The impact of global warming and snail susceptibility to schistosomiasis

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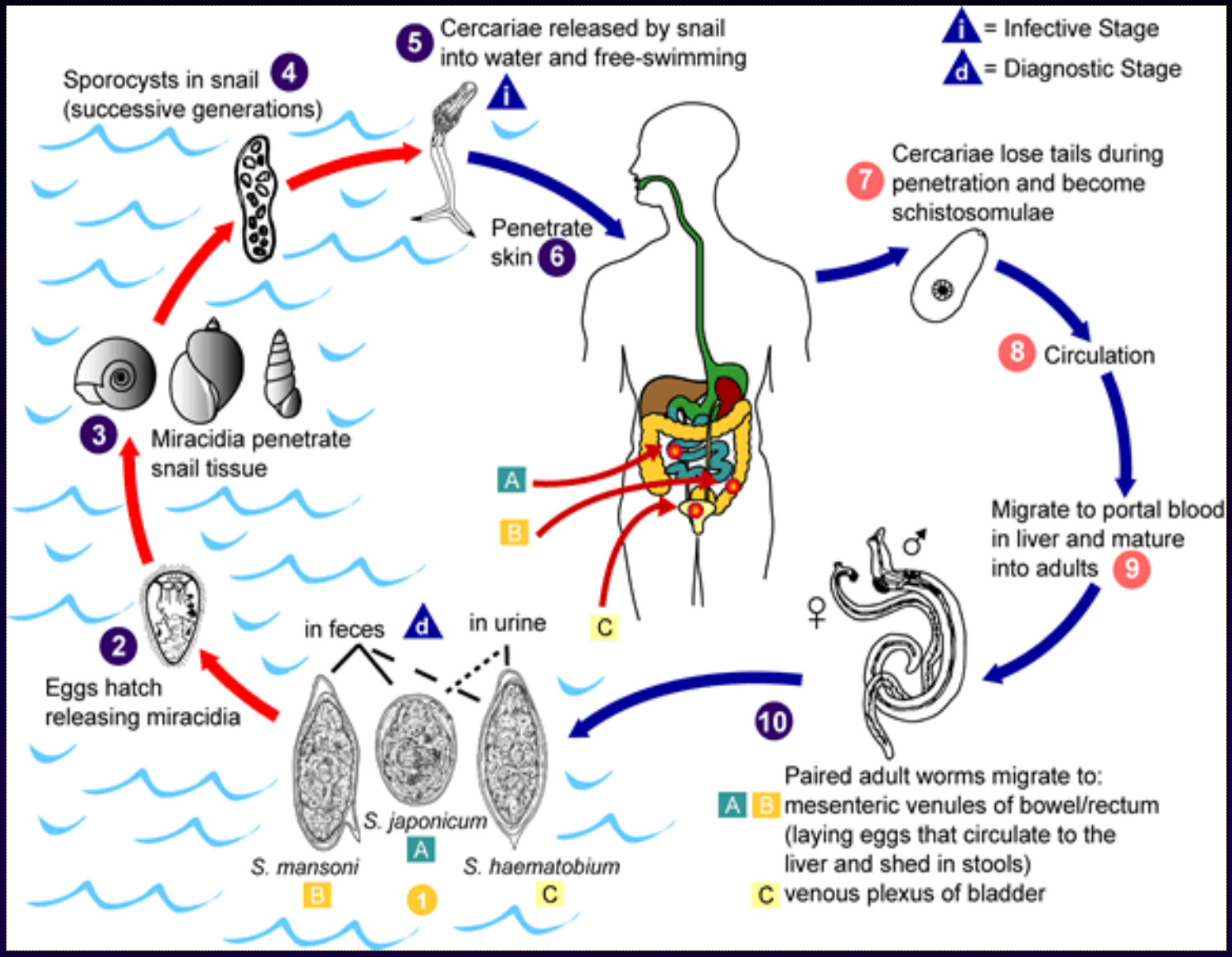
Life cycle of *Schistosoma spp*.



Who is most risk?





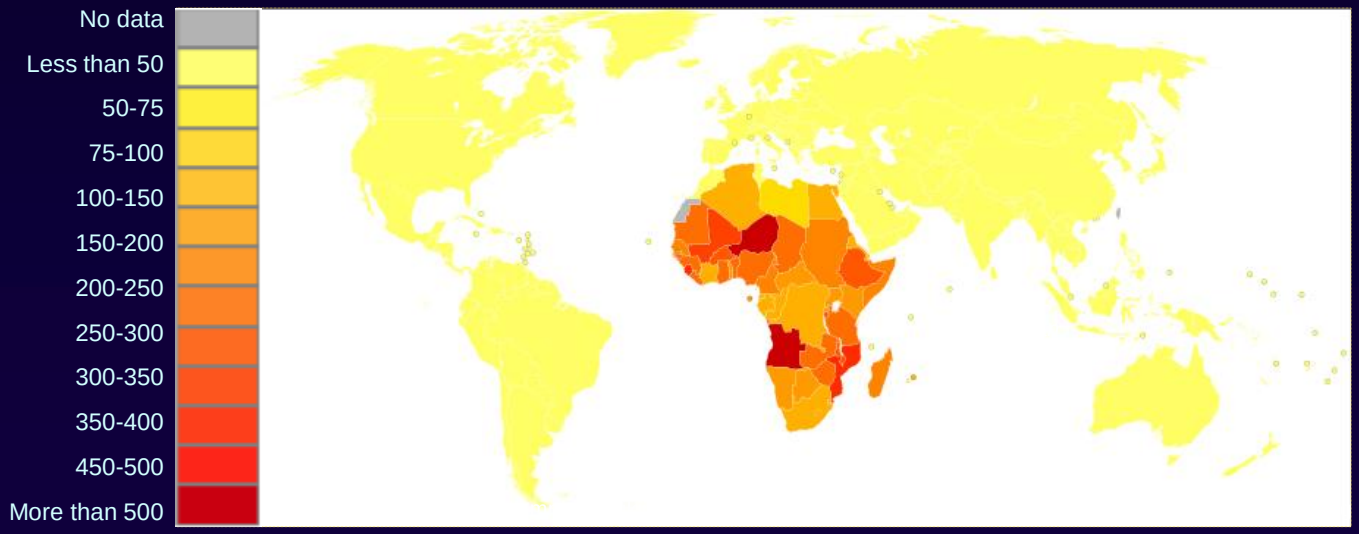

- School age children: between the age of about 6 and 15 years old, who swim and play in nearby lakes and irrigation channels.
- Women: who carry out the household work of collecting water and washing clothes and cooking utensils are also in contact with water and therefore more likely to become infected.
- Fishermen/irrigation workers: whose occupations involve contact with water should be treated as a high-risk group.
- Tourism

Global Distribution of Schistosomiasis

- Schistosomiasis is focally located around local water bodies which contain the appropriate snail vectors.

- Water-resource developments, dams and irrigation channels aggravate the transmission of schistosomiasis as they provide the perfect habitat for snails.

- Between 1950 and 1990, the number of dams worldwide increased dramatically from about 5000 to 36, 000, with a consequent rise in schistosomiasis in sub-Saharan Africa.



Age-standardized disability-adjusted life year (DALY) reate from Schistsomiasis by country (per 100,000 inhabitants).

- A genetic basis exits for the susceptibility of the snail *B.glabrata* to *S. mansoni* infection.
- Both snail and parasite genes affect the outcome of the snail/parasite encounter.
- Identifying those genes that render a snail resistant to infection may aid the future of genetic transformation of susceptible snails with those factors that will render them resistant to infection.

S. mansoni infection and outcome in the snail

Adult male & female → Eggs → Miracidium → Mother sporocyst

Susceptible snail:

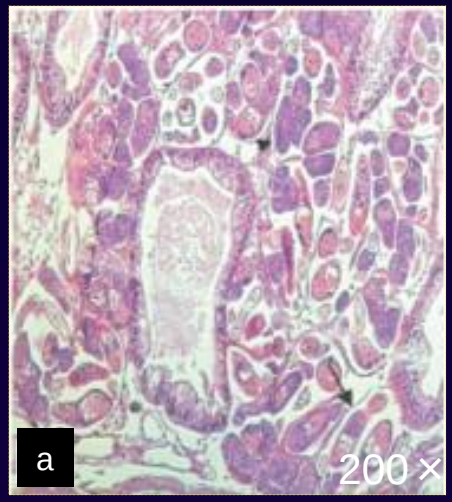
a)Numerous sporocysts (arrow) and developing cercariae (arrow) with digestive glands in the absence of tissue reaction.

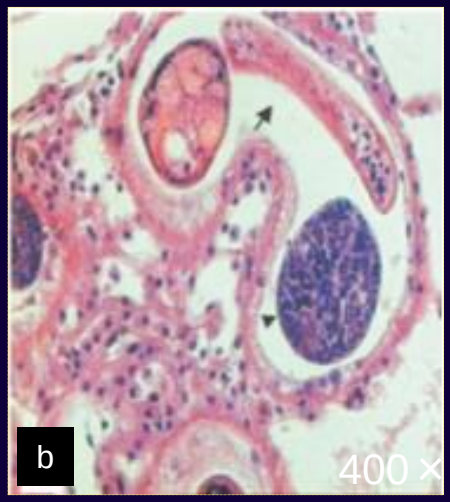
b)The parasite forms (arrow) in the snail digestive glands, with a mild hemocytic infiltration around.

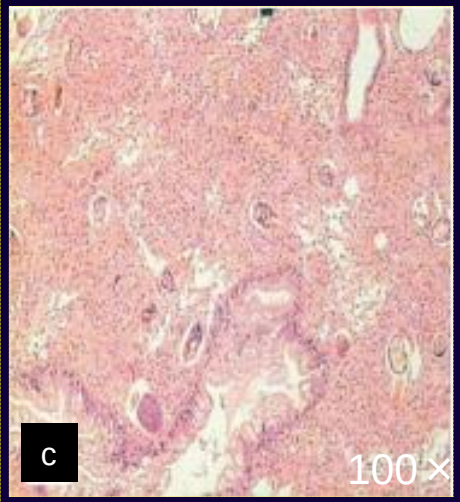
Resistant snail:

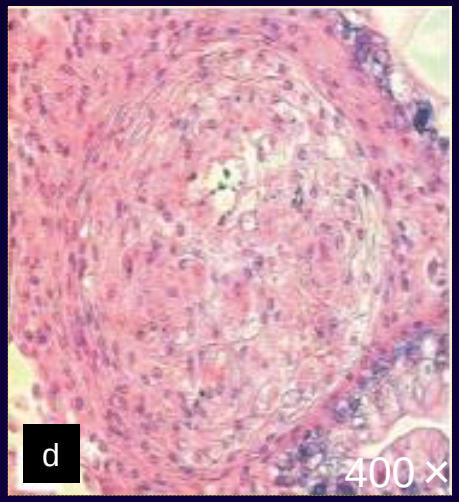
c) An extensive proliferation of hemocytes dissociating the digestive glands and encircling isolated parasite forms within 72 hrs.

d) A granuloma-like structure formed by hemocytes.









All pictures from sections stained with hematoxylin and eosin[Borges et al., 2006]

Alternative control strategies are needed!

- None of the current candidate vaccine antigens indicated that they will provide levels of protection required to help control the disease.

- Although chemotherapy and molluscicides have helped to curb transmission, long-term control of schistosomiasis remains elusive.

- Parasite-resistance to the only effective drug, PZQ, remains a concern

- Earlier studies showing that transmission could be reduced if existing susceptible *B. glabrata* snails in an area were displaced by less-susceptible secondary snails (*B. straminea)* gave credence to using resistant snails to replace susceptible snails as a form of biological control of schistosomiasis (Jordan, 1972).

- Alternative methods for controlling schistosomiasis based on novel tools that will specifically target the invertebrate snail stage of the parasites' life cycle has been suggested (Jordan *et al*., 1980).

Studies of early differential gene regulation between R and S snails in response to *S. mansoni*

Rationale:

- Identification of genes that are highly expressed in either S or R snails upon early infection should enable us to identify pathways that lead either to parasite survival (in S snail), or its destruction (in R snail)

- Early time post exposure (PE) timed from within the first 10 hours PE should enable us to identity differences in the timing of gene expression between R and S snails

Goals

- Elucidate the molecular basis of the snail host and schistosome parasite relationship.
- Develop markers for identifying parasite resistant from susceptible snails.
- Develop alternative control methods aimed at using resistant genes to block parasite transmission: create genetically modified (GM)snails

Why a Snail Genome Project?

- Freshwater snails of the genus *Biomphalaria* (*B. glabrata)* are important intermediate snail hosts for the widespread transmission of schistosomiasis in humans.

- Genome sequences of the three organisms that are pertinent to transmission of schistosomiasis -the parasite, the intermediate snail host, and the human definitive host will be useful.

- The genome of *B. glabrata* is estimated to be around 950 Mb, (Gregory, 2003) The chromosomes (haploid number = 18) are small, and relatively monomorphic.

- Various *B. glabrata*, gene libraries (cDNA, genomic, cosmid, BAC) are available.The full-length mitochondrial genome sequence (13,670 nt) has also been obtained (DeJong *et al*., 2004).

- The snail genome project that is already underway will help determine whether or not molecular co-evolution has allowed these two organisms (parasite and snail) to adapt to each other and to the human host.

The *B. glabrata* genome project meeting held May 2010; *projected publication in 2015*

Funded by NIH- NHGRI

Strategy adopted

- Suppressive Subtractive Hybridization (SSH) cDNA cloning strategy for identification of differentially expressed genes between R and S snails

Ittiprasert et al, Mol Biochem Parasitol. Jan;169(1):27-39, 2010. Epub 2009 Oct 6.2010

Experimental Parasitology 123 (2009) 203–211

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Schistosoma mansoni infection of juvenile *Biomphalaria glabrata* induces a differential stress response between resistant and susceptible snails

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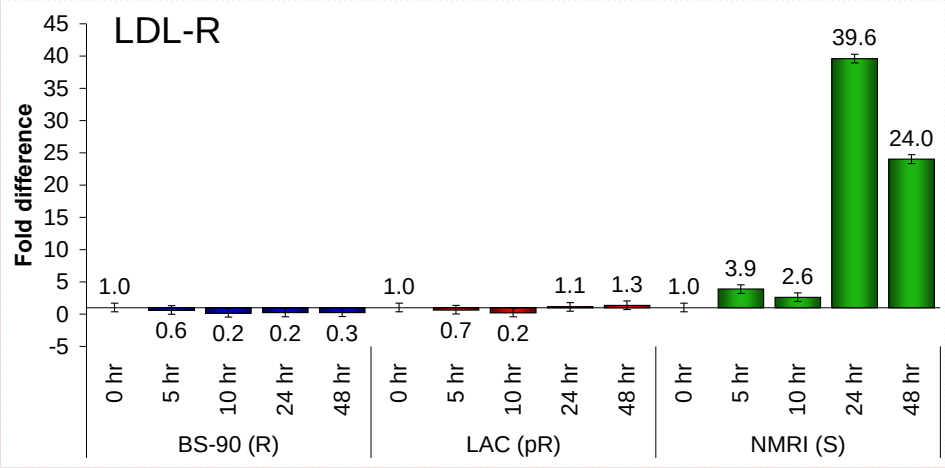
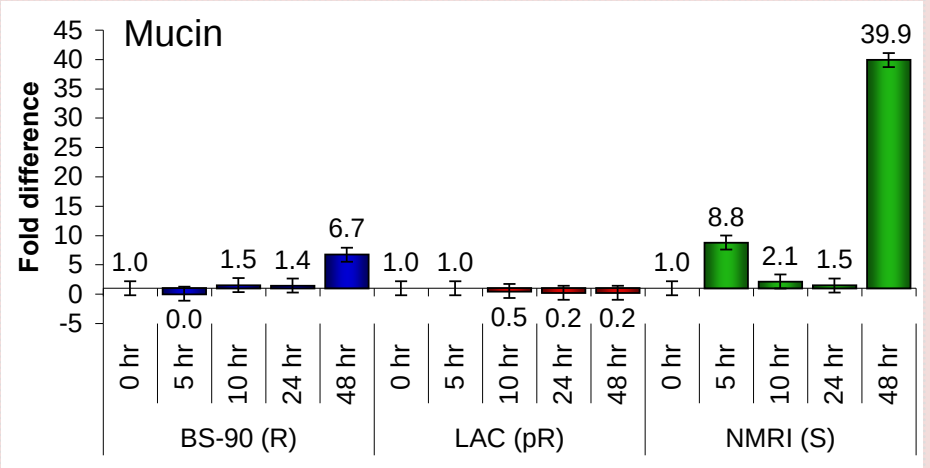
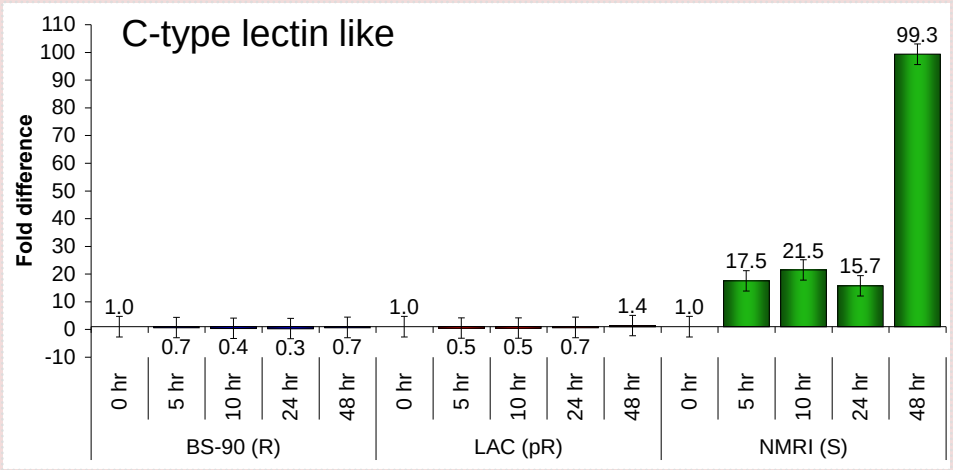
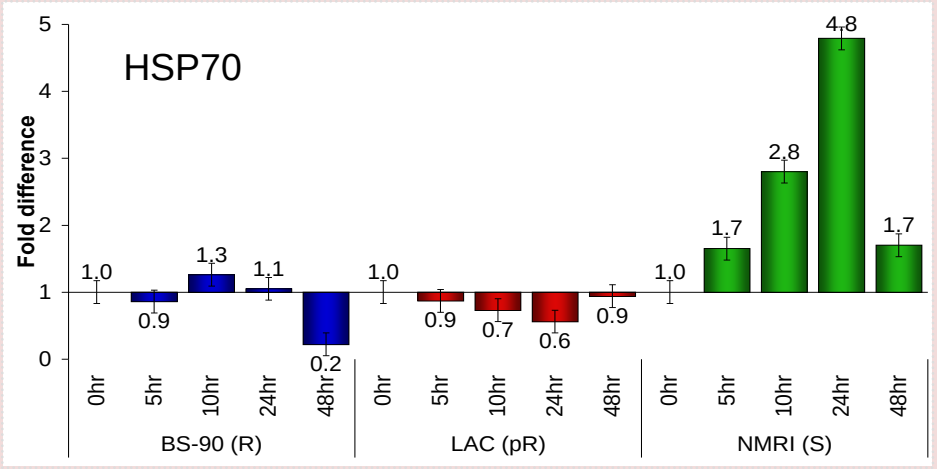
Fold change expression during the anti-parasite response in R and S *B. glabrata*









(A value of 1.0 equals gene expression in the unexposed snail)

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International Journal for Parasitology 37 (2007) 1307–1318
www.elsevier.com/locate/ijpara

Nimbus (*BgI*): An active non-LTR retrotransposon of the *Schistosoma mansoni* snail host *Biomphalaria glabrata* ☆,☆☆

Nithya Raghavan [a], Hervé Tettelin [b], André Miller [a], Jessica Hostetler [b], Luke Tallon [b], Matty Knight [a,*]

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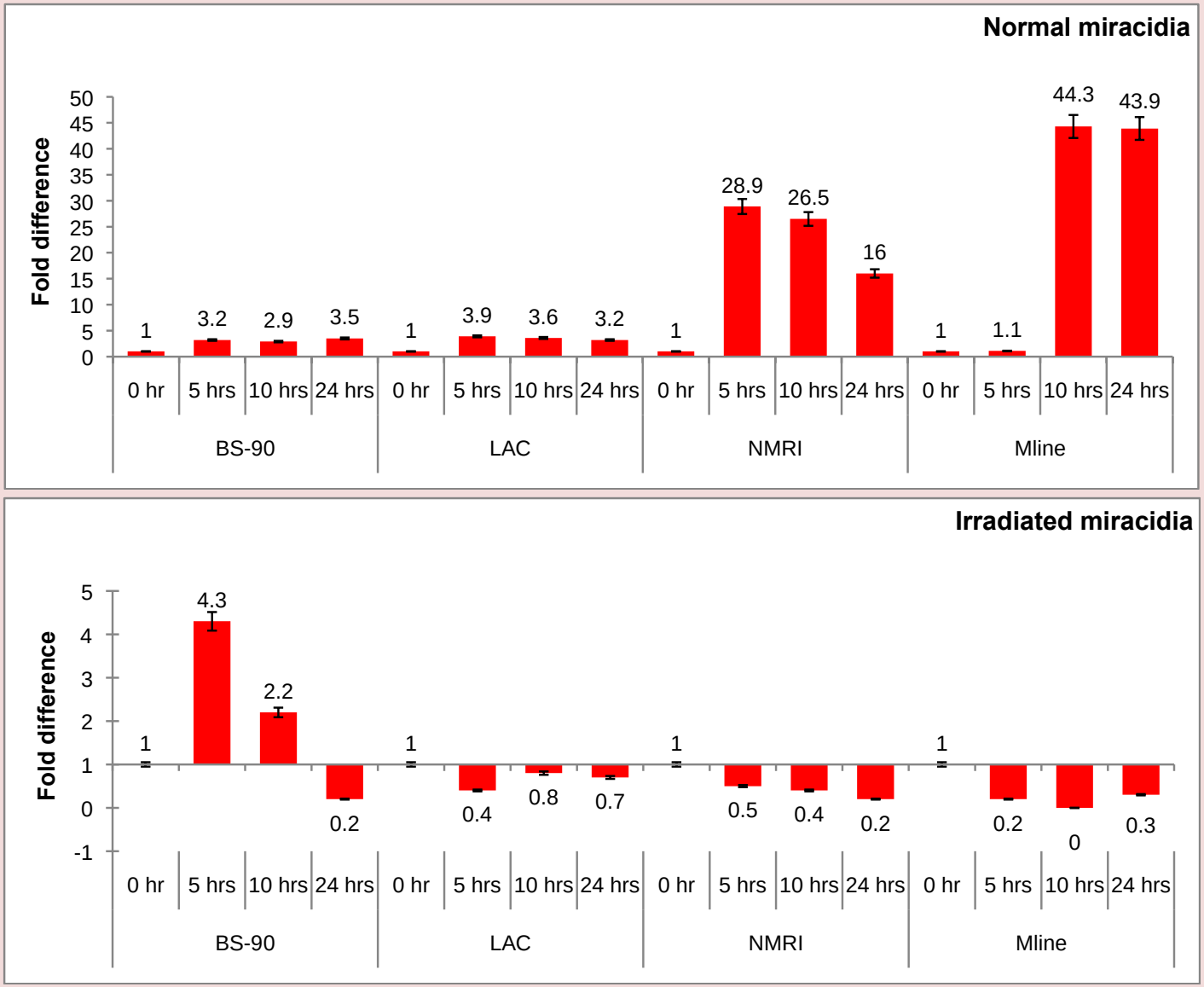
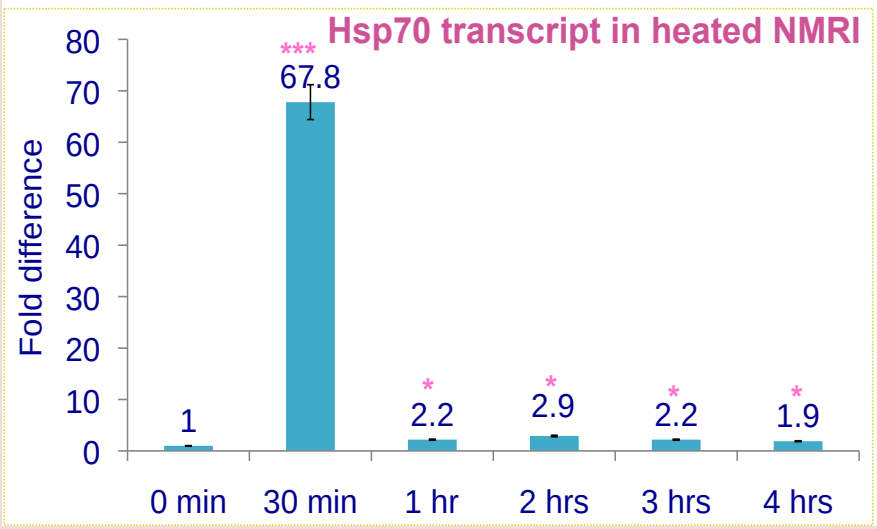
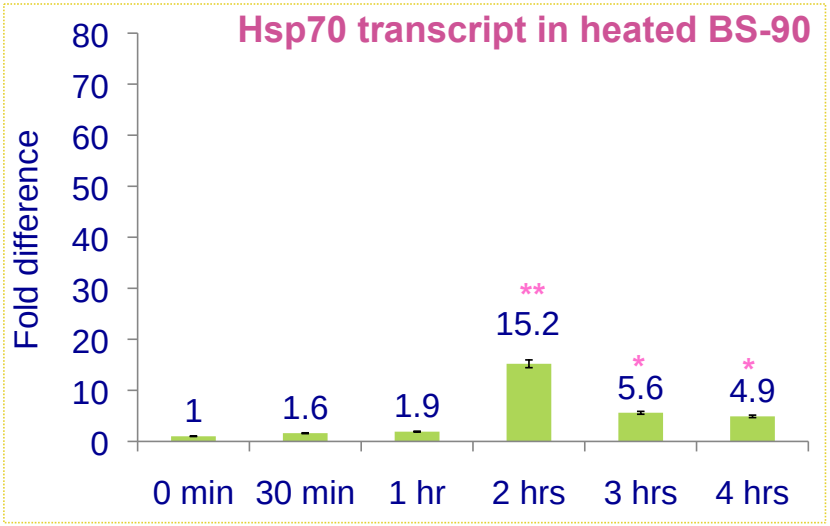
Received 16 February 2007; received in revised form 29 March 2007; accepted 5 April 2007

Kinetic of induction of *nimbus* RT upon infection of snails to normal *vs* attenuated *S. mansoni* miracidia



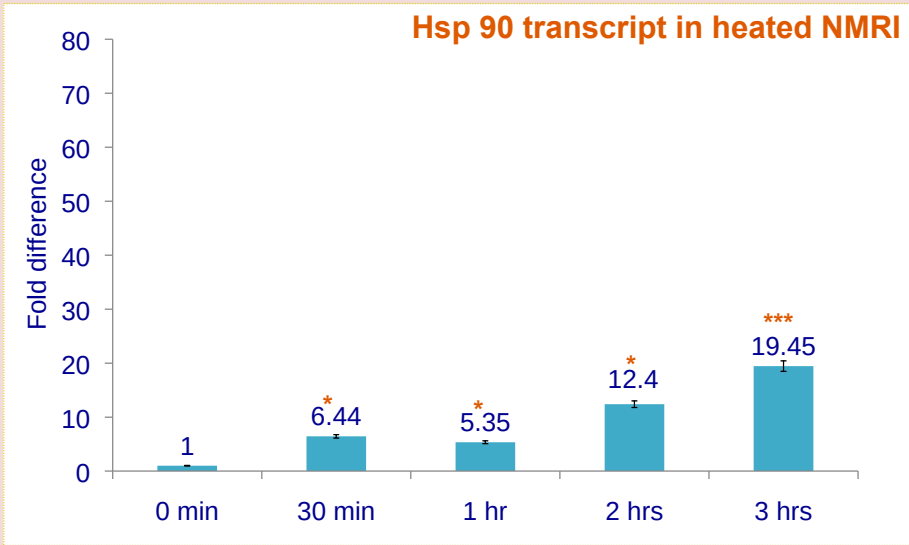
Real time PCR analysis of the differential expression of **Hsp70** in BS-90 & NMRI snails in response to heat shock (32° C) for various time periods (30 min-4 hrs).

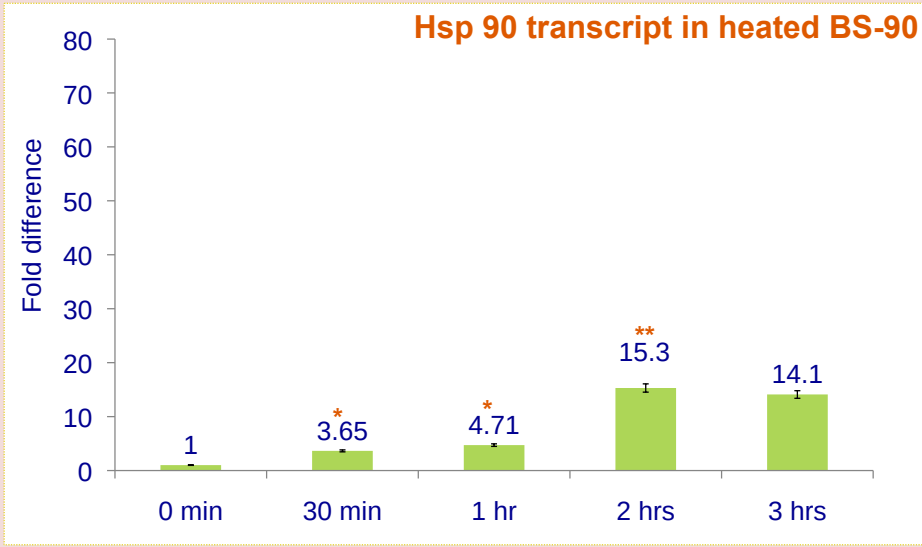




Significant *P*-values of < 0.05 and < 0.01 are indicate by * and **, respectively to show the significance of gene expression determined using Student's *t*-test.

Real time PCR analysis of the differential expression of **Hsp90** in BS-90 & NMRI snails in response to heat shock (32° C) for various time periods (30 min-3 hrs).





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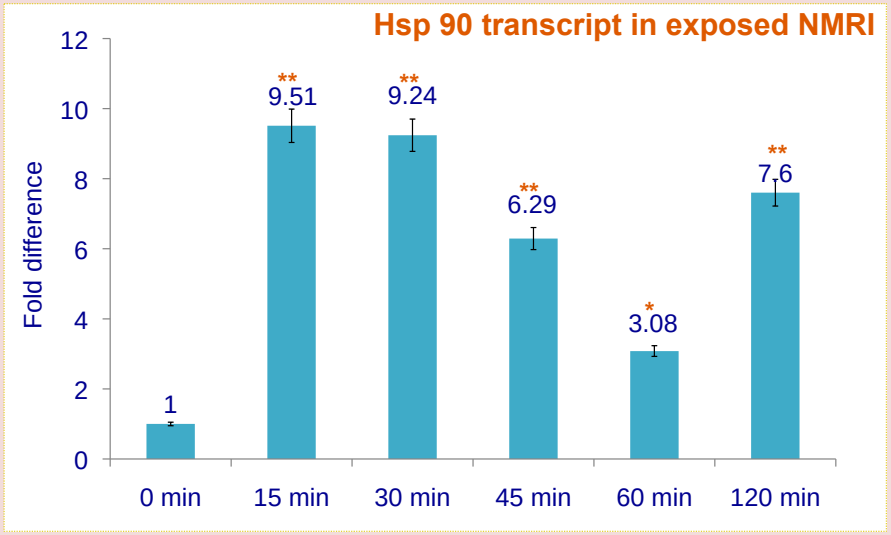
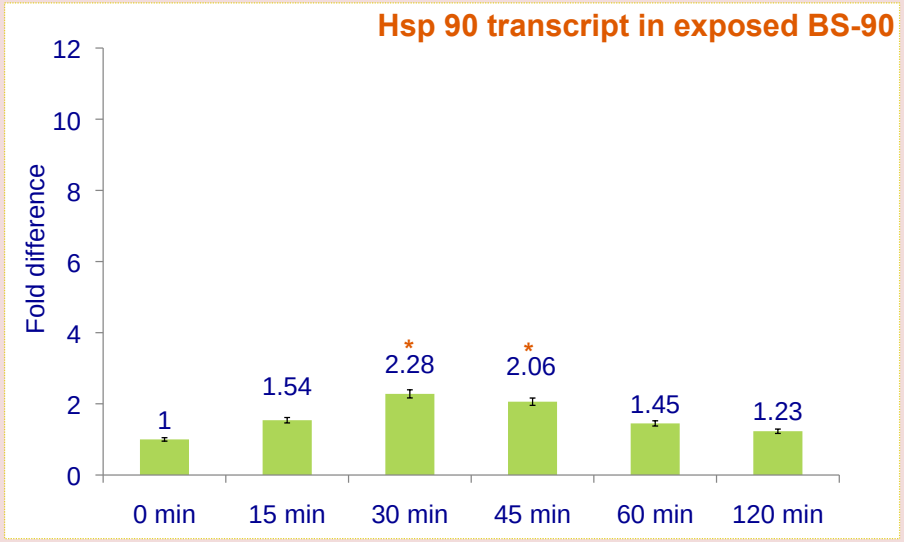
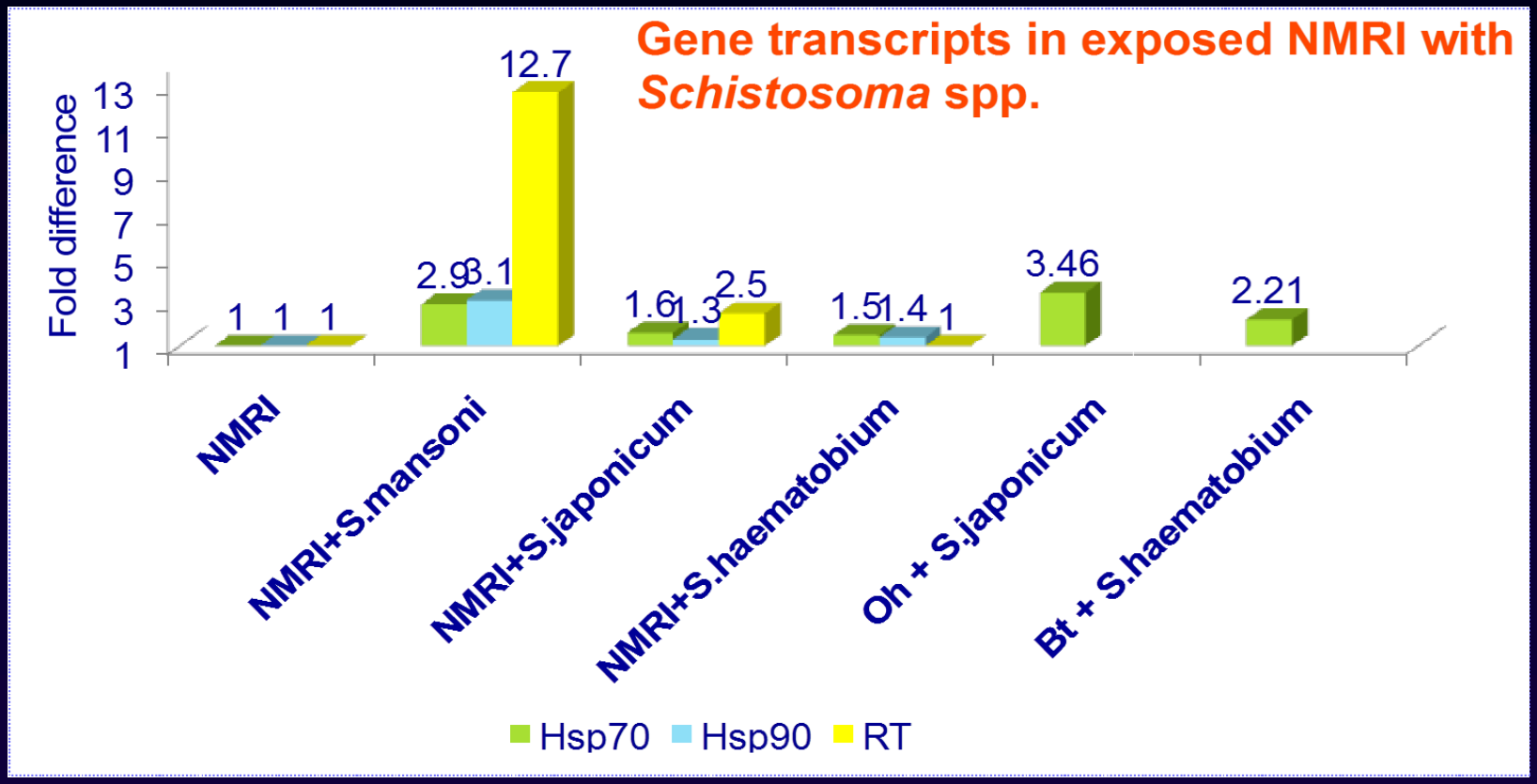
Real time PCR analysis of the differential expression of **Hsp 90** in BS-90 & NMRI snails in response infection at various time periods (15-120 min)



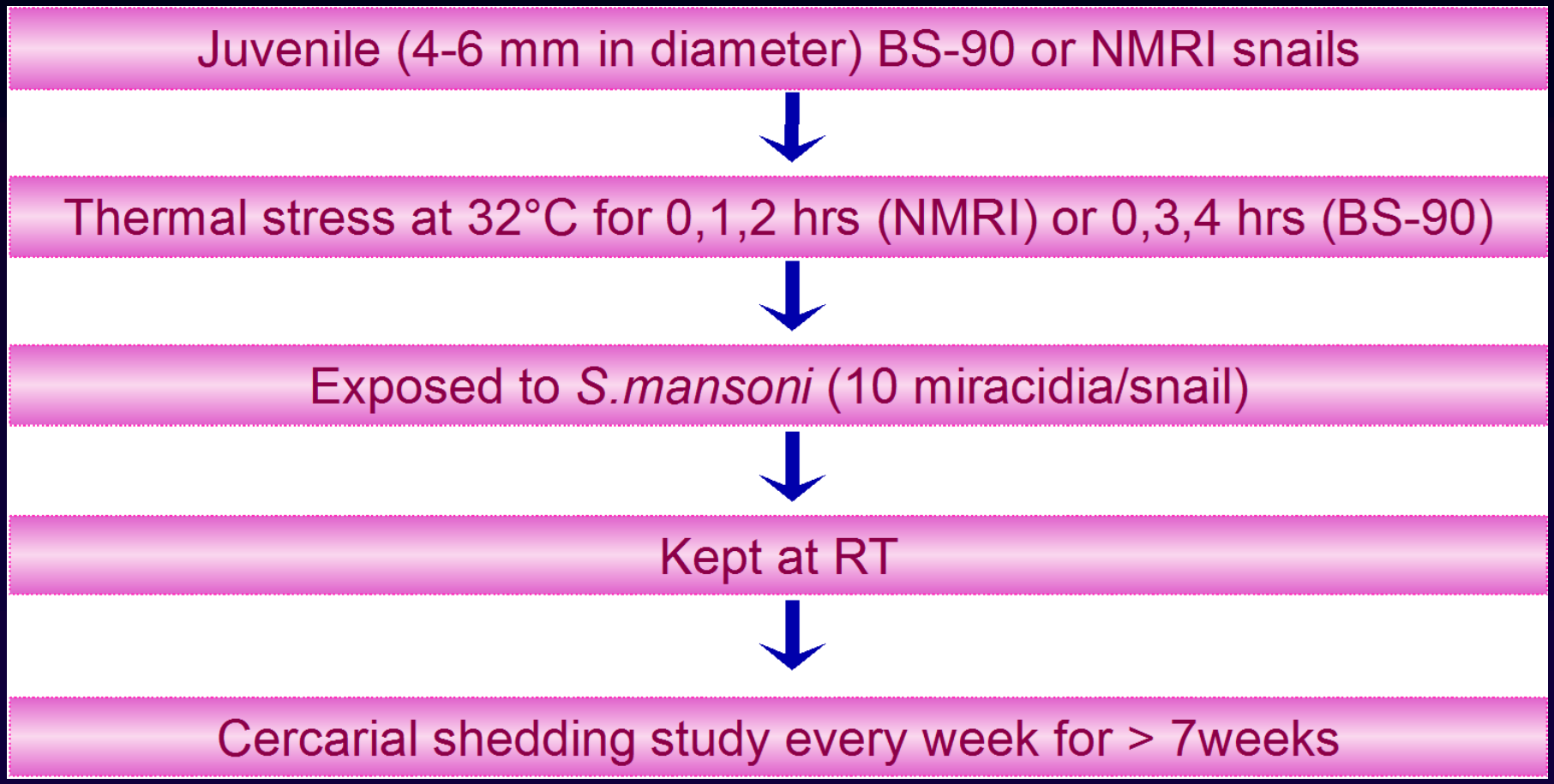


Significant *P*-values of < 0.05 and < 0.01 are indicate by * and ** respectively to show the significance of gene expression determined using Student's *t*-test.

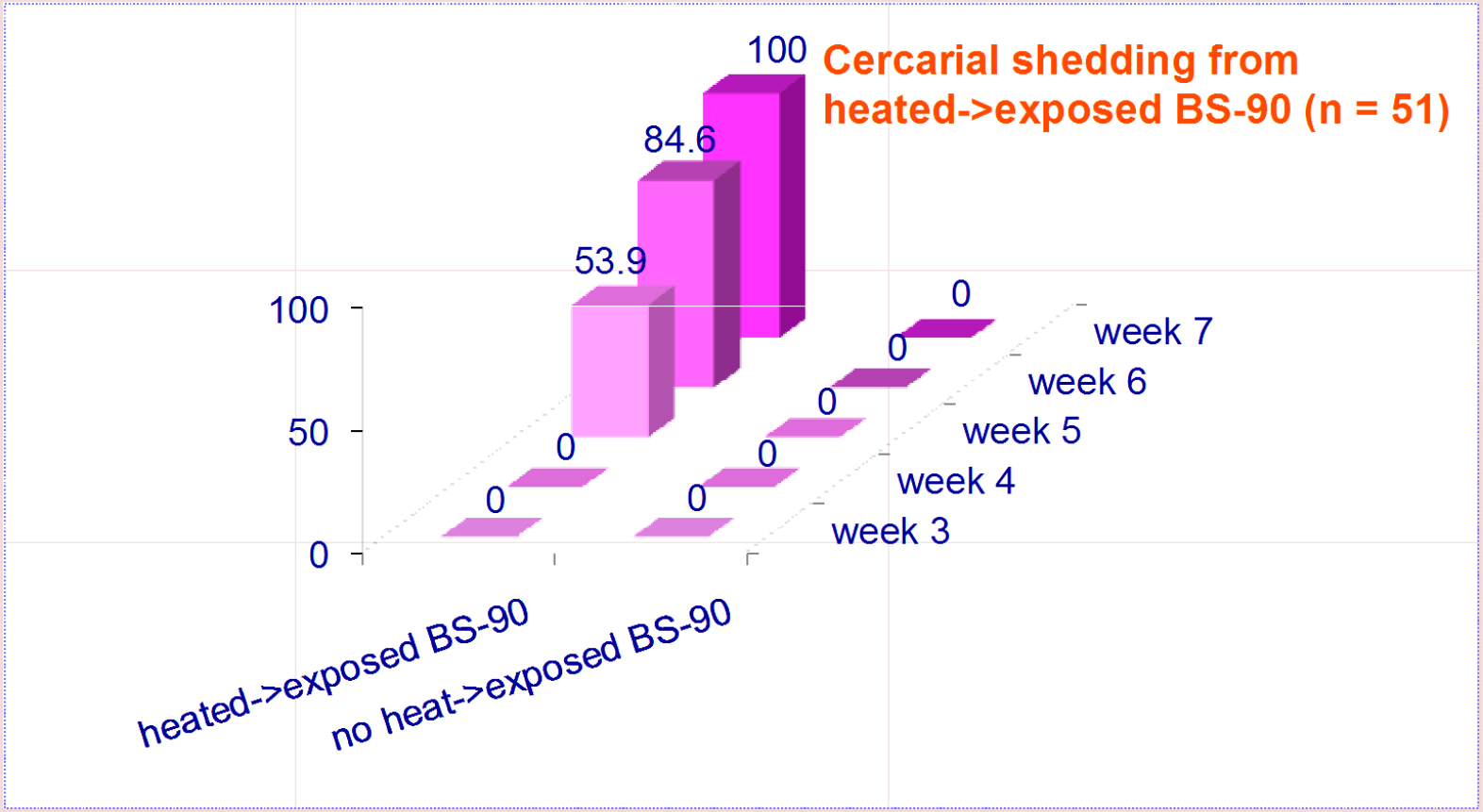
Real time PCR analysis of the differential expression of Hsp70, Hsp90 and *nimbus* RT upon exposure with *S. mansoni, S. japonicum, S. haematobium*



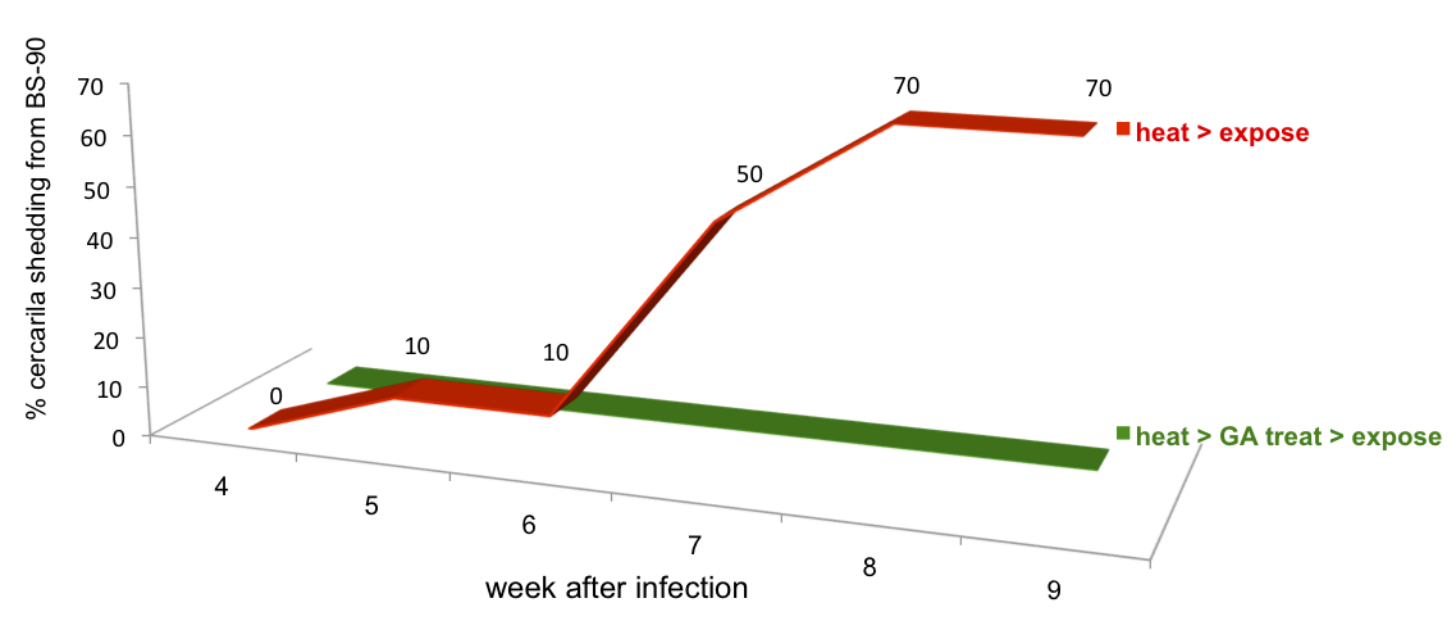
Heat–pulse reversal of refractory phenotype



