UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Expressão de hsp70 e hsp83 no desenvolvimento de *Drosophila* em resposta ao estresse químico causado por disseleneto de difenila e paraquat

RONALDO MEDEIROS GOLOMBIESKI

Prof. Dra. Vera Lúcia da Silva Valente Gaiesky

Prof. Dr. Élgion Lúcio da Silva Loreto

Porto Alegre, Janeiro de 2008.

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Expressão de hsp70 e de hsp83 no desenvolvimento de *Drosophila* em resposta ao estresse químico causado por disseleneto de difenila e paraquat

RONALDO MEDEIROS GOLOMBIESKI

Prof. Dra. Vera Lúcia da Silva Valente Gaiesky

Prof. Dr. Élgion Lúcio da Silva Loreto

Porto Alegre, Janeiro de 2008.

Drosophila

Drosophila *LabDros*

Agradecimentos

Drosophila

Drosophila

Drosophila

Drosophila *LabDros*

RESUMO

EXPRESSÃO DE Hsp70 E DE 83 NO DESENVOLVIMENTO DE *Drosophila* EM RESPOSTA AO ESTRESSE QUÍMICO CAUSADO POR DISSELENETO DE DIFENILA E PARAQUAT

Drosophila

Drosophila

melanogaster *willistoni* *D. willistoni*

D. equinoxialis, D. paulistorum, D. insularis *D. tropicalis.*

Drosophila melanogaster

willistoni. *D. melanogaster,*

willistoni *D.*

melanogaster

D. willistoni

D. melanogaster

hsp83 *Northern blot*

willistoni *hsp70*

Real Time PCR

P

Drosophila melanogaster *D. willistoni*

ABSTRACT

Hsp70 and 83 EXPRESSION IN *Drosophila* DEVELOPMENT IN RESPONSE TO CHEMICAL STRESS CAUSED BY DIPHENYL DISELENIDE AND PARAQUAT

Drosophila

Drosophila melanogaster

willistoni *D. willistoni* *D. equinoxialis* *D. willistoni* *D. paulistorum* *D. insularis* *D. tropicalis*

Drosophila melanogaster *willistoni* *D. melanogaster*

willistoni
D. melanogaster

D. willistoni

D. melanogaster

hsp83
willistoni *hsp83* *hsp70*

P

Drosophila melanogaster *D. willistoni*

SUMÁRIO

Drosophila

Drosophila

P

Real Time PCR

willistoni

Drosophila

willistoni

willistoni

Sepia

white *Drosophila willistoni*

Sepia *white* *D. willistoni*

Abreviaturas, símbolos e unidades

hsp70

o

Polymerase chain reaction

μ

μ

Lista de figuras

hsp83

Real Time PCR β *D. willistoni Sepia*

Real Time PCR β *D. willistoni white*

Real Time PCR *D. willistoni Sepia*

Real Time PCR *D. willistoni white*

Real Time PCR *P*

Lista de Tabelas

white *D. willistoni*

white *D. willistoni*

D. equinoxialis

D. equinoxialis

D. equinoxialis

D. equinoxialis

D. insularis

D. insularis

D. insularis

D. insularis

D. paulistorum

D. paulistorum

D. paulistorum

D. paulistorum

D. tropicalis

D. tropicalis

Capítulo I

Introdução Geral

white

Drosophila

Drosophila melanogaster

Drosophila

Drosophila

et al

Drosophila

Drosophila

willistoni *Drosophila*

et al

Drosophila melanogaster

I.1. Subgrupo da *Drosophila willistoni*

D. willistoni

Drosophila
Sophophora
willistoni
willistoni
willistoni *Drosophila*

willistoni *Sophophora,*
willistoni
D. willistoni, D. equinoxialis, D. tropicalis, D. insularis, D. pavlovskiana
D. paulistorum
et al,

et al

D. melanogaster
et al

et al.

willistoni *Drosophila*

in situ

Hsp83, Hsp27, Hsr-ômega *Ubi*

Drosophila willistoni

Drosophila willistoni *D. melanogaster*

P *D. willistoni*

Drosophila willistoni

et al

et al

Drosophila willistoni

Drosophila melanogaster

º ± º

Drosophila melanogaster

Drosophila willistoni,

I.2. Adaptação às variações climáticas

et al.,

Hsp90

et al.,

fitness

et al.,

Drosophila

et al.,

et al., *et al*

et al.

et al.,

et al *et al.,*

et al.,

et al

et

al.,

et al.

Agentes indutores de estresses químicos

D. melanogaster

Drosophila *et al* *et al* *et al*

(

et al.,

et al.,

Drosophila

et al

°

et al

º

et al

D. melanogaster

et al

et al

D. melanogaster

et

al *et al*

Drosophila

I.3. Proteínas de Choque Térmico

Drosophila busckii

Drosophila

hsps

Drosophila

et al

et al

Drosophila

et al

Drosophila melanogaster o

o

o *et al* o

o

Mycobacterium leprae *M. tuberculosis* *Coxiella burnetti* *Plasmodium falciparum* *Schistosoma mansoni* *Brugia malayii* *Trypanosoma cruzi* *Leishmania major* *et al*

heat-shock,

heat-shock cognate

I.3.1. Hsp27

D. melanogaster

D. willistoni

et al

I.3.2. Hsp60

like *E. coli*

et al

E. coli *et al*

in vitro

et al

et al

I.3.3. Hsp70

et al.,

et al., *et al.,*

hsp70

Drosophila *Escherichia coli* *et*

et al

KAR2 *et al* *et al*

Saccharomyces cerevisiae *et al* *Euglena gracilis* *et al* *Trypanosoma cruzi* *et al* *et al* *S. cerevisiae* *SSC1* *et al*

et al

et al *et al*

I.3.4. Hsp90

α β

Drosophila,

et al

Drosophila

I.4. Elementos transponíveis e estresse

et al

et al

transcriptase reversa.

Long

terminal repeats

Ty1-copia BEL, DIRS

Ty-3 gypsy,

long interpersed nuclear elements *short interpersed nucleat elements*

hAT hobo, Activator, Tam-3 *mariner-like*

P

IS

E. coli operons

IS *fitness*

operons β *glucoside*

IS β

Tnt1 *Nicotiana tabacum*

et al

Drosophila simulans *412*

mariner

et al *et al.,*

Objetivos

Objetivo geral:

Drosophila.

Objetivos específicos:

1)

Drosophila melanogaster

Hsp83

2 *willistoni*

Drosophila

3

hsp70 *hsp83* *Sepia* *white*

Drosophila willistoni

4

P *D. willistoni.*

Capítulo II

Over-activation of the *Drosophila melanogaster hsp83* gene by selenium intoxication

Trabalho aceito para publicação na revista

Genetics and Molecular Biology, 2008

Parte dos resultados deste trabalho foram obtidos durante o Mestrado em Bioquímica na UFSM e parte durante o Doutorado na UFