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UNIVERSITY
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Normalization of real-time RT PCR data using an external RNA control

Stian Ellefsen

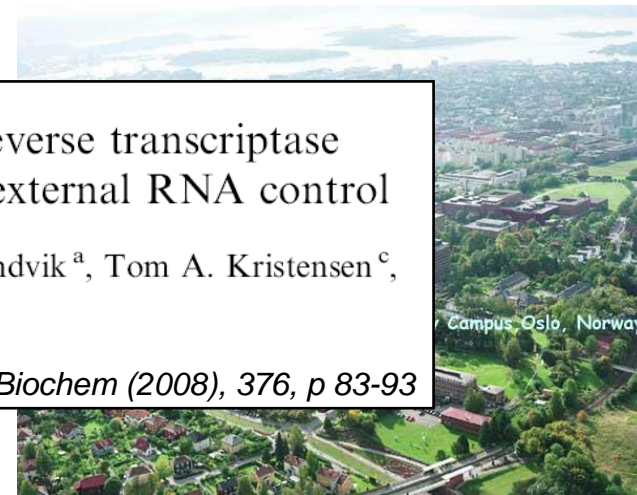
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Improved normalization of real-time reverse transcriptase
polymerase chain reaction data using an external RNA control

Stian Ellefsen^{a,*}, Kåre-Olav Stensløykken^b, Guro K. Sandvik^a, Tom A. Kristensen^c,
Göran E. Nilsson^a

Anal Biochem (2008), 376, p 83-93



Introduction



Crucian carp
(*Carassius carassius*)



A vertebrate with an extraordinary advantage:

it produces ethanol!

This enables survival without oxygen (anoxia)

Introduction



Introduction



What has anoxia tolerance to do with normalization of real-time RT PCR data?

a balanced ATP-budget is a prerequisite for anoxic survival

1)
ATP production ~~=~~ ATP consumption

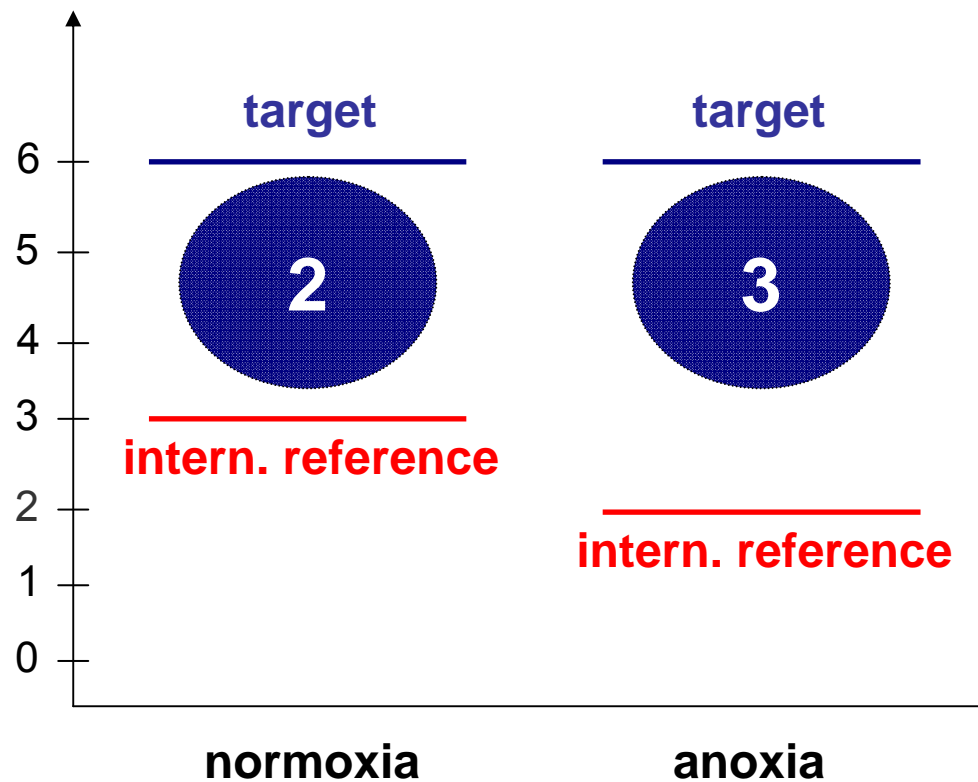
2)
ATP production ~~=~~ ATP consumption

3)
ATP production ~~=~~ ATP consumption

Introduction



Anoxia is an extreme physiological challenge, and "everything" must be expected to change

