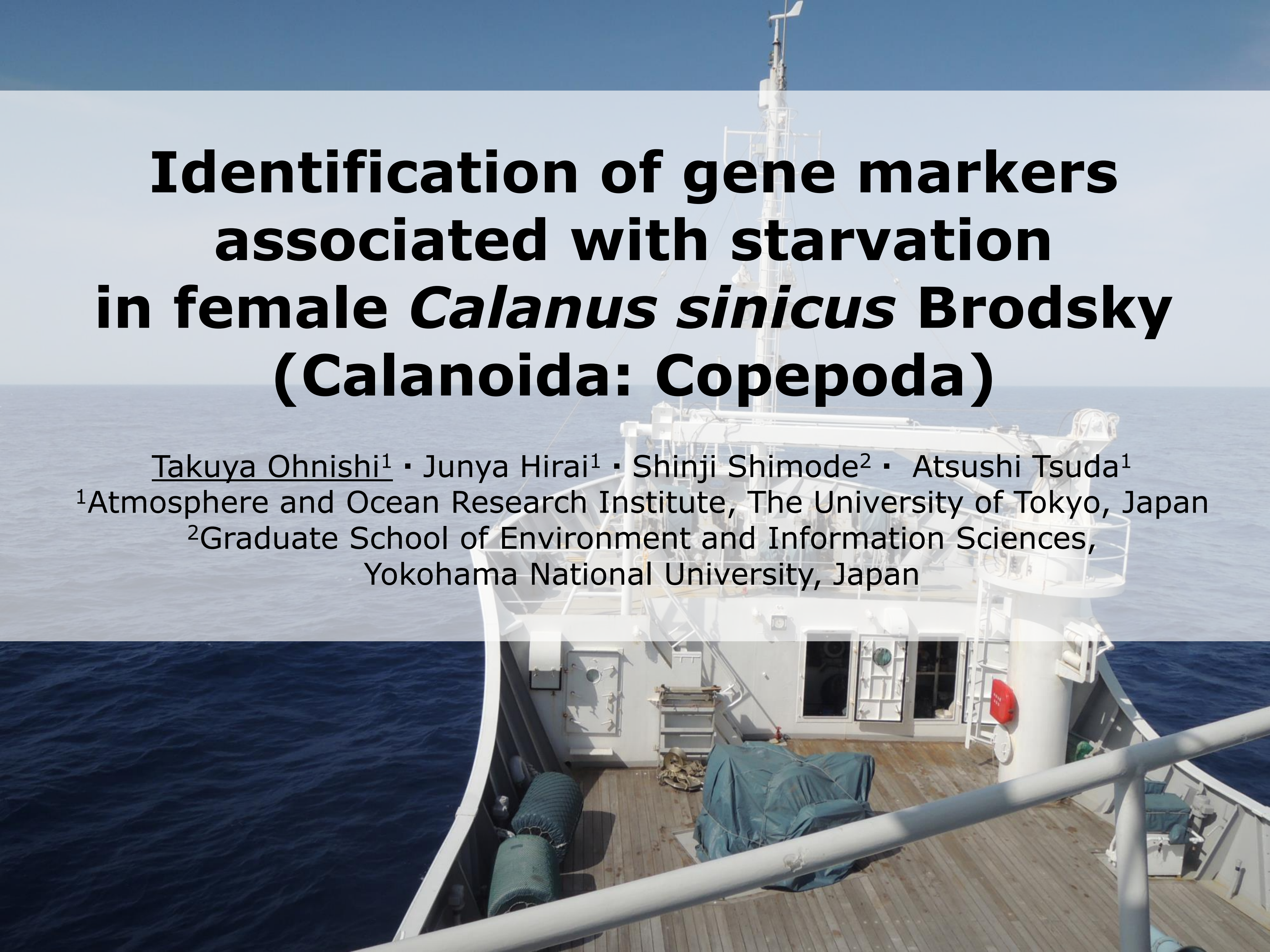

Identification of gene markers associated with starvation in female *Calanus sinicus* Brodsky (Calanoida: Copepoda)

