University of Connecticut

Gene expression analysis of time series collections of *Calanus finmarchicus* in the Gulf of Maine, NW Atlantic

by

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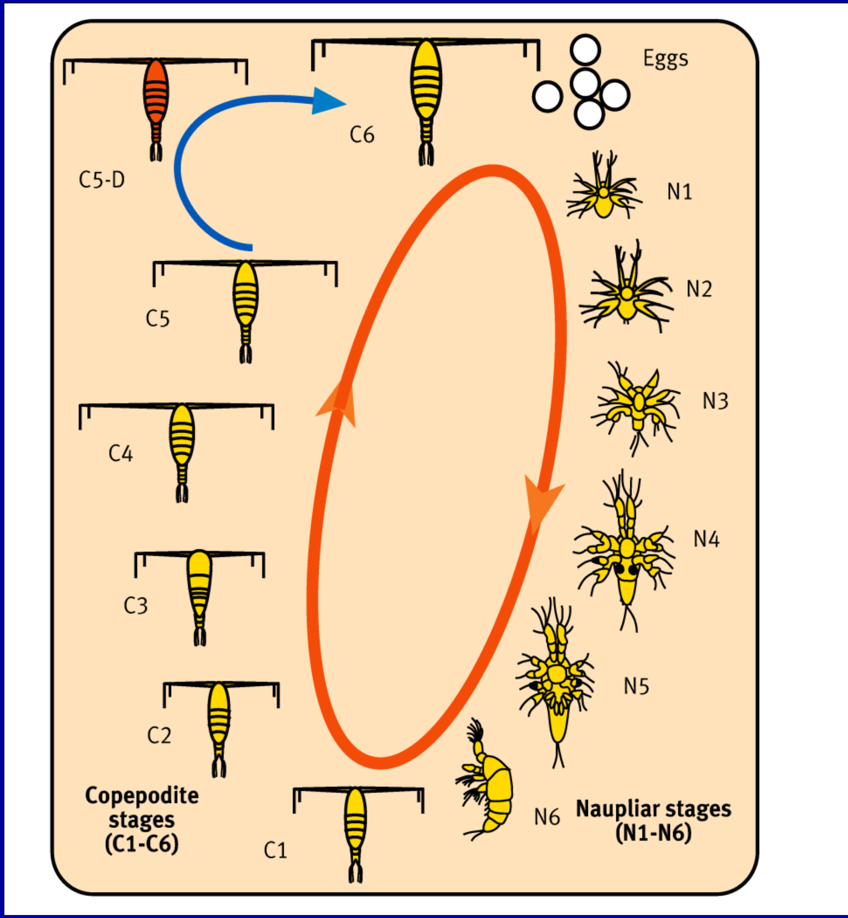
Calanus finmarchicus
Life cycle

- Seasonally dominant zooplankton in North Atlantic (50-80%).
- Pivotal sp in ocean food webs, model organism
- Diapausing C5 copepodites in Winter (lipid rich)
- Adult C6 stage in Spring, reproduction
- Non-diapausing population
- Low genetic diversity



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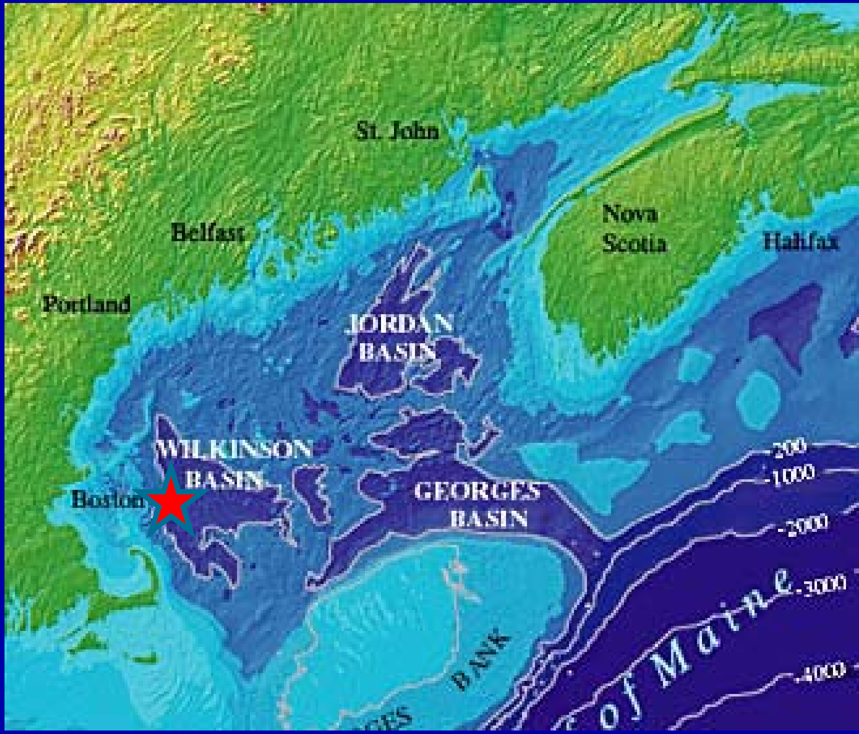
Gulf of Maine

- Semi-enclosed sea, 3 major deep basins, isolated > 200m
- Highly productive food web, commercial fishery
- In winter: Vertically well-mixed
  April-November: Stratification
- Influenced by NAO