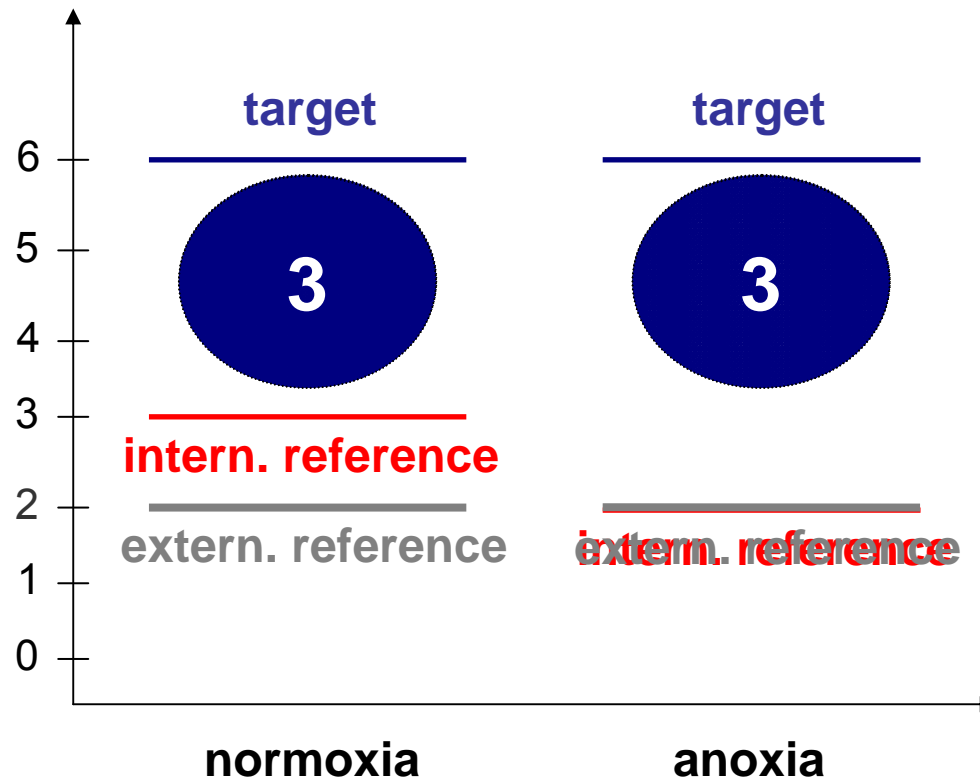


Introduction



Aim of project:

to develop a procedure for accurate assessment of gene expression in anoxic crucian carp





This is the first study to introduce an external RNA control gene prior to RNA extraction on a “per unit weight of tissue” basis, and to use it for normalization of real time RT PCR data.

mw2060

mw = *Microcystis cf. wesenbergi*

2060 = number of nucleotides

External RNA control gene:
an *in vitro* synthesized mRNA strand that does not have analogs in the experimental system of interest