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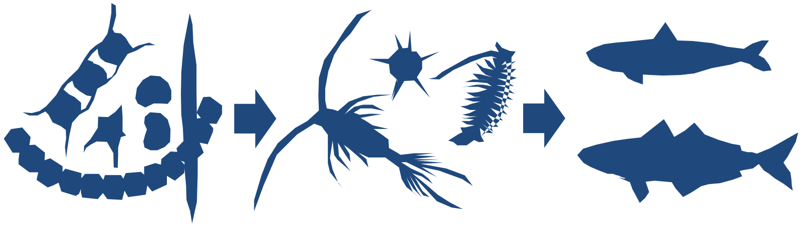
Background

Need a new approach to evaluate starvation

- Indicator for conditions of marine ecosystems

  Copepods provide a crucial trophic link between primary producer and fish. This energy flow determines the amount of energy available to higher trophic levels.

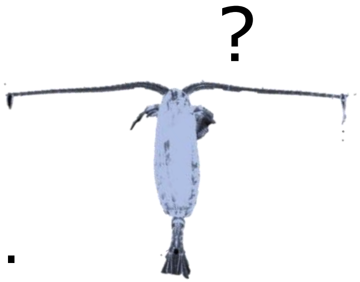


- Starvation effects zooplankton production

  The physiological state of copepods is largely influenced by variable food availability in the ocean.

- Difficulty in identifying starved individuals

  The food availability is much more difficult to evaluate than physical factors such as temperature.



Background

By identifying differentially expressed genes,
We develop a new method to evaluate starvation.

■ This study focused on gene expression analysis

Physiological changes appear in gene expression.

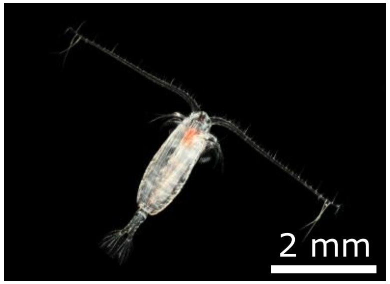
Some genes expression will be indicator of starvation.

■ What kinds of gene expression are changed?

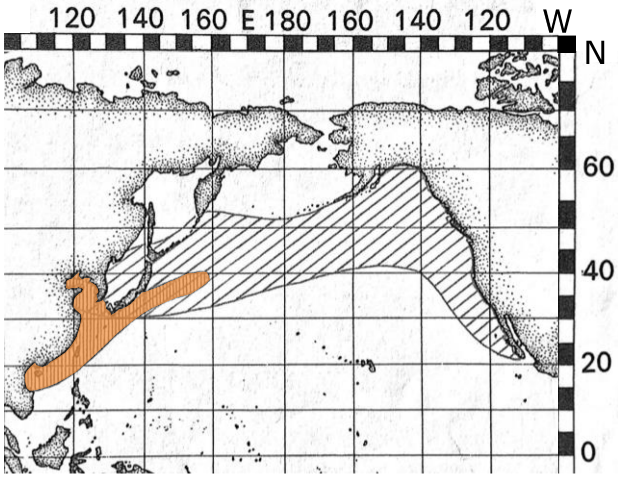
Physiological changes at starvation includes:

- decreasing reproduction rate
- inhibiting somatic growth
- decreasing respiration rate

■ About *Calanus sinicus*



- ecologically important species
- relatively large body size
- warm-temperate species



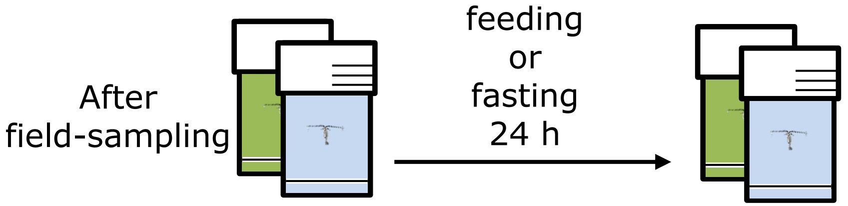
●: Distribution of *C. sinicus*
(Spoel&Heyman 1983)