- Sample collection:

  Surface tow & deep tow (WB7)

  Live individual copepods flash frozen in liquid nitrogen

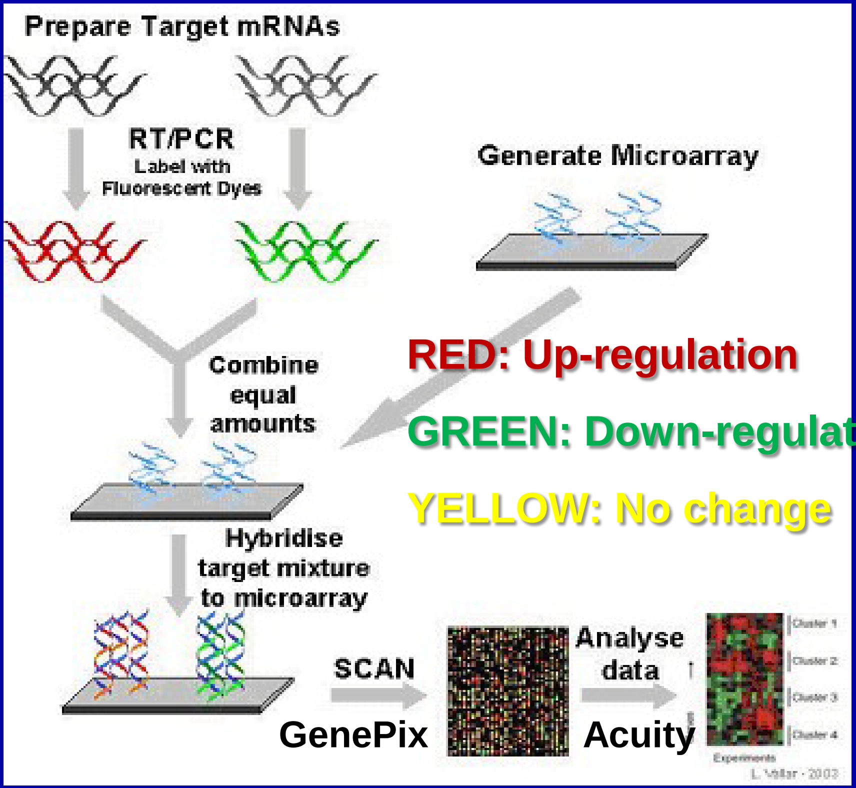
EST microarray assay

Expressed Sequence Tags (ESTs) : Small DNA sequences identified from the cDNA (complementary DNA) libraries

Microarray: A set of short probes generated from ESTs printed on a slide.

DNA microarray assay: Detects transcription at mRNA level based on the principle of base-pairing hybridization.

Thousands of genes at a time!



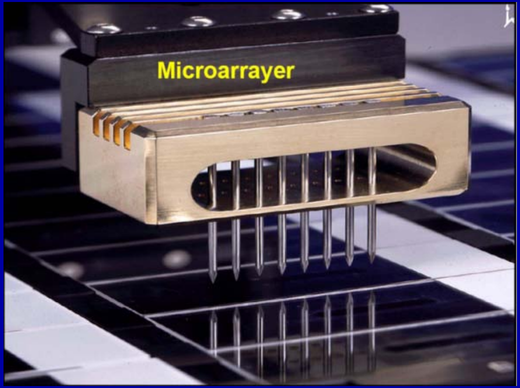
RED: Up-regulation

GREEN: Down-regulation

YELLOW: No change

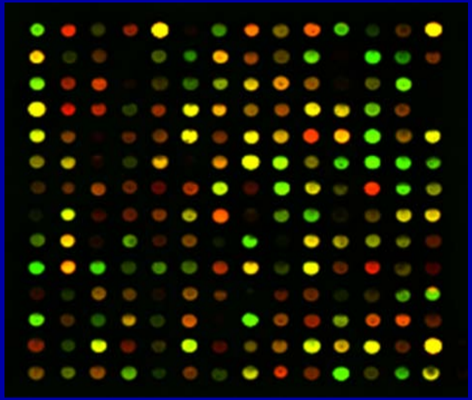
GenePix

Acuity



Experimental design

- **Array printing:** 1,000 ESTs of known function *"Calanus physiology microarray"*.
- **Array hybridization:** 15 arrays, 4 comparisons, normalized (Lowess).



Comparison	RED dye (635 nm)	GREEN dye (532 nm)	Sampling date	Replicate array #
A	Deep female	Surface female	April, 2008	4
	Surface female	Deep female	April, 2008	2 (Dye-swap)
B	Surface female	Deep CV	April, 2008	3
	Deep CV	Surface female	April, 2008	2 (Dye-swap)
C	Deep CV	Surface CV	Oct, 2006	2
D	Deep unstaged	Surface female	Oct, 2005	2

Mount Desert Island Biological Laboratory (MDIBL), Maine, USA

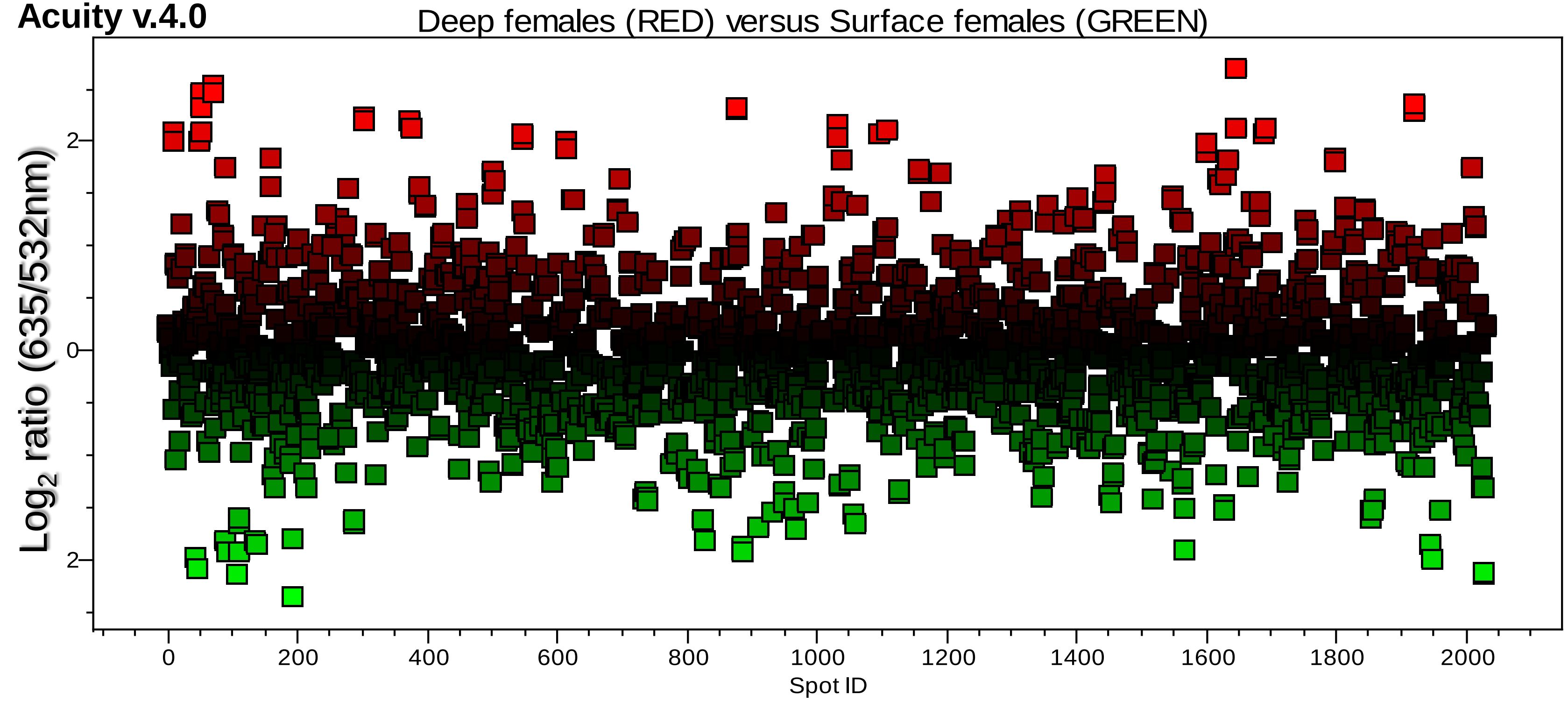
Results

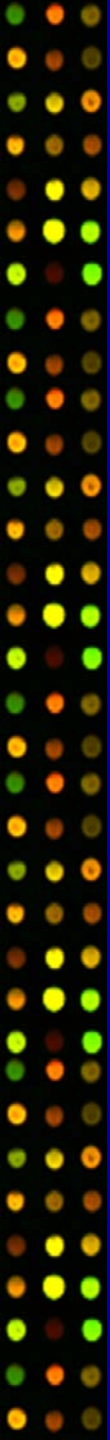
Fold-change analysis of relative fluorescence

Median log ratio fluorescence (Log_2): Index of fold change in activity

Negative: A decrease in activity (Down-regulated)

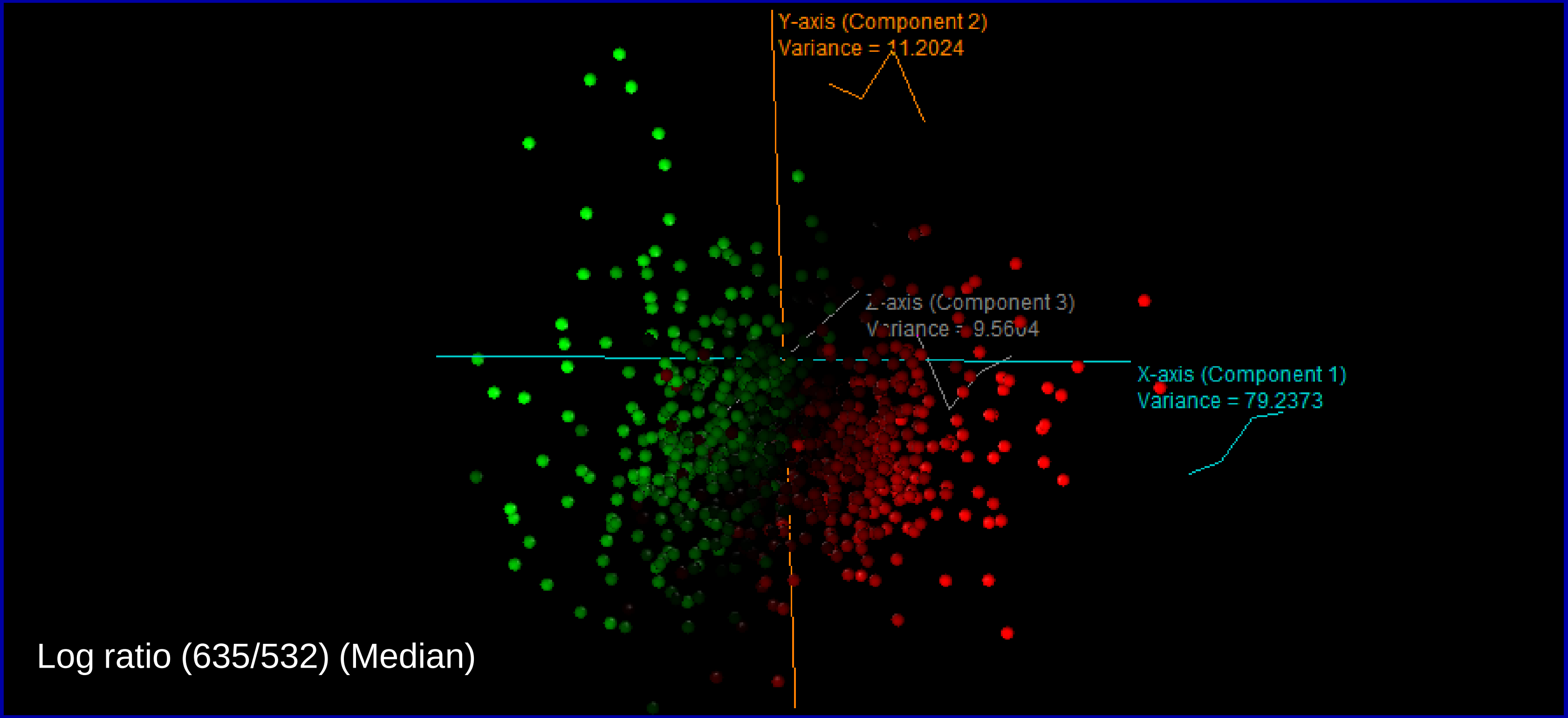
Positive: An increase in activity (Up-regulated)



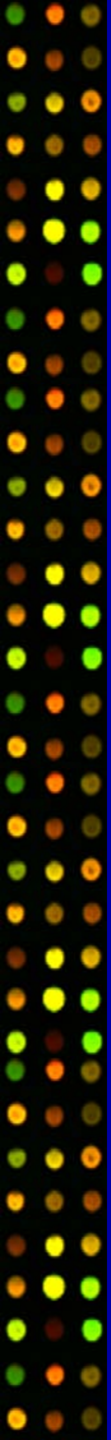


Gene Discovery

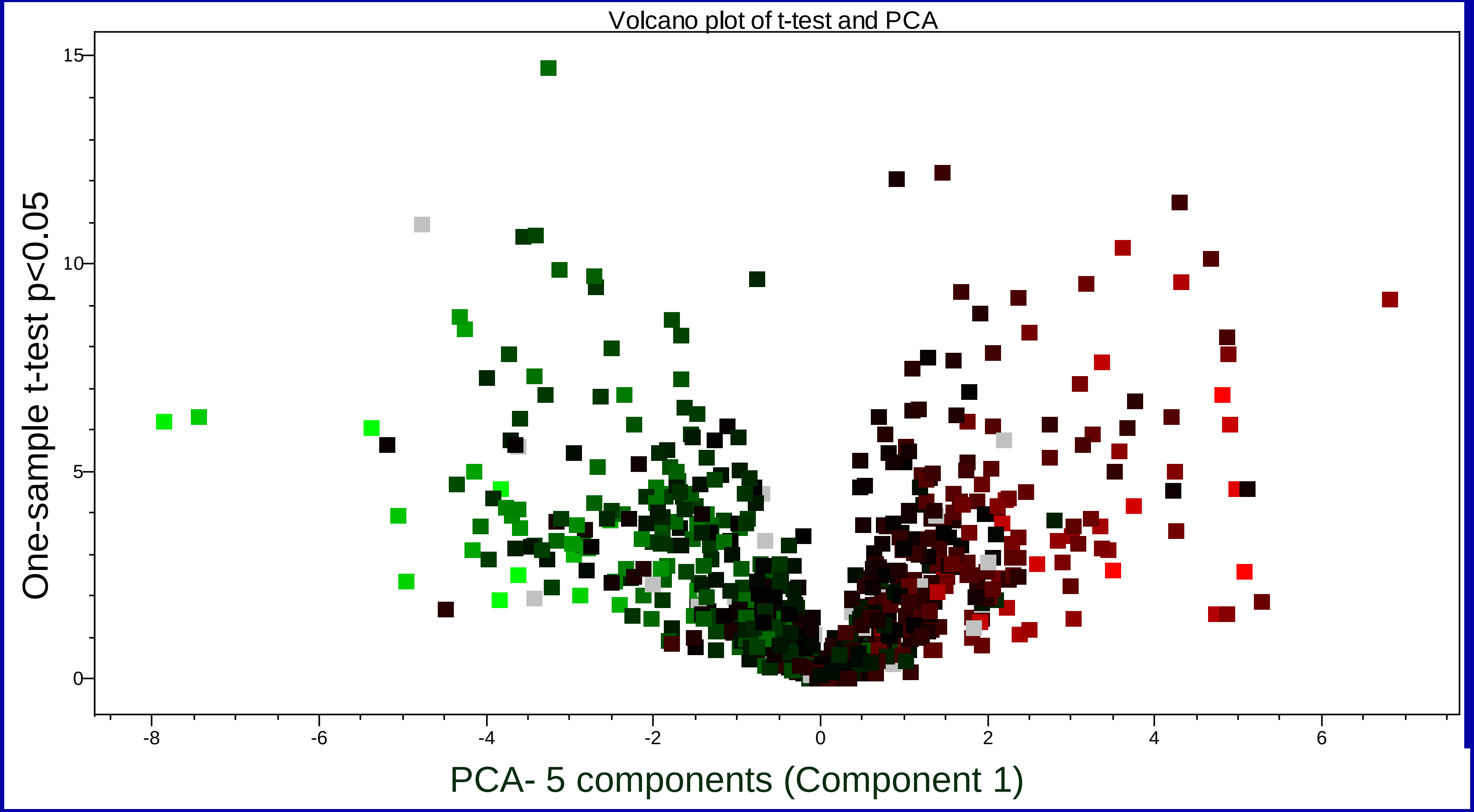
Principle Component Analysis (PCA)