Standard curve

- using *one* batch of mw2060
- using *one* pool of homogenized brains

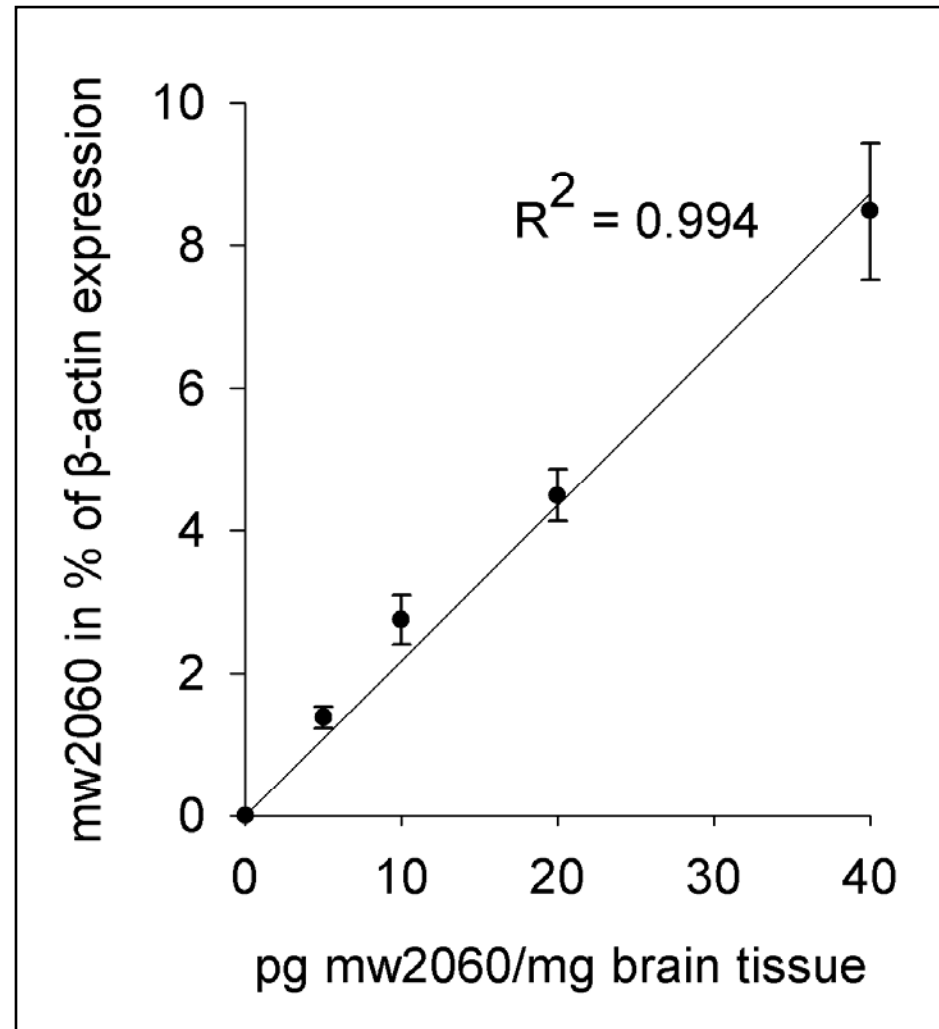


Fig. 1



Anoxia exposures

- Two anoxia experiments:
 1. 8 °C
 2. 13 °C

- Four oxygen regimes in each experiment:
 1. *Normoxia 7 days (N7)*
 2. *Anoxia 1 day (A1)*
 3. *Anoxia 7 days (A7)*
 4. *Anoxia 7 days/Normoxia 3 or 7 days (A7N3/A7N7)*

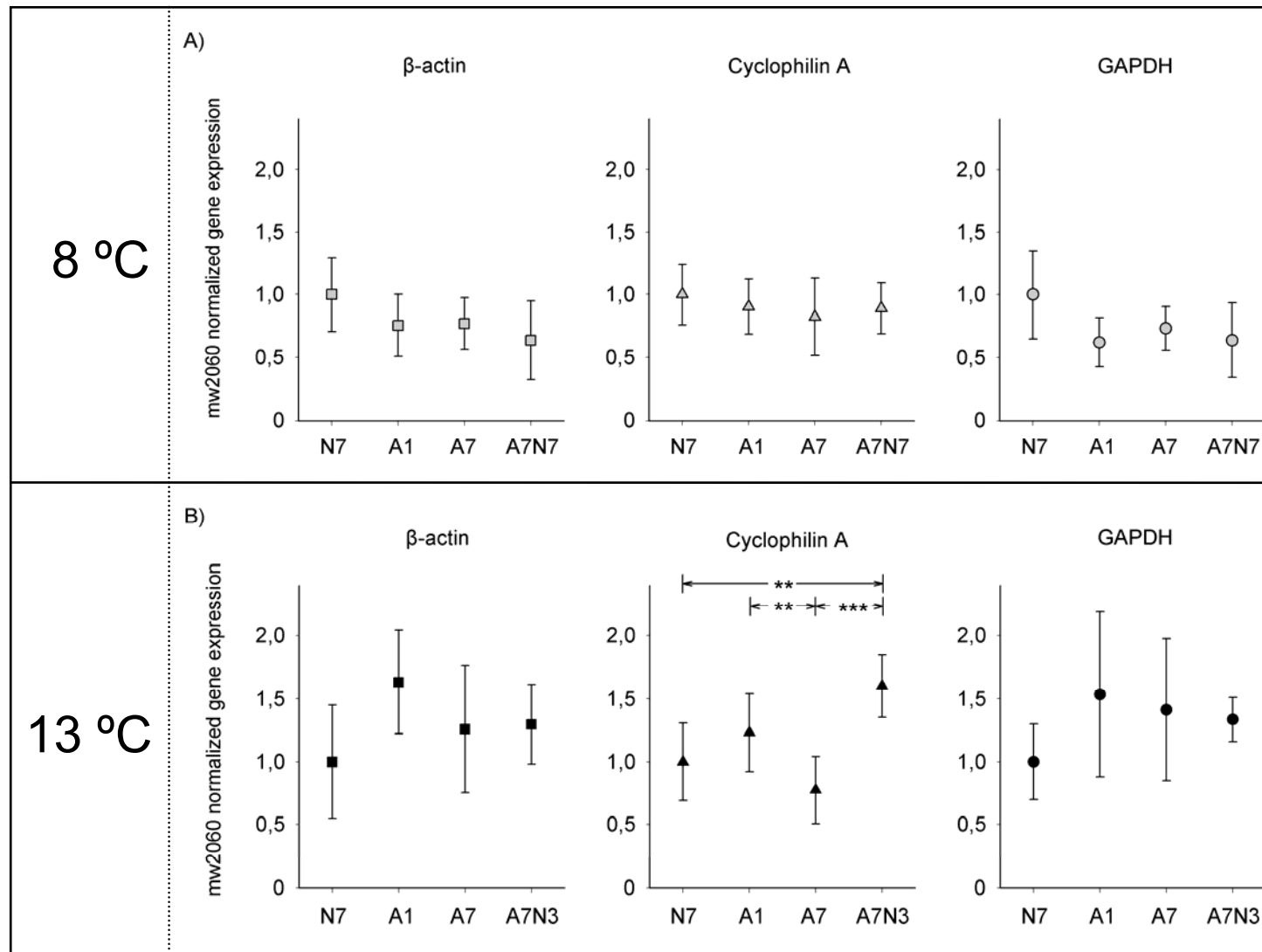
- Brain and heart were sampled for real-time RT PCR analyses