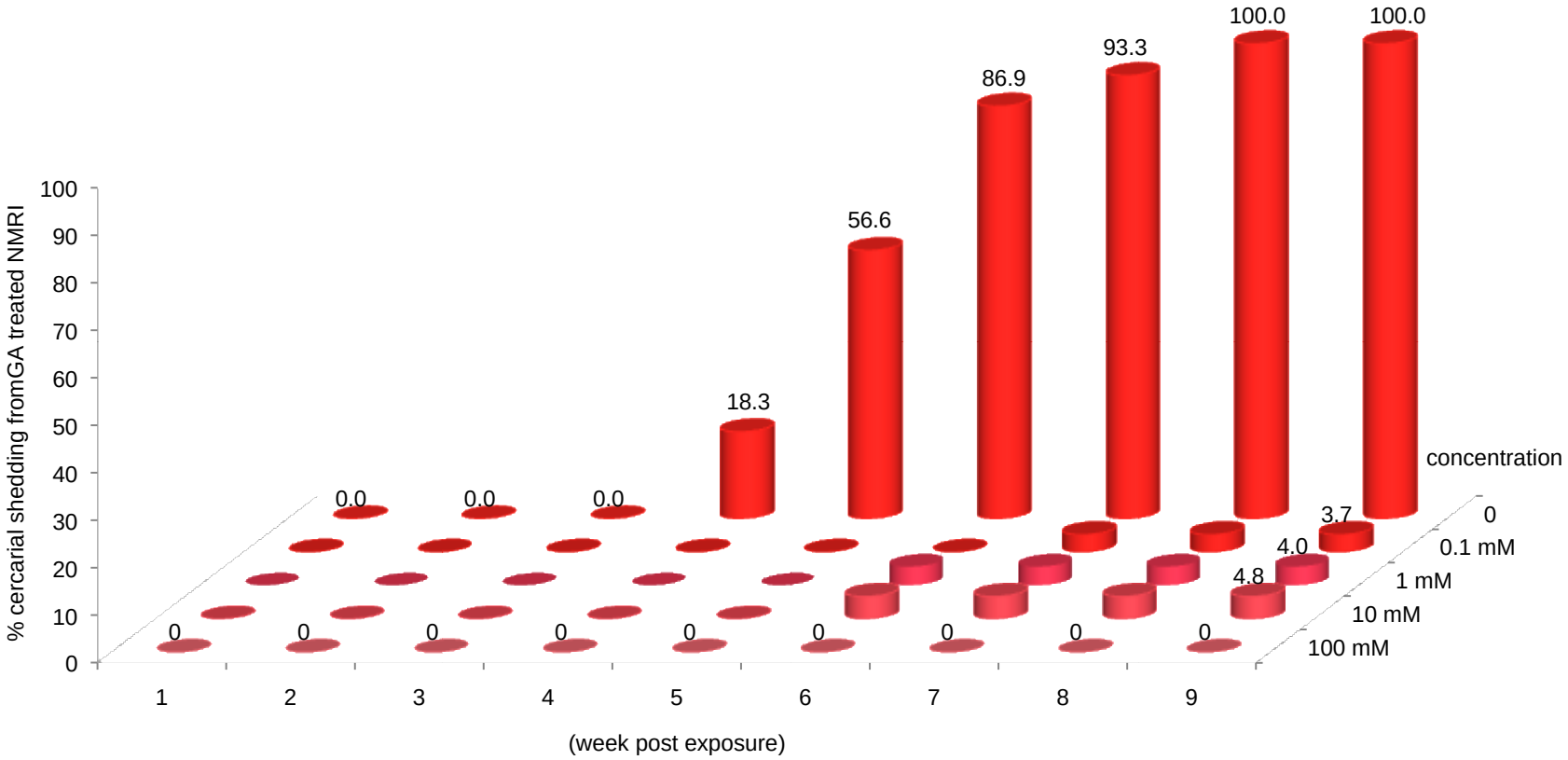
Heat-pulse reversal of refractory phenotype



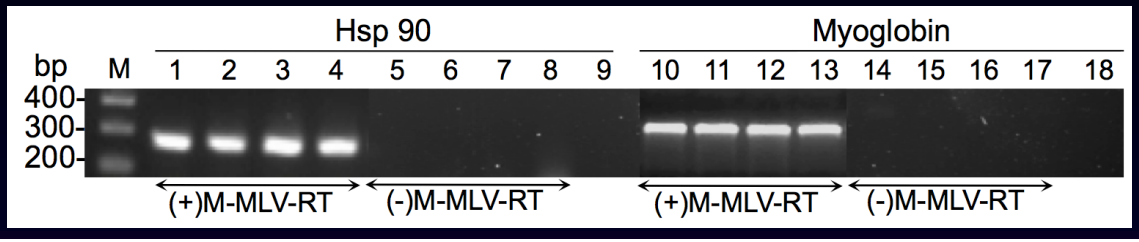
Percentage of cercarial shedding from BS-90

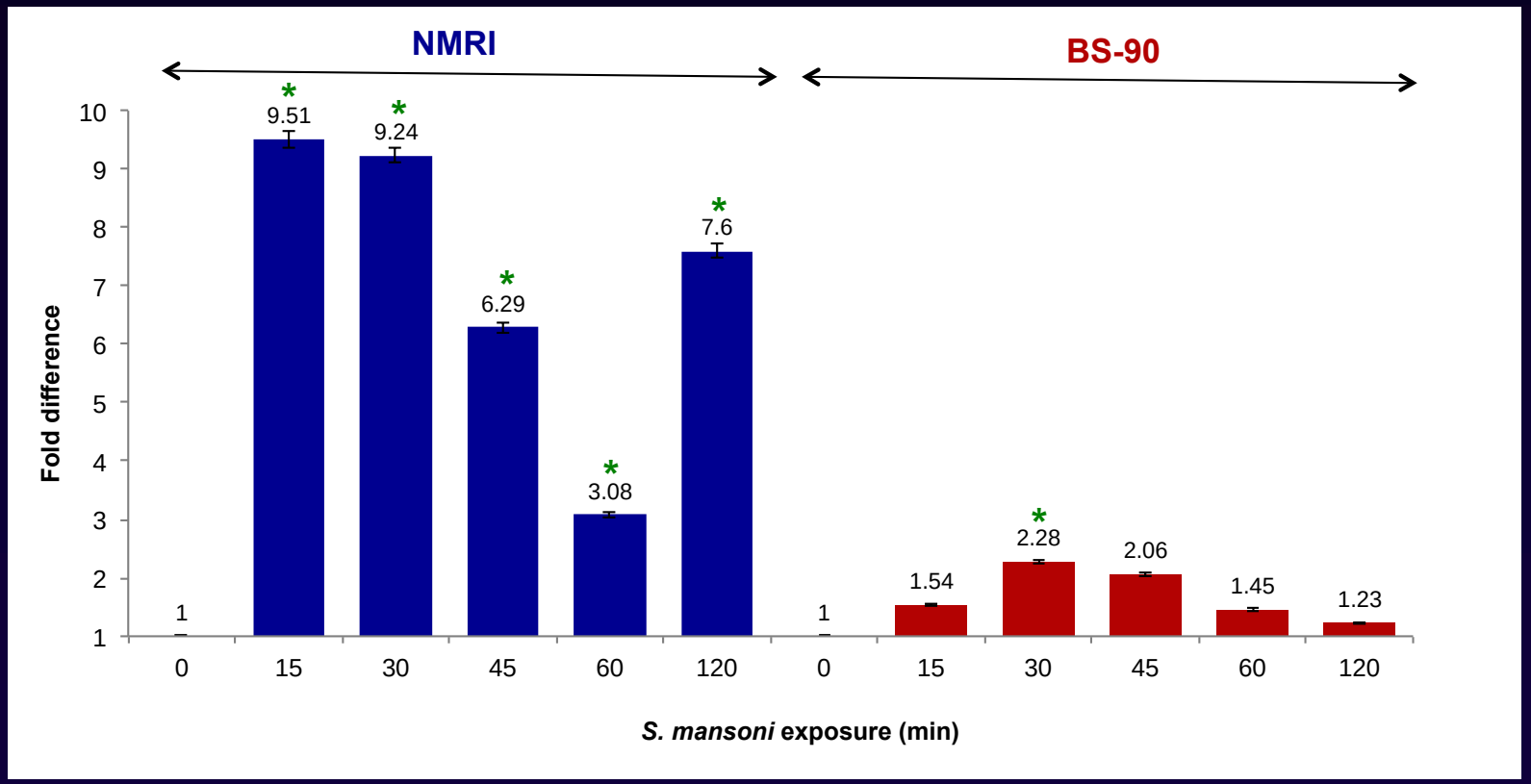


Hsp -90 inhibitor drug, Geldanamycin, pre-treatment of S snail renders them non-susceptible



