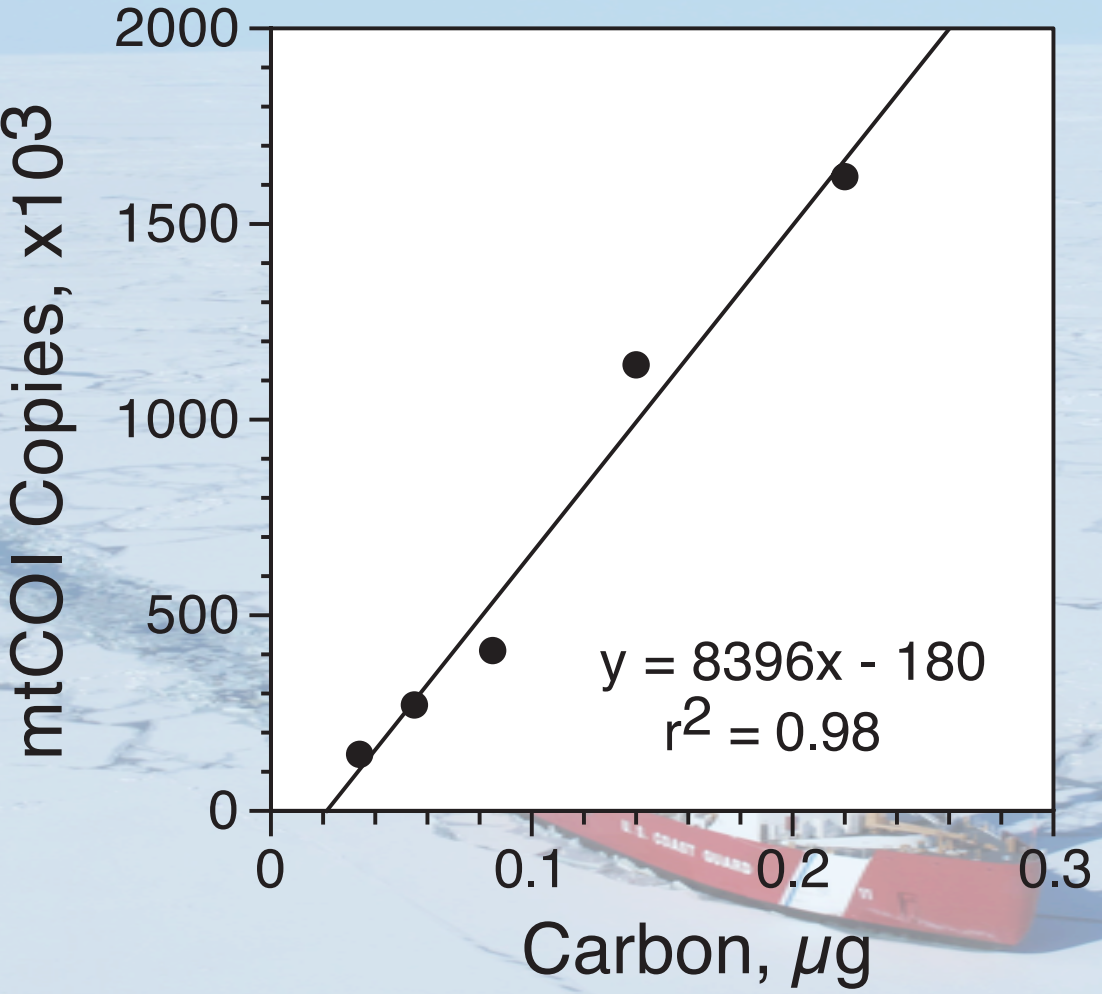
- The DNA method looks promising. Good qualitative results
- Quantitative use needs work.
- Need more measurements of 18S copy number per cell and carbon per cell of phytoplankton, and DNA digestion rates
- Need to further evaluate presence of non-consumed DNA

Back to the beginning

- Could we have answered the initial question about predation on nauplii in the field that started us down this route?
- Possibly but not easily