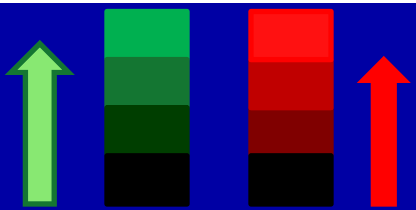
- Low-dimensional data analysis. Genes are plotted in a space defined by the components that are derived from the data.
- Each axis (i.e. Principle Component) represents an expression profile that explains variance as Median Log ratio (635/532).



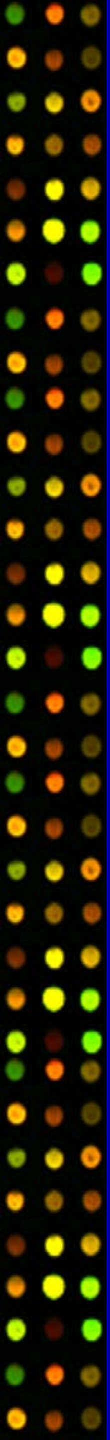
Volcano plot of t-test and PCA



Down-regulation

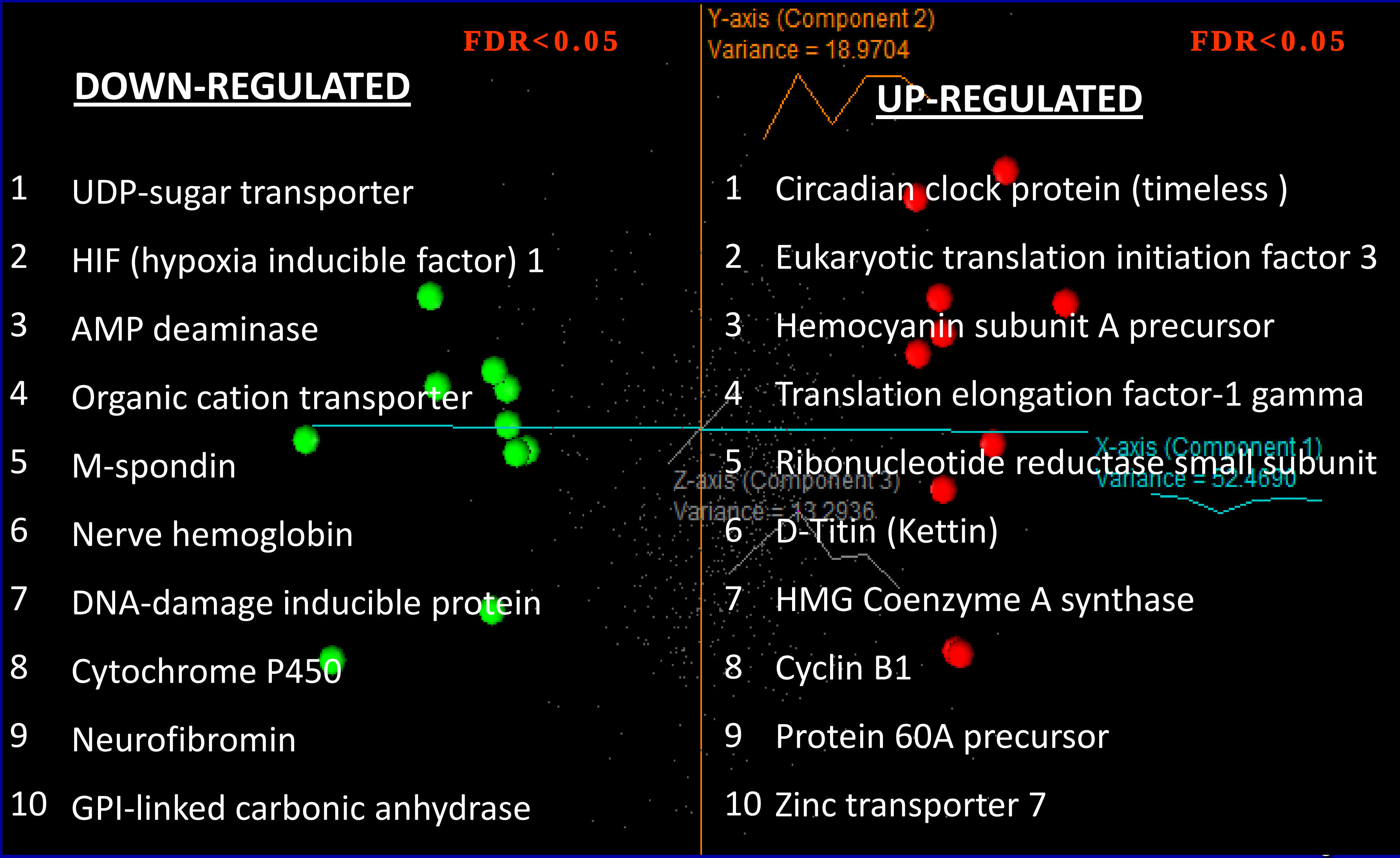


Up-regulation

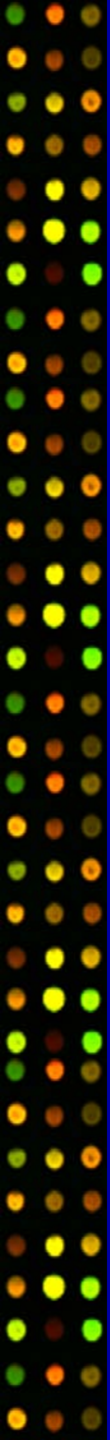


Differentially expressed genes

Comparison A: Deep female - Surface female