Internal RNA control genes in the crucian carp **brain**



mw2060-normalized data:

Fig. 2



Results

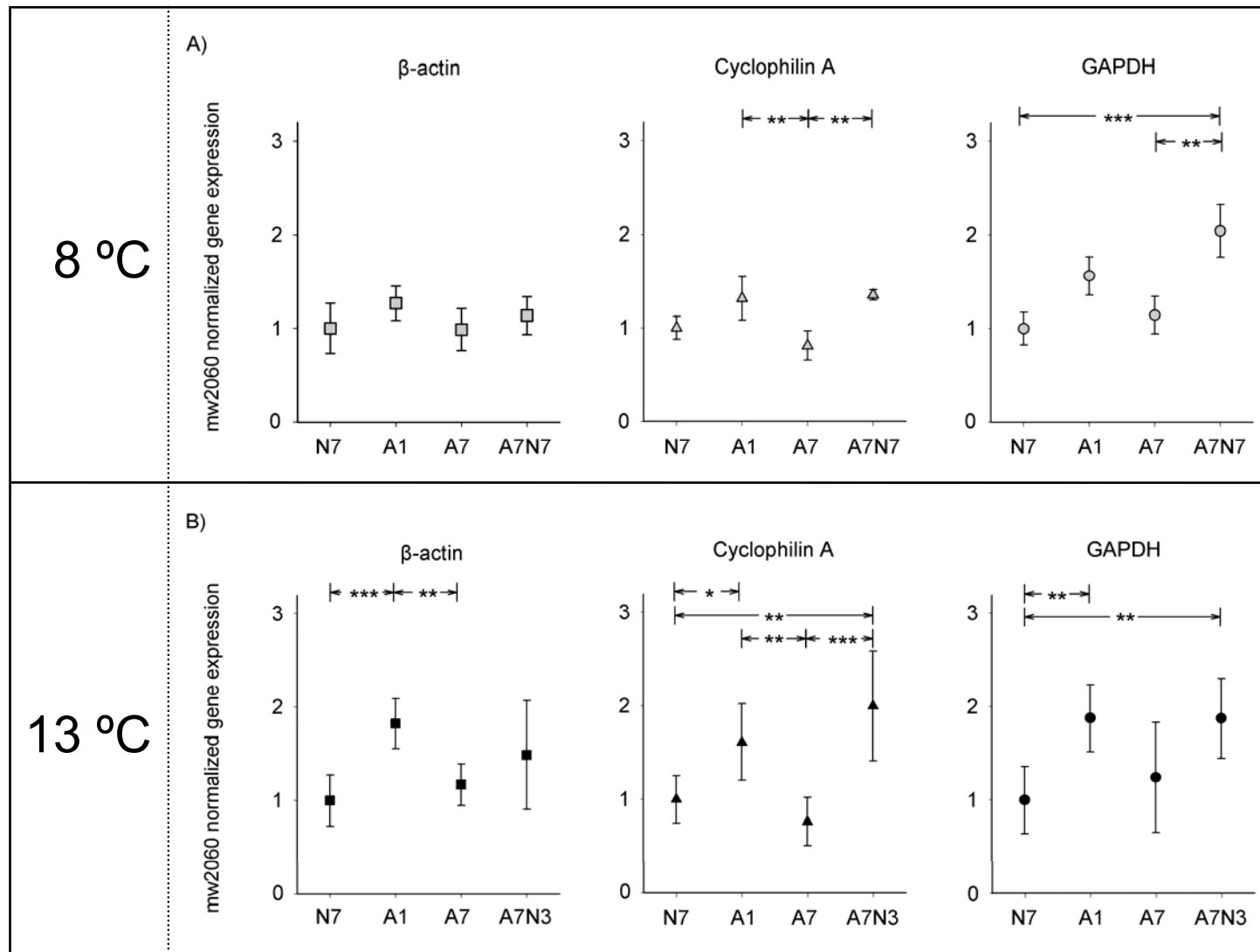
Internal RNA control genes in the crucian carp *heart*