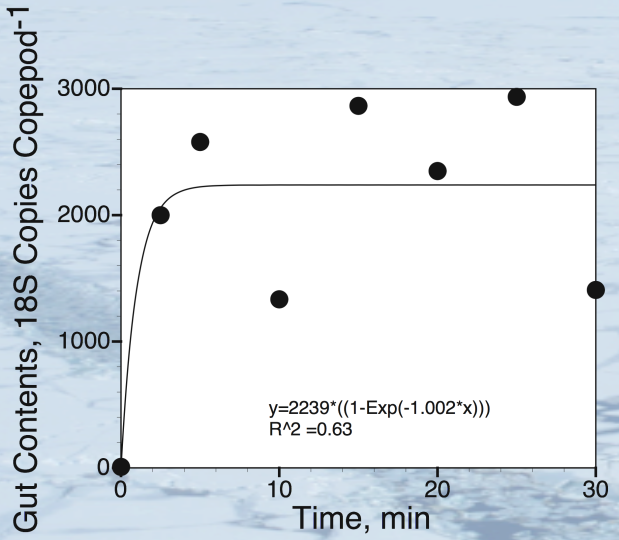
But perhaps we can estimate total copepod carbon ingested

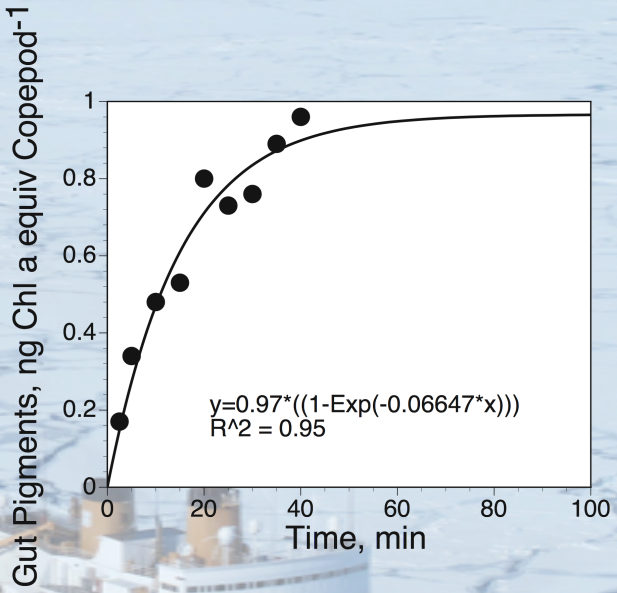


Relationship between mtCOI copy number and carbon copepod^{-1} for *Acartia tonsa* stages N2 to N6. The analyses were carried out with copepods with the October/ February haplotype. Carbon data are for laboratory reared *A. tonsa* (Berggreen et al. 1988). From: Durbin et al 2007.


Questions?

3. Only the rate of digestion influences the gut filling time (assuming exponential processes)





Acartia tonsa fed *Thalassiosira weissflogii*: Gut filling rates for DNA (18S copies) and gut pigments.

Note: The effect of R (digestion or disappearance rate) on the rate of gut filling. Gut filling time is independent of feeding rate

Acknowledgements

This research was supported by NSF Biological Oceanography grants 078963 and 909415, and Polar Programs grant No 909415. This research is based in part upon work conducted using the Rhode Island Genomics and Sequencing Center which is supported in part by the National Science Foundation (MRI Grant DBI-0215393 and EPSCoR Awards 0554548 & 1004057)

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Assessment of Copepod feeding *in situ*