Differential Expression of Hsp 90 between susceptible and resistance snails with and without *S. mansoni*





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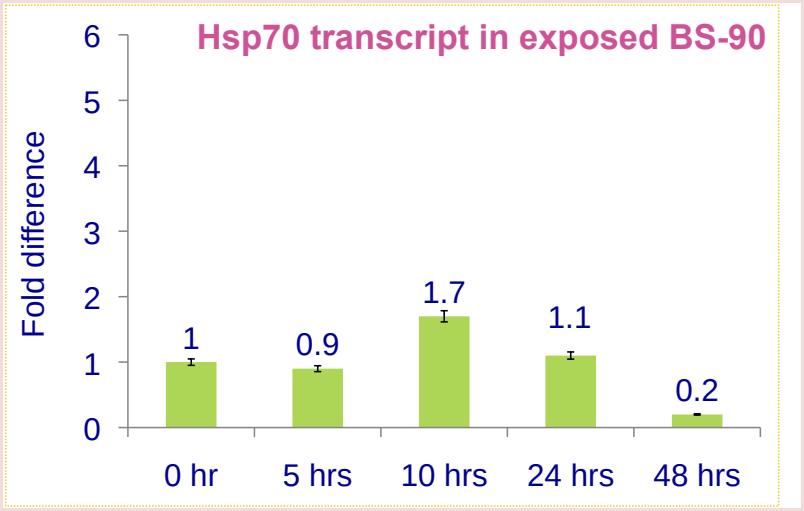
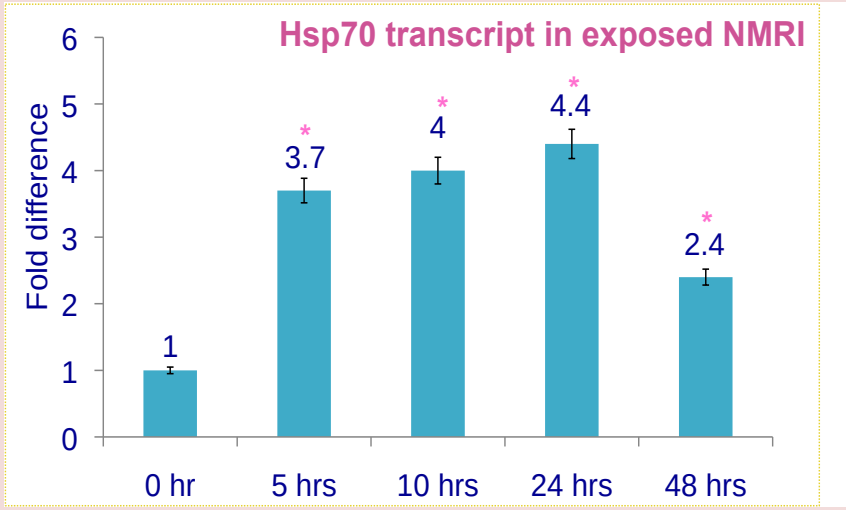
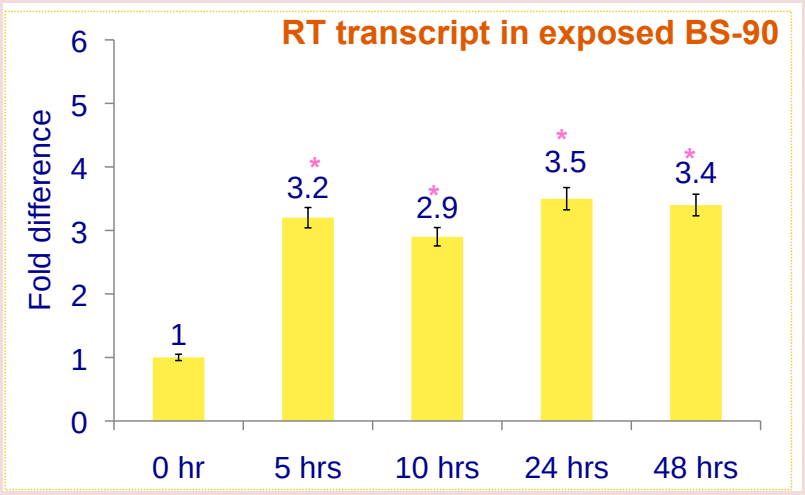
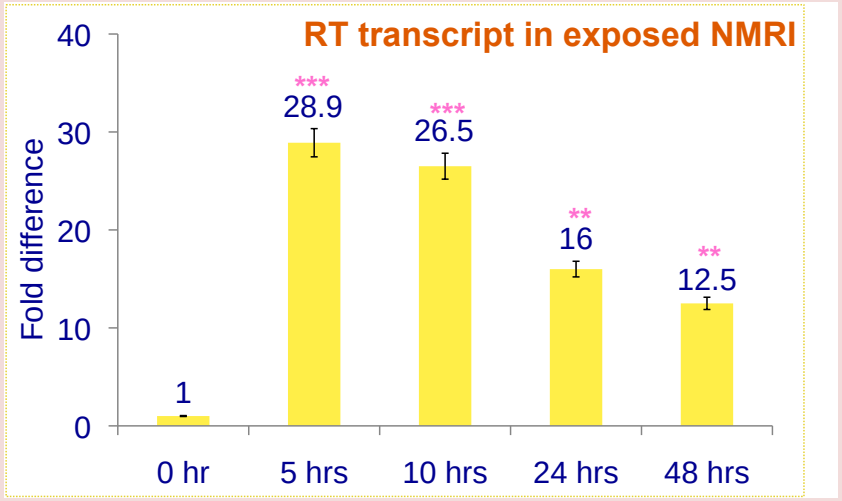
Real time PCR analysis of the differential expression of **Hsp70** & ***nimbus* RT** in BS-90 & NMRI snails in response infection at various time periods (5-48 hrs)