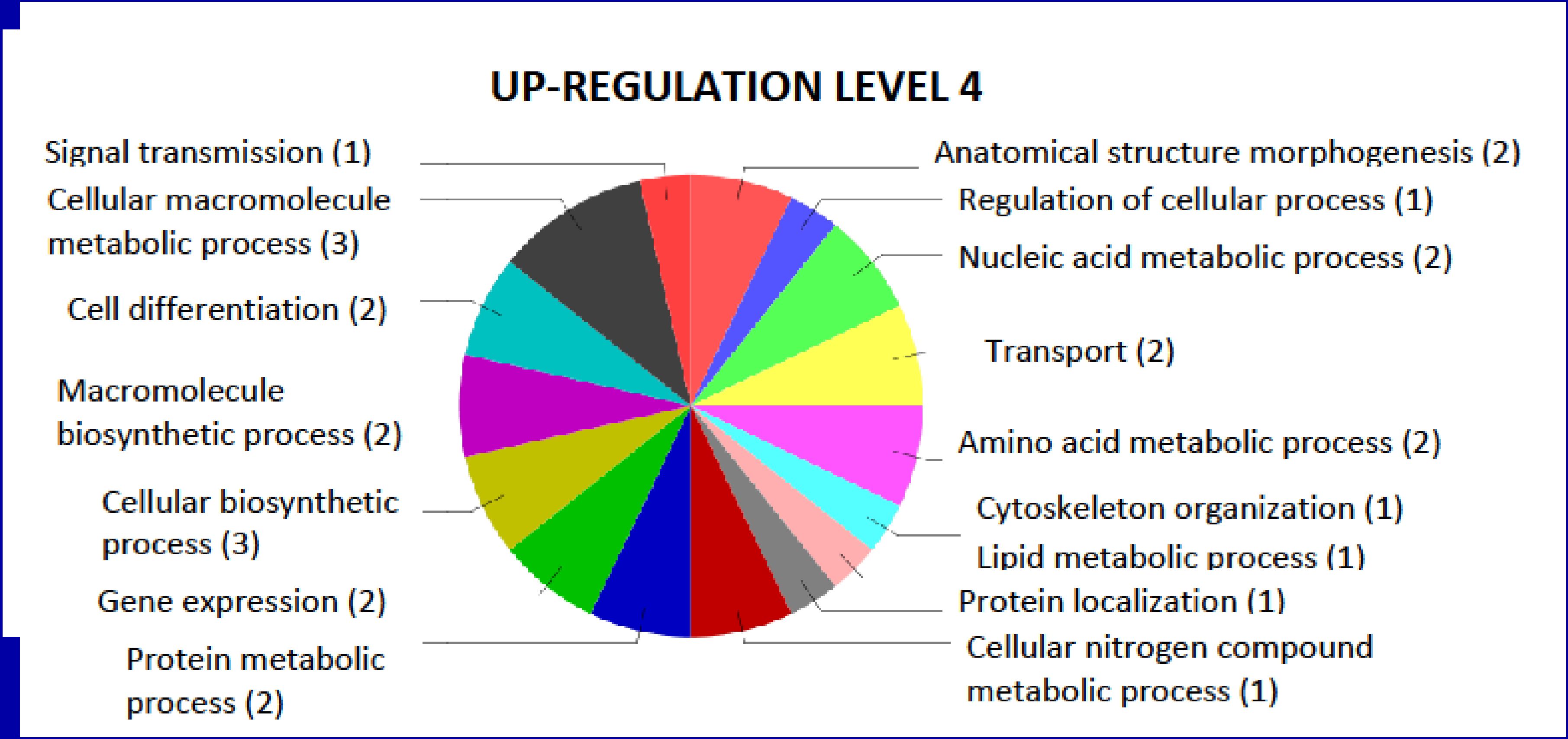


	FDR<0.05		**FDR<0.05**
	DOWN-REGULATED		**UP-REGULATED**
1	UDP-sugar transporter	1	Circadian clock protein (timeless)
2	HIF (hypoxia inducible factor) 1	2	Eukaryotic translation initiation factor 3
3	AMP deaminase	3	Hemocyanin subunit A precursor
4	Organic cation transporter	4	Translation elongation factor-1 gamma
5	M-spondin	5	Ribonucleotide reductase small subunit
6	Nerve hemoglobin	6	D-Titin (Kettin)
7	DNA-damage inducible protein	7	HMG Coenzyme A synthase
8	Cytochrome P450	8	Cyclin B1