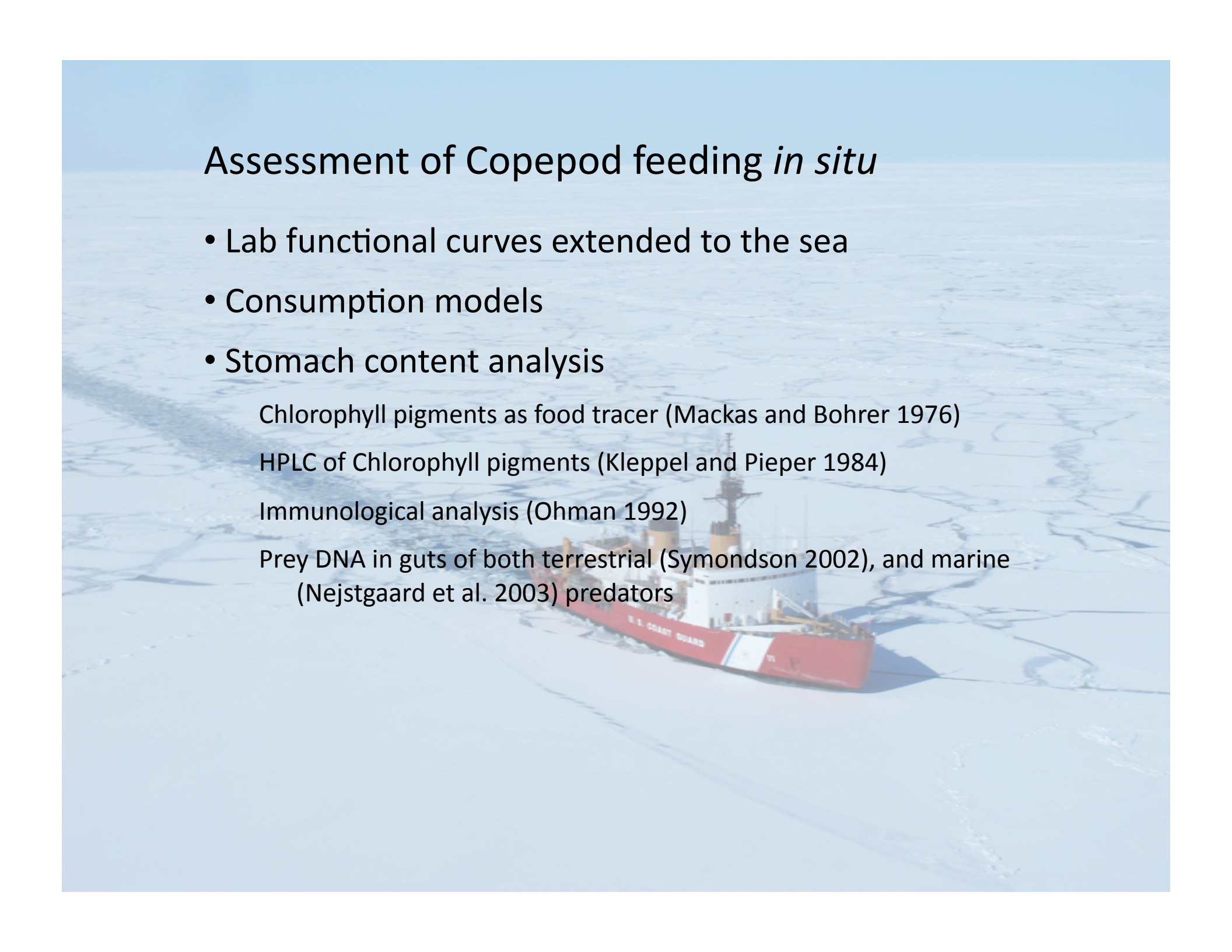
- Lab functional curves extended to the sea
- Consumption models
- Stomach content analysis

  Chlorophyll pigments as food tracer (Mackas and Bohrer 1976)

  HPLC of Chlorophyll pigments (Kleppel and Pieper 1984)

  Immunological analysis (Ohman 1992)

  Prey DNA in guts of both terrestrial (Symondson 2002), and marine (Nejstgaard et al. 2003) predators