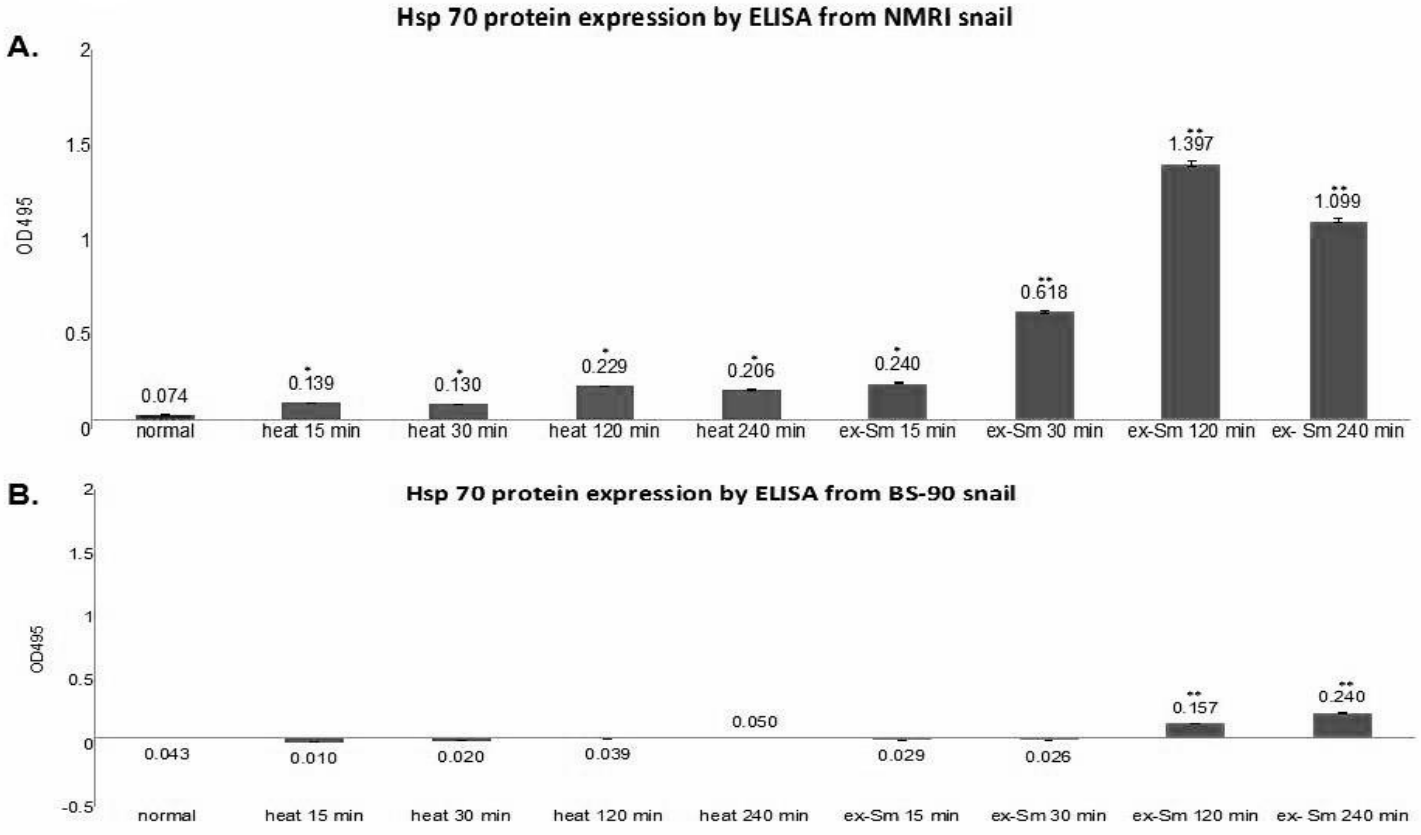




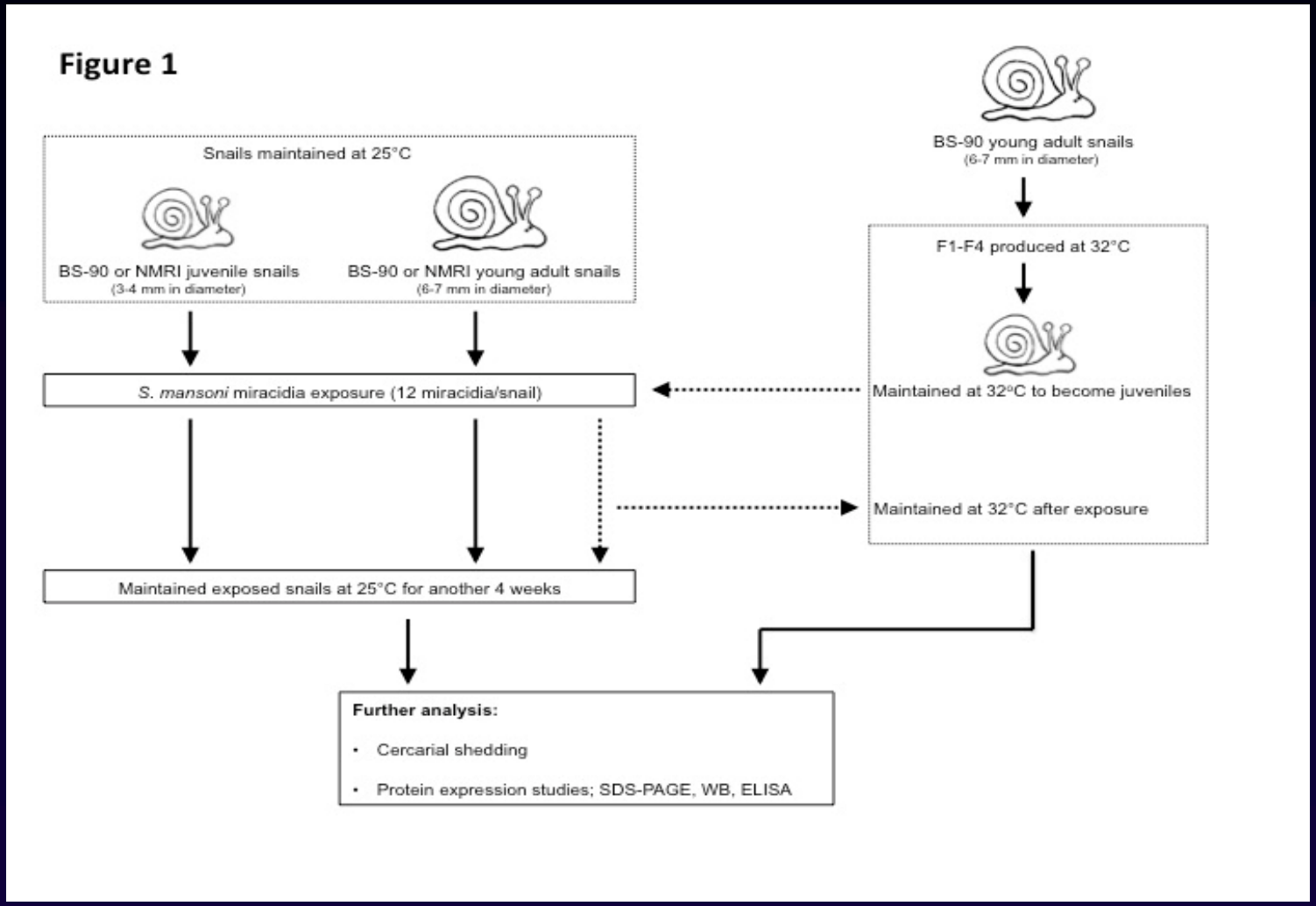


Significant *P*-values of < 0.05, < 0.01 and < 0.001 are indicate by *, ** and ***, respectively to show the significance of gene expression determined using Student's *t*-test.

Figure 4


A.
Hsp 70 protein expression by ELISA from NMRI snail
OD495
2
1.5
1
0.5
0
0.074
0.139
0.130
0.229
0.206
0.240
0.618
1.397
1.099
normal
heat 15 min
heat 30 min
heat 120 min
heat 240 min
ex-Sm 15 min
ex-Sm 30 min
ex-Sm 120 min
ex- Sm 240 min
B.
Hsp 70 protein expression by ELISA from BS-90 snail
OD495
2
1.5
1
0.5
0
-0.5
0.043
0.010
0.020
0.039
0.050
0.029
0.026
0.157
0.240
normal
heat 15 min
heat 30 min
heat 120 min
heat 240 min
ex-Sm 15 min
ex-Sm 30 min
ex-Sm 120 min
ex- Sm 240 min

Experimental design: simulating global warming by maintaining and breeding snails at 32ºC

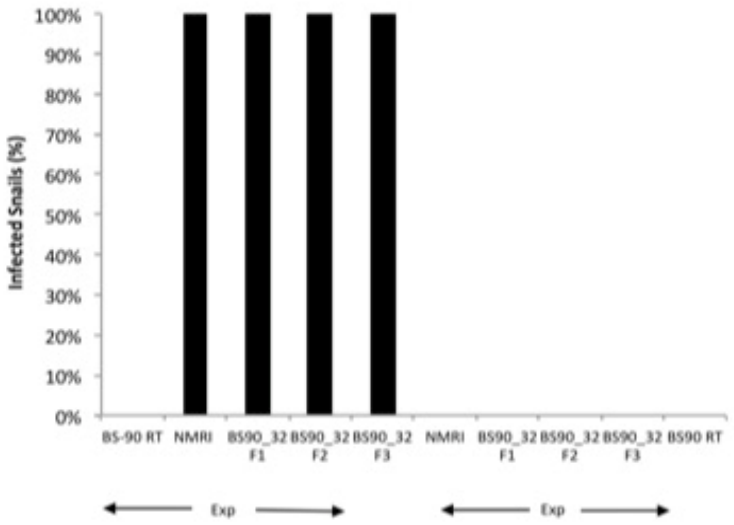


The progeny of resistant snails produced at warmer temperature are susceptible

Progeny (F_1 to F_3) of resistant snails produced at 32^{o}C are susceptible when infected at room temp

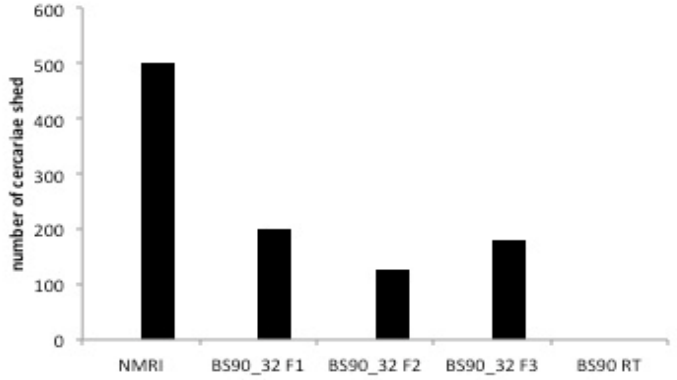
Figure 3

A.



The progeny of resistant snails produced at warmer temperature shed ceracariae

Numbers of cercariae shed from progeny of resistant snails bred at 32^{o}C



Summary

- stress genes; Hsp 70, 90 and *nimbus* RT are expressed early in *B. glabrata* in response to *S. mansoni* depending on their susceptibility phenotype
- Susceptibility can be reversed by prior treatment of susceptible snails with Hsp 90 inhibitor drug; geldanamycin
- Resistance can be reversed by prior non-lethal heat shock treatment of snails at non-lethal temperature
- Progeny of resistant snails maintained at warmer temperature (32^{o}C) are susceptible when infected at ambient temperature (25^{o}C) an indication that global warming will make transmission of schistosomiasis difficult to control

Schistosomiasis reaches Europe

Figure: **Focus of freshwater transmission of urogenital schistosomiasis frequented by tourists in Corsica**
This river is visited by 3000–5000 people every day during the summer season.

Jérôme Boissier, Hélène Moné, Guillaume Mitta M Dolores Bargues, David Molyneux, Santiago Mas-Coma

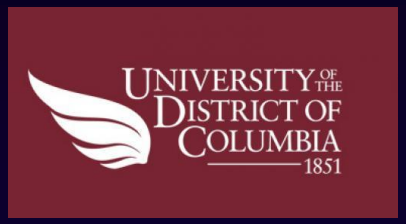
The Lancet vol.15 July 2015

Acknowledgement

George Washington University

- ✓ Dr. Paul Brindley
- ✓ Dr. Gabriel Rinaldi
- ✓ Dr. Victoria Mann

Funded by NIH RO1: AI63480



University of the District of Columbia

- ✓ Dr. Carolyn Cousin
- ✓ Michael Smith
- ✓ Oumsi Elhelu

Brunel University, London, UK

- ✓ Dr. Joanna Bridger

Biomedical Research Institute

- ✓ Dr. Wannaporn Ittiprasert
- ✓ André Miller