mw2060-normalized data:

Fig. 3

NB!
N7 vs. A1



Results

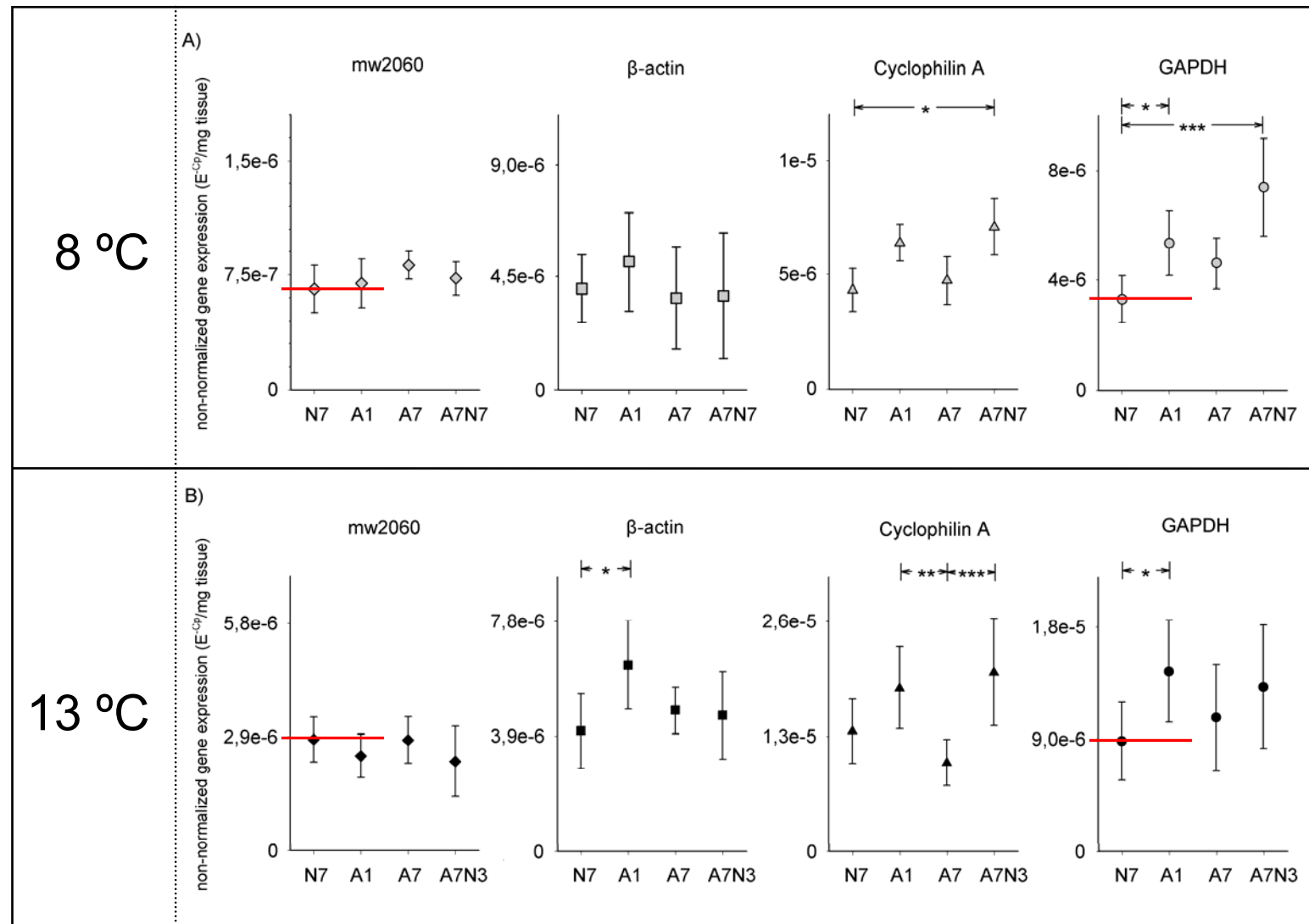
Internal RNA control genes in the crucian carp **heart**