Spectacled eider

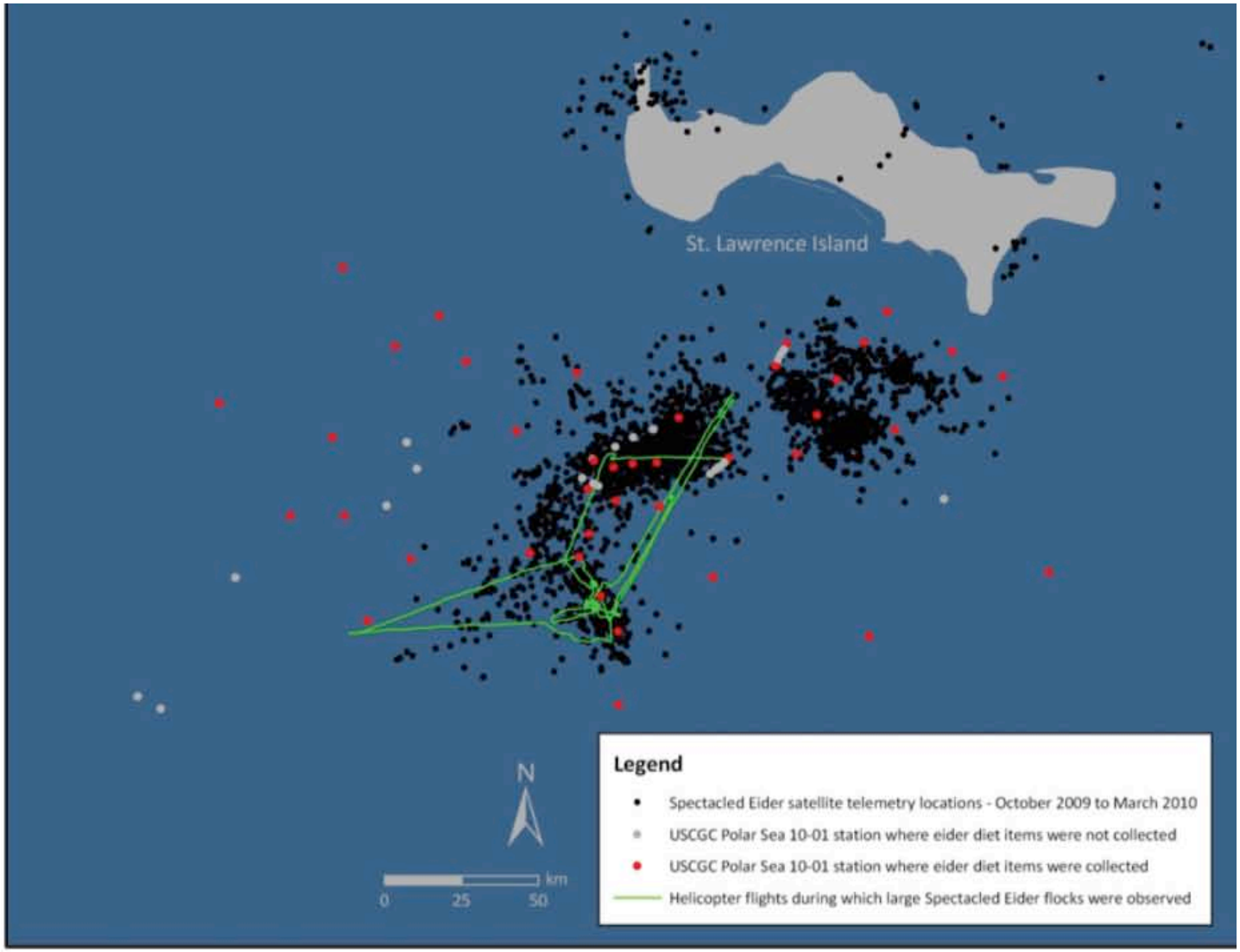
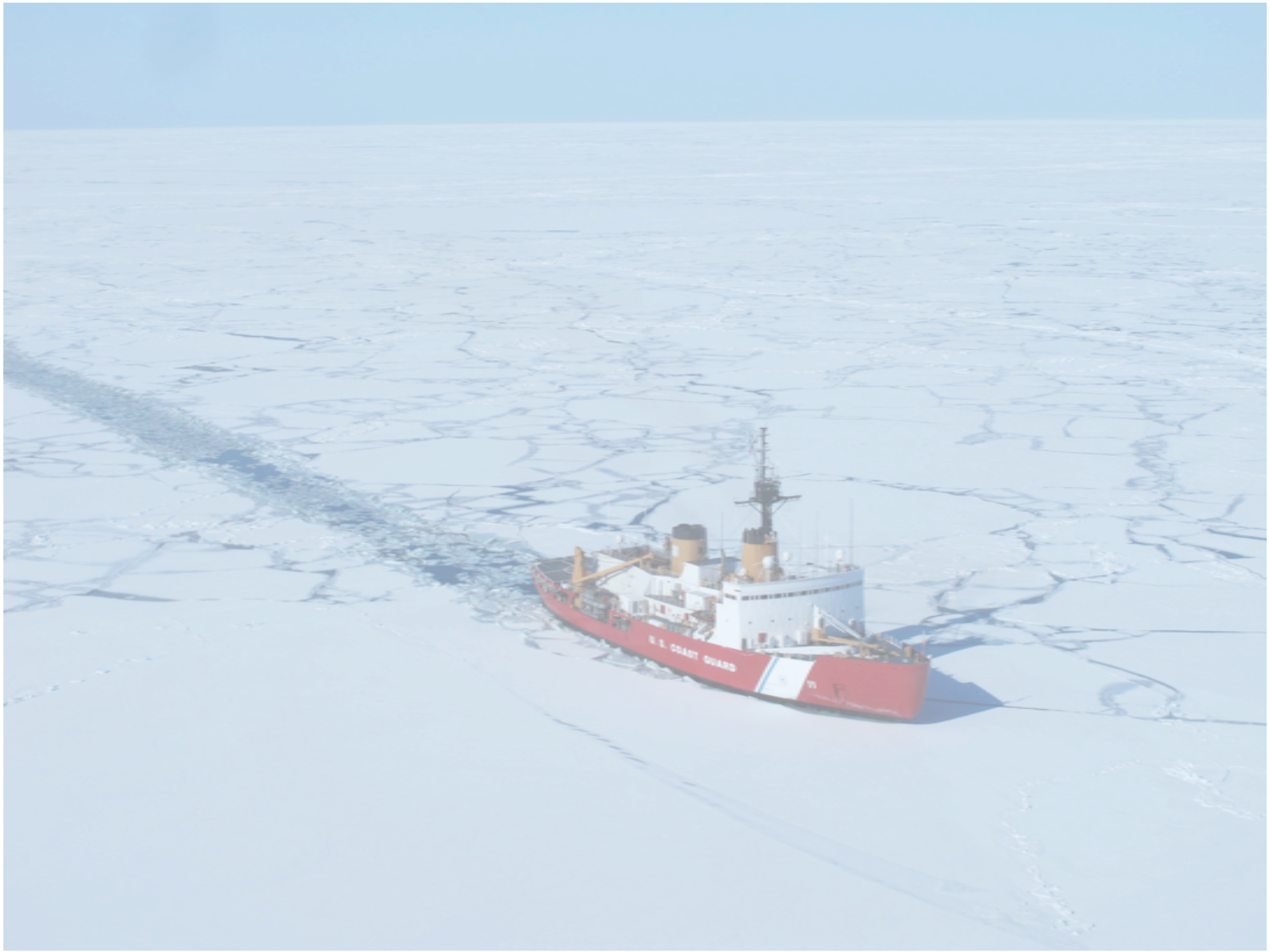


Figure 1. Spectacled Eider satellite telemetry locations south of St. Lawrence Island, Alaska from October 2009 - March 2010. USCGC Polar Sea 10-01 benthic sampling stations (gray and red) and helicopter flights (green) where large concentrations of Spectacled Eiders were observed are shown.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