9	Neurofibromin	9	Protein 60A precursor
10	GPI-linked carbonic anhydrase	10	Zinc transporter 7



Gene Ontology (Blast2GO)

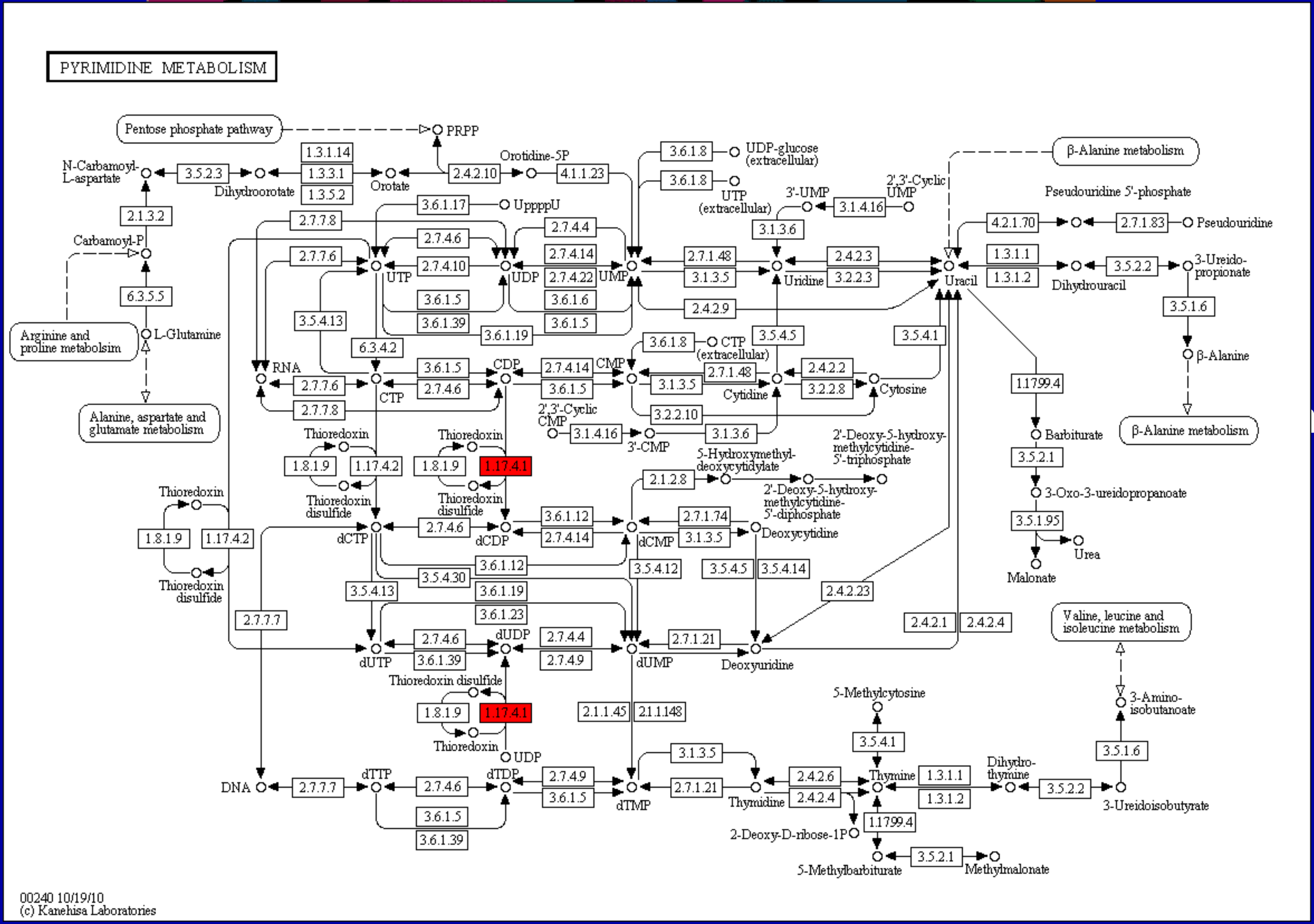
Biological Process



Comparison A: Deep female - Surface female

KEGG

Kyoto Encyclopedia of Genes and Genomes



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Target Gene Approach

- Genes identified in physiological studies of *Calanus finmarchicus** :

  Lipid catabolism (1, 2), krebs cycle (3), electron transport chain (4), anaerobic metabolism (5), amino acid catabolism (6).

1. Hydroxyacyl-Coenzyme A dehydrogenase

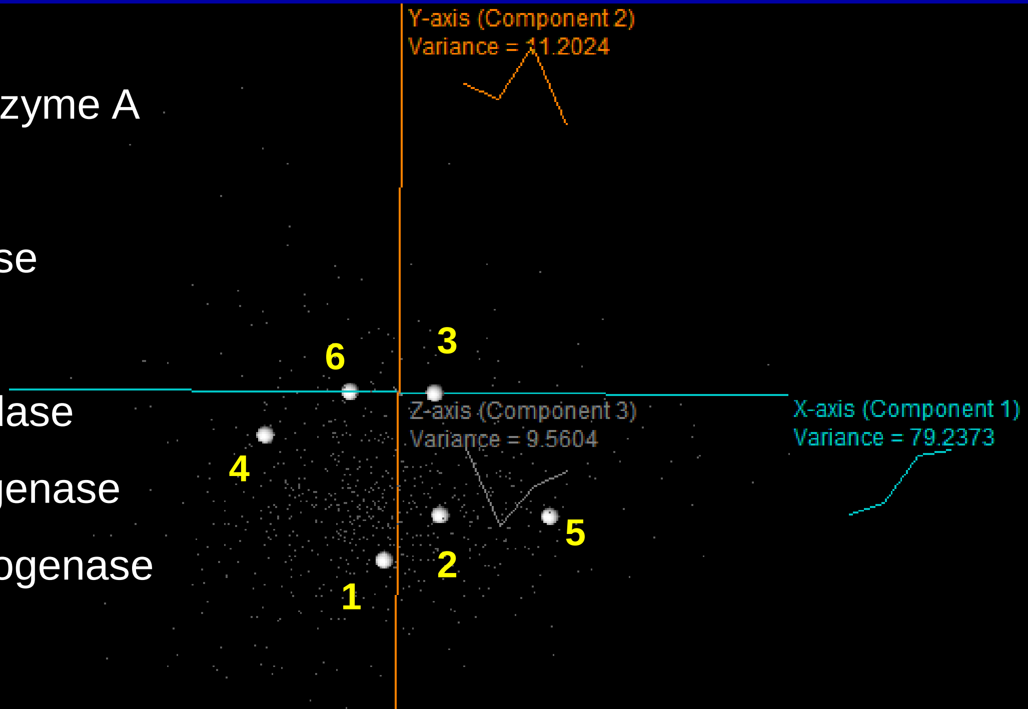
2. Palmitoyltransferase

3. Citrate synthase

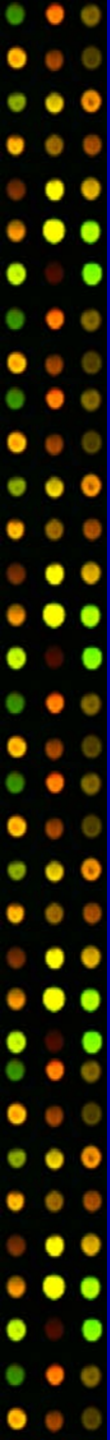
4. Cytochrome c oxidase

5. L-lactate dehydrogenase

6. Glutamate dehydrogenase



* Hassett, R.P. 2006. . Limnol. Oceanogr. 51: 997-1003.

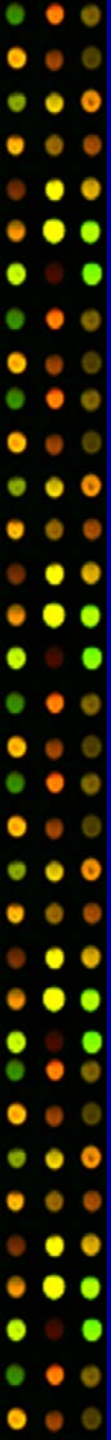


Target Gene Approach

An example

Function	Gene name	Comp A Mean Log	Comp B Mean Log	t-test p-value
Lipid catabolism	Hydroxyacyl-CoA dehydrogenase	0.396	-0.902	0.0210
	Catalase	0.972	-0.206	0.020
Krebs cycle	Citrate synthase	0.090	-0.581	n.s.
Electron transport chain	Cytochrome-c oxidase I	-0.369	1.378	n.s.
Anaerobic metabolism	Lactate dehydrogenase	-0.628	-0.273	0.0003
Molting / development	Ecdysteroid receptor	0.566	-0.784	0.019
Egg production	Vitellogenin receptor	0.769	-0.674	0.048
Digestive enzymes	Exo-1,3-beta-glucanase	-0.962	1.408	0.0051
	Beta galactosidase	-0.639	0.518	0.031
	Trypsin	0.539	-0.111	0.009

Comparison A: Deep female - Surface female
Comparison B: Surface female – Deep cV



Functional analysis

C. finmarchicus life history

Up-regulation in deep females:

DNA replication, developmental processes

Molting from CV to female?

Embryonic development

Egg maturation?

Up-regulation in surface females:

Carbohydrate transport

Feeding status?

Stress response

Environmental pressure?

Overall conclusions

- Genome-wide patterns of gene expression using DNA microarrays provide powerful means of identifying high responder genes among thousands of candidate genes.
- DNA microarray technology accelerates the search for indicators of the key biochemical and physiological processes responsive to environmental change.
- Microarrays can also test hypothesis of differential expression of target genes chosen based on their physiological function.
- Microarray analysis of time series collections of *Calanus finmarchicus* in the Gulf of Maine is identifying genes that may control critical physiological life history processes.

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