Differential gene expression analysis by RNA–seq

Following objects:

- construction of reference sequence
- identifying candidate gene markers

■ Starvation experiment



Fasting period: 24 h

Temperature: 18°C

Food condition: ambient surface seawater (particle size >20 µm)

■ Comprehensive differential gene expression analysis

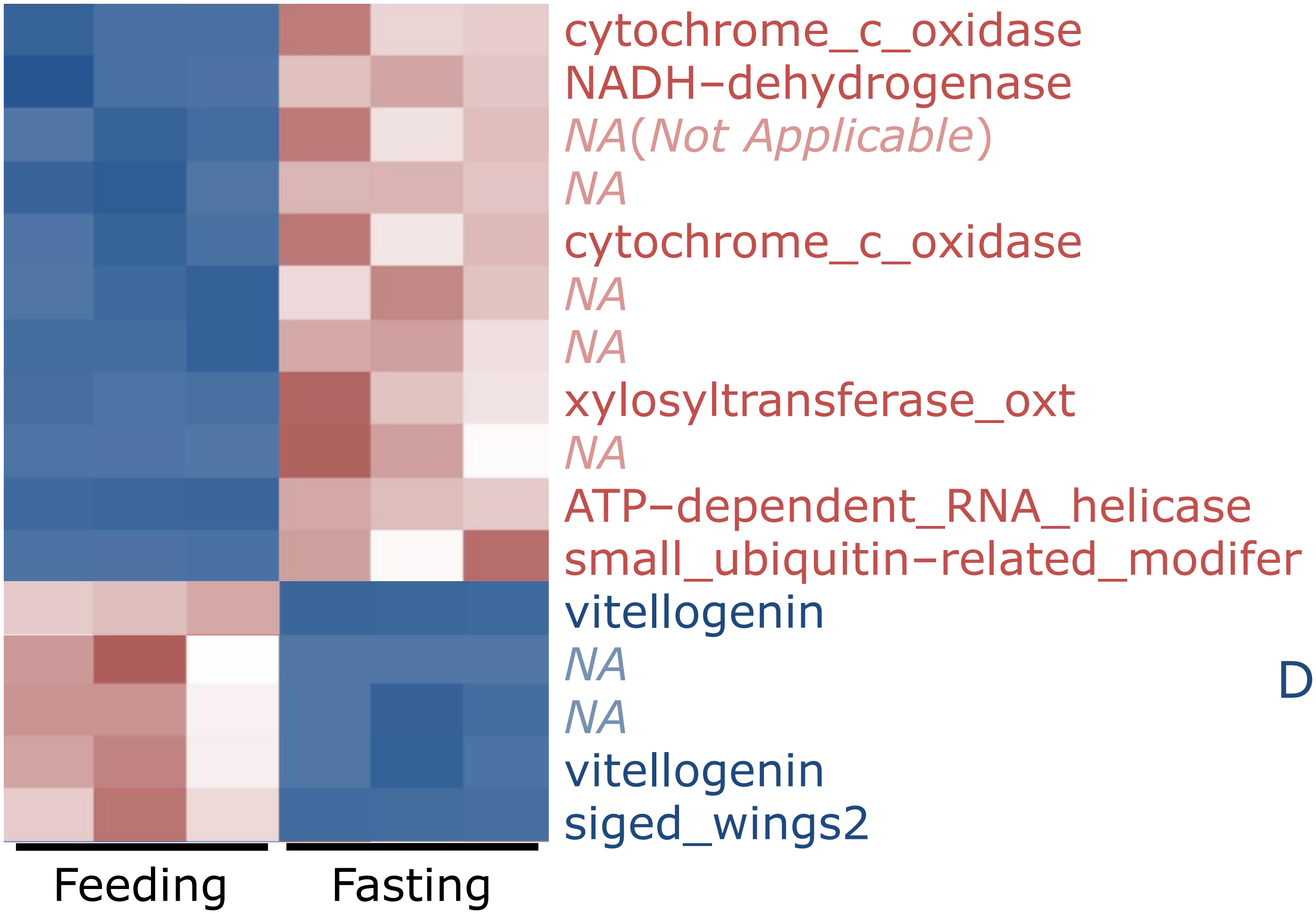
Steps includes:

- mRNA extraction
- cDNA synthesis
- next generation sequencing
- *de novo* assembly
- gene expression analysis

Results & Discussions

We identified 16 candidates for gene marker.

In reconstructed 84,095 reference sequences, 16 sequences have significant difference between feeding and fasting.
（multiple test corrected *p*-value<0.01, fold change≧2）



Up-regulated
11 genes
in fasting

Down-regulated
5 genes
in fasting

Results & Discussions

Some metabolic process were changed.

Expected roles:	Up-regulated genes:
Respiration	NADH–dehydrogenase Subunit of the respiratory chain cytochrome_c_oxidase Component of the respiratory chain
Glucose metabolism	xylosyltransferase_oxt Involved in biosynthesis of glycosaminoglycan
Protein synthesis	ATP–dependent_RNA_helicase Involved in alteration of RNA small_ubiquitin–related_modifier Cellular protein modification process
	Down-regulated genes:
Egg production	vitellogenin Precursor of egg-yolk proteins
Somatic growth	singed_wings_2 Indirect control of ecdysone genes

Second experiment Materials & Methods

Quantifying gene expression by real-time PCR

Following objects:

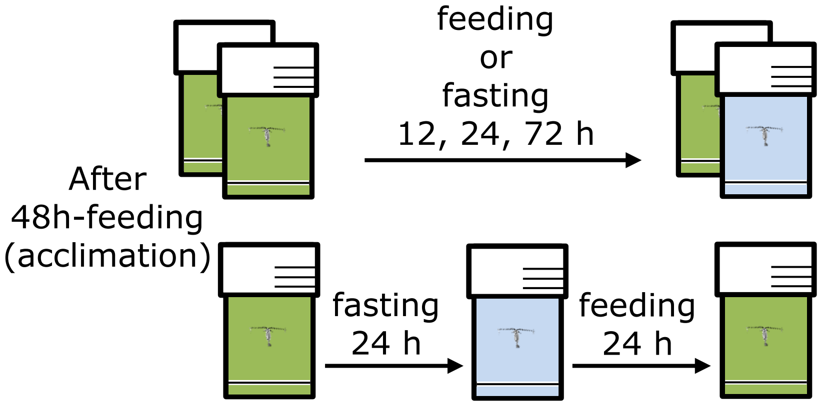
- validating results of RNA–seq
- evaluating temporal changes of each genes

■ Starvation experiment

Fasting period: 12, 24, 72 h

Temperature: 18°C

Food condition: *Tetraselmis* sp.
(~8,000 cells/ml)


After
48h-feeding
(acclimation)