Non-normalized data:

Fig. 4

NB!
N7 vs. A1

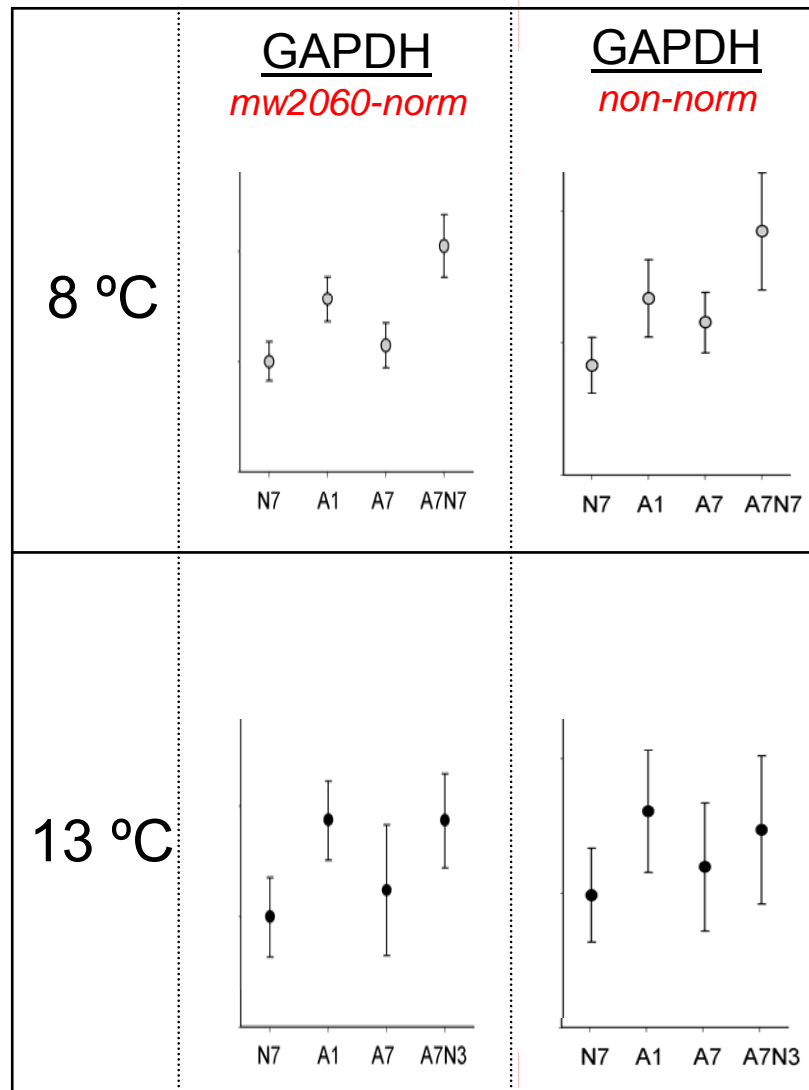


Results

Internal RNA control genes in the crucian carp **heart**



Comparing mw2060-normalized
and non-normalized data:



Results

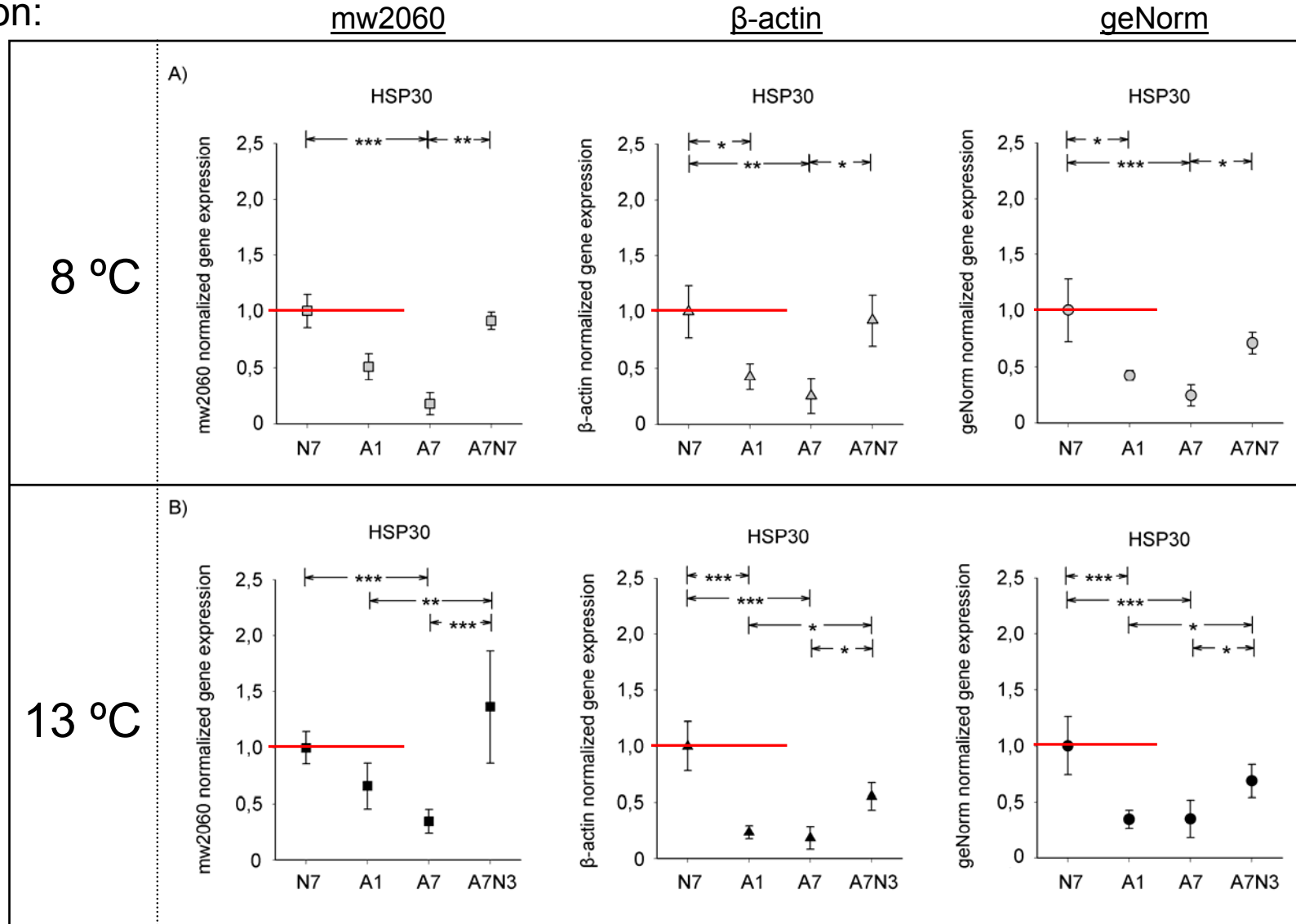
Normalization of target gene expression in crucian carp *heart*: **HSP30**



Normalization:

Fig. 5

NB!
N7 vs. A1



Results

Normalization of target gene expression in crucian carp *heart*: **HSC70**



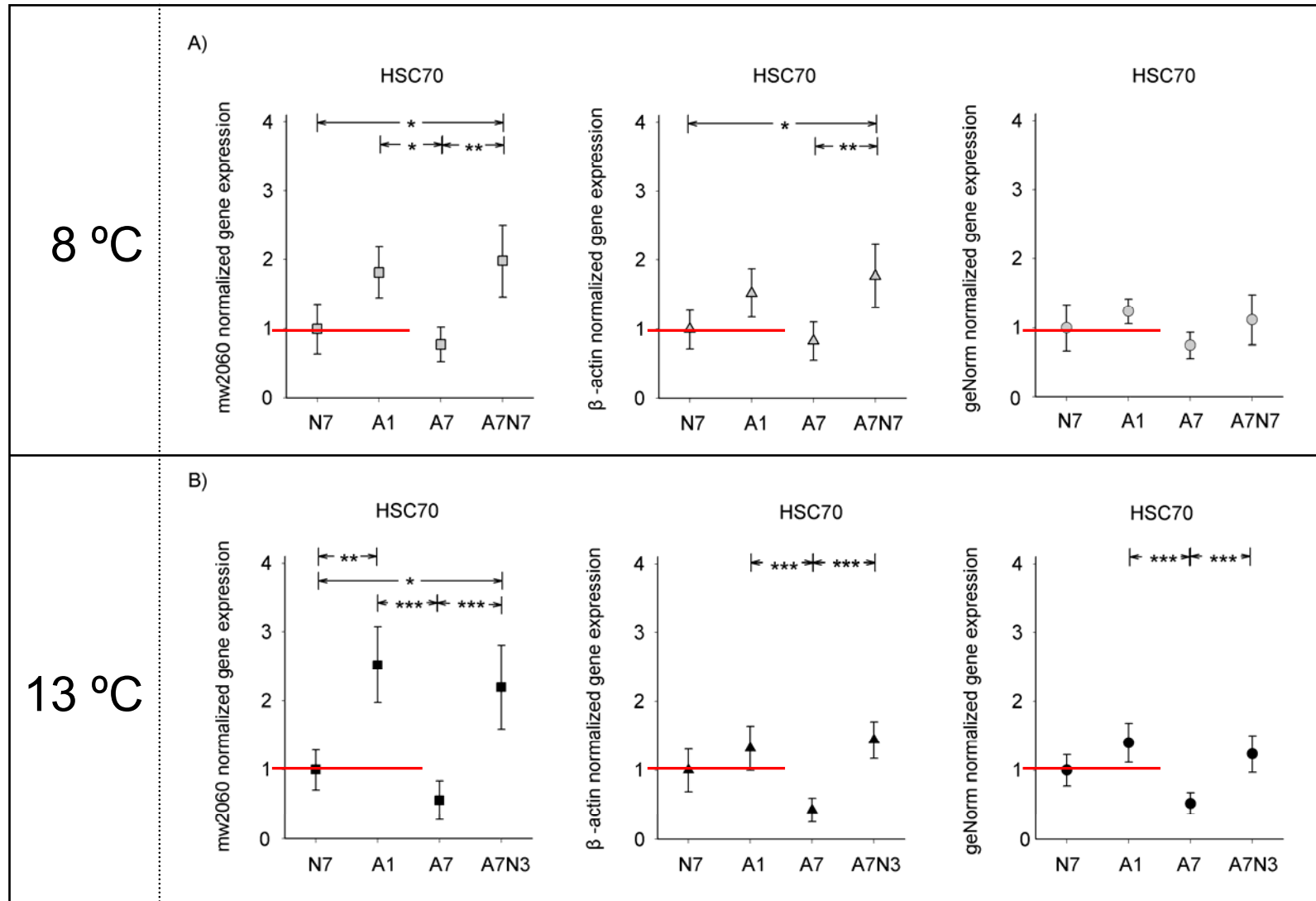
Normalization:

mw2060

β -actin

geNorm

Fig. 6



NB!
N7 vs. A1

Observed changes in
internal RNA control gene
expression had large
consequences for target gene
normalization



Pros

- standard curve
- intra-experimental reproducibility (*low* Coeff. of var.)
- inter-experimental reproducibility (*similar* expression profiles between experiments)
- non-normalized data supports normalized data
- changes in mw2060-normalized gene expression could not be explained from changes in RNA yields

Cons

- batch-to-batch variation
- complete cell lysis?

Our external RNA control gene approach seems to enable accurate normalization of real-time RT PCR data

Expression of genes involved in excitatory neurotransmission in anoxic crucian carp (*Carassius carassius*) brain

Stian Ellefsen,^{1,2} Guro K. Sandvik,¹ Helene K. Larsen,¹ Kåre-Olav Stensløkken,³ Dag Are S. Hov,¹ Tom A. Kristensen,⁴ and Göran E. Nilsson¹

Physiol Genomics 35: 5–17, 2008

Expression of genes involved in GABAergic neurotransmission in anoxic crucian carp brain (*Carassius carassius*)

Stian Ellefsen,^{1,2} Kåre-Olav Stensløkken,³ Cathrine E. Fagernes,¹ Tom A. Kristensen,⁴ and Göran E. Nilsson¹

Physiol Genomics 36: 61-68, 2009

Differential regulation of AMP-activated kinase and AKT kinase in response to oxygen availability in crucian carp (*Carassius carassius*)

Kåre-Olav Stensløkken,^{1,2} Stian Ellefsen,^{3,4} Jonathan A. W. Stecyk,³ Mai Britt Dahl,² Göran E. Nilsson,³ and Jarle Vaage¹

Am J Physiol Regul Integr Comp Physiol 295: R1803-R1814, 2008



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