feeding
or
fasting
12, 24, 72 h

fasting
24 h

feeding
24 h

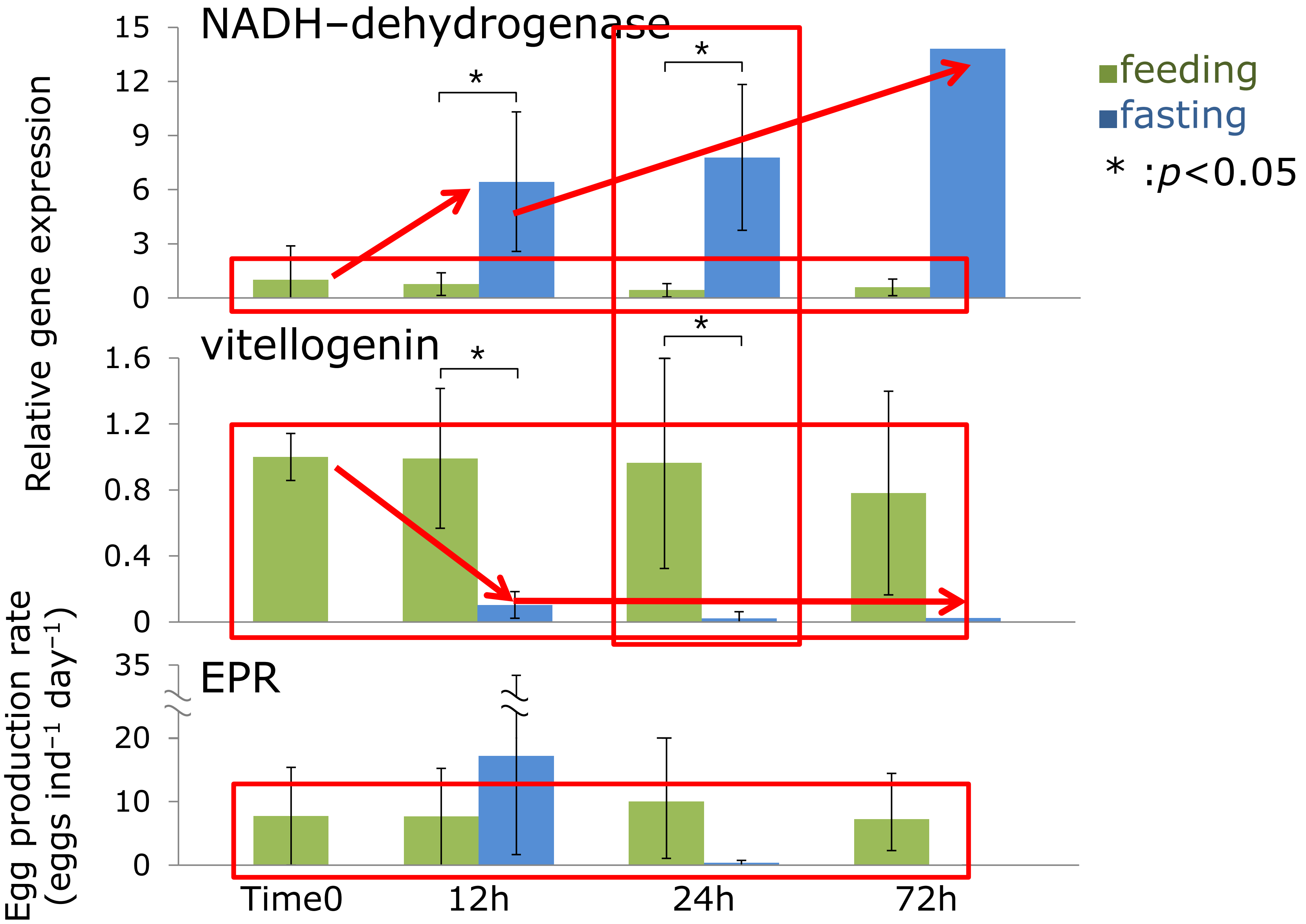
■ Quantitative real–time PCR analysis

Steps includes:

- total RNA extraction
- cDNA synthesis
- relative gene expression analysis

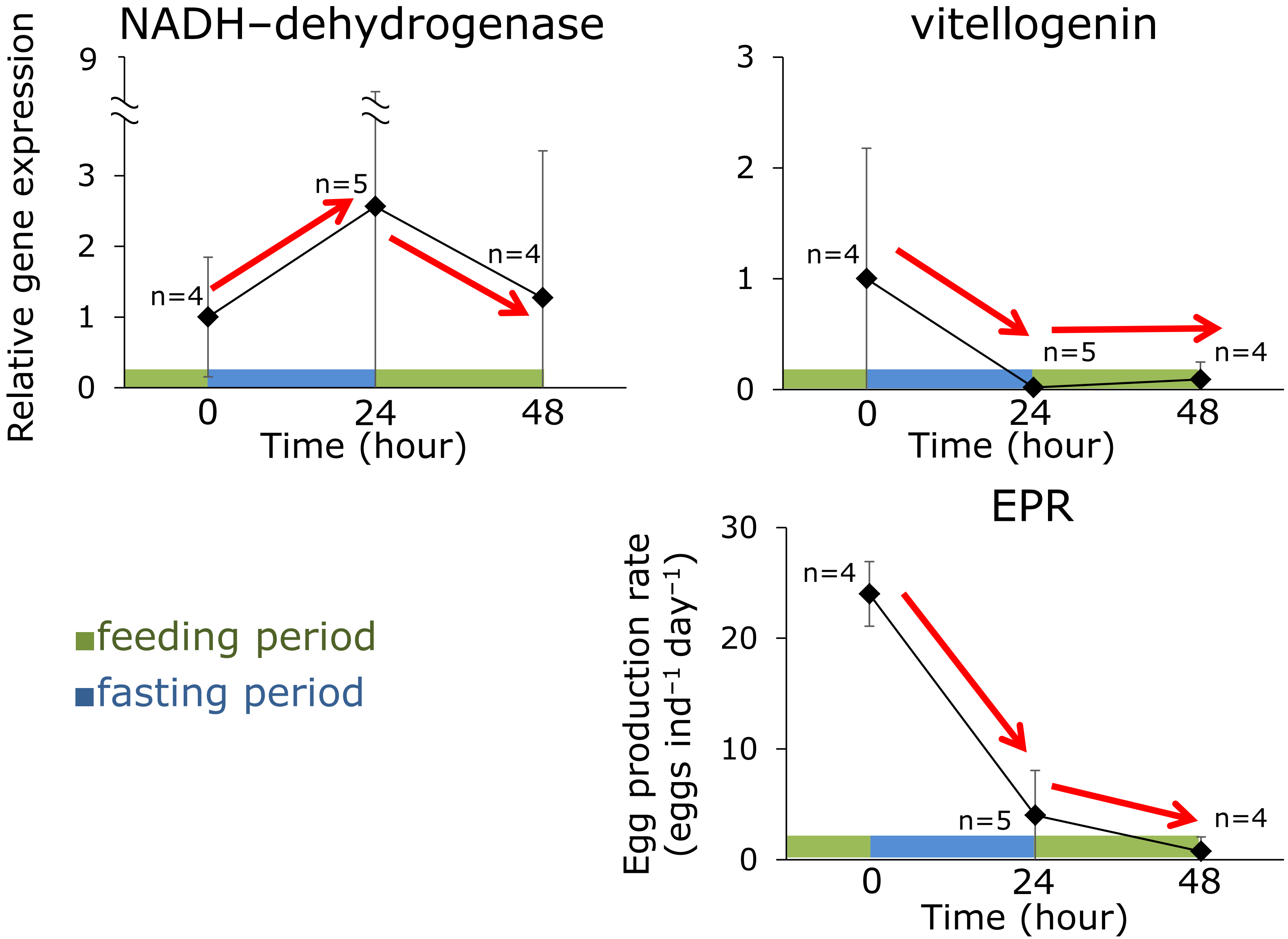
Results & Discussions

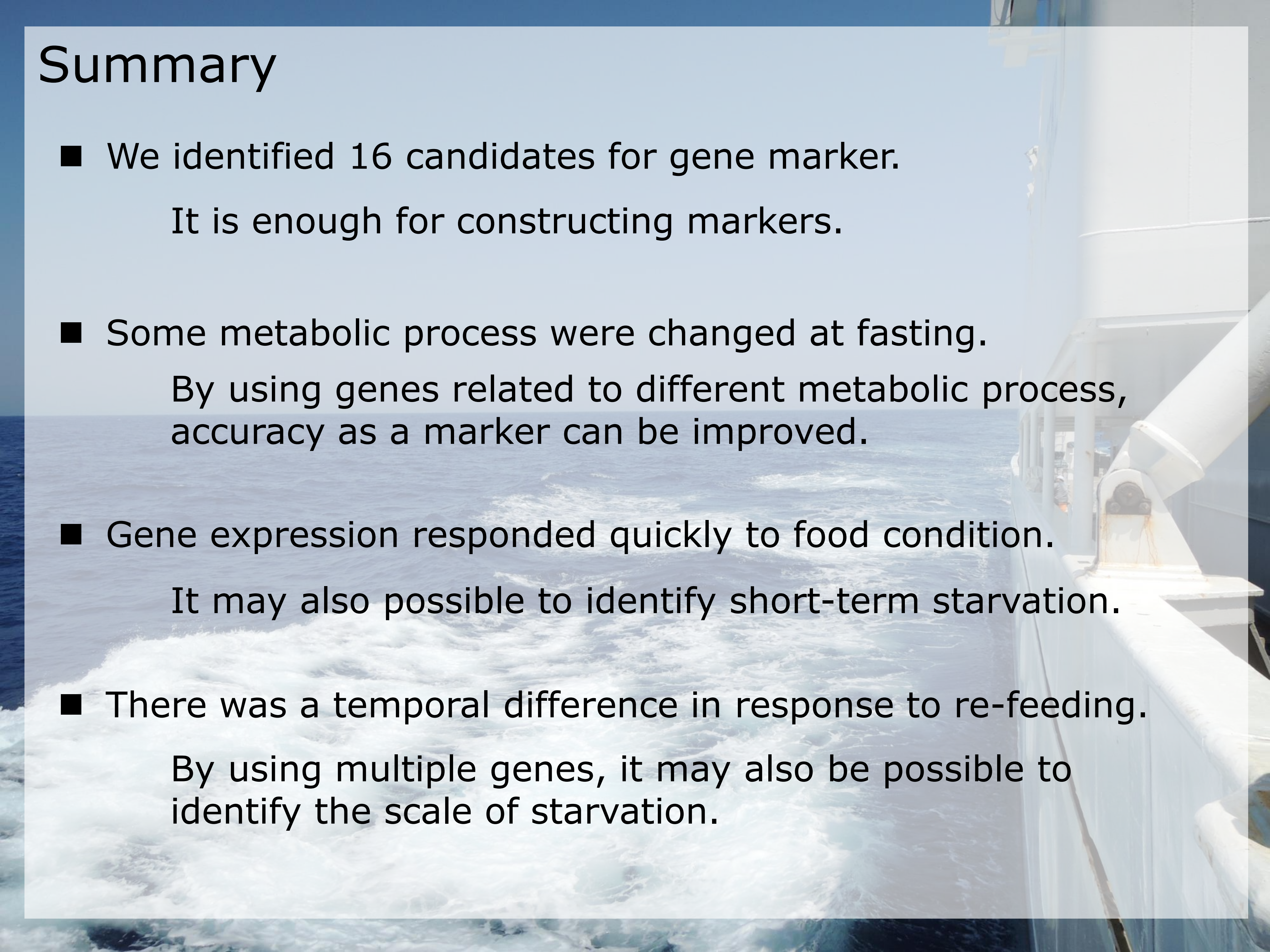
Each gene expression responded quickly.



Results & Discussions

Temporal difference in response to re-feeding





Summary

- We identified 16 candidates for gene marker.

  It is enough for constructing markers.

- Some metabolic process were changed at fasting.

  By using genes related to different metabolic process, accuracy as a marker can be improved.

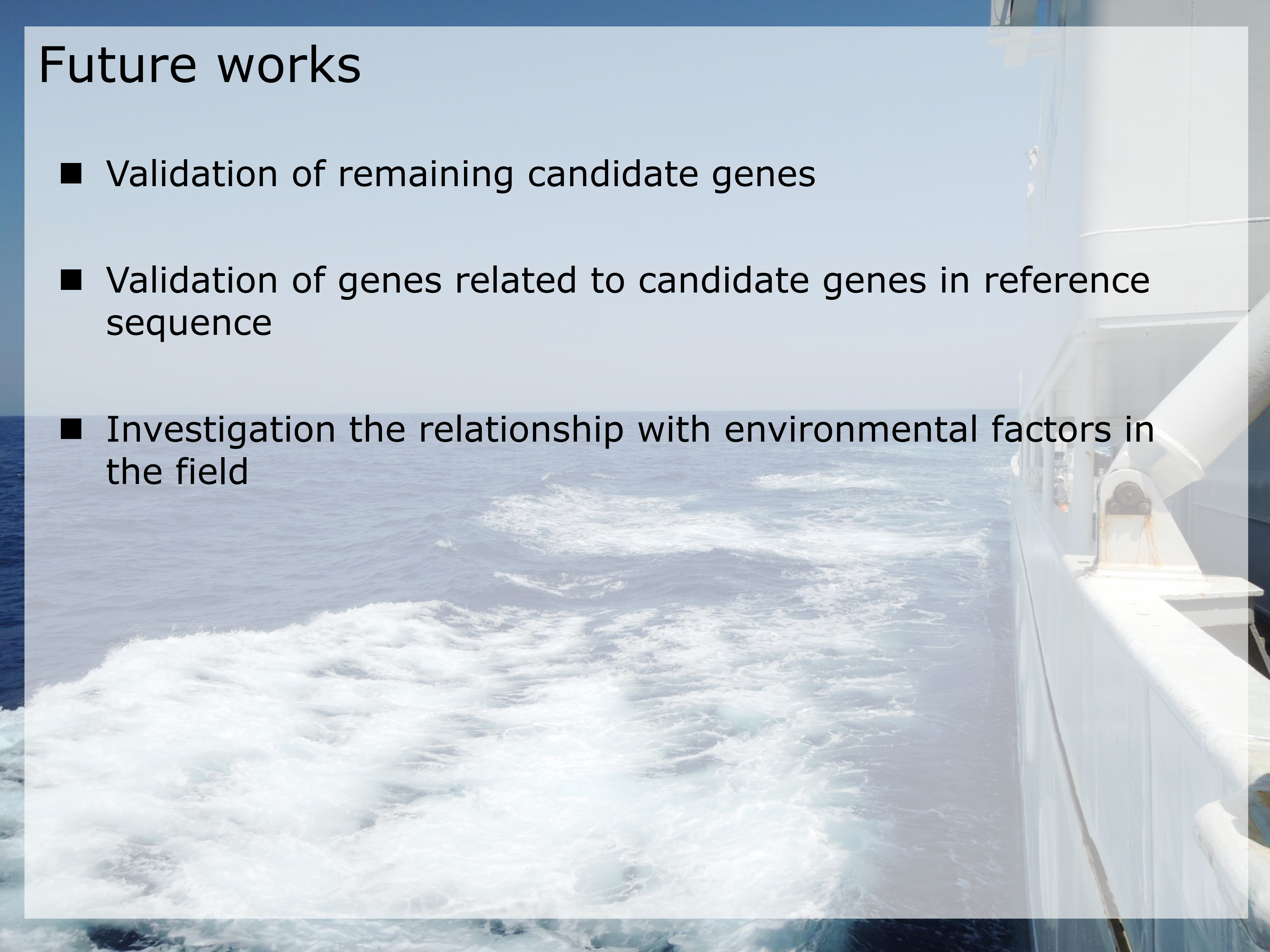
- Gene expression responded quickly to food condition.

  It may also possible to identify short-term starvation.

- There was a temporal difference in response to re-feeding.

  By using multiple genes, it may also be possible to identify the scale of starvation.

Future works



- Validation of remaining candidate genes
- Validation of genes related to candidate genes in reference sequence
- Investigation the relationship with environmental factors in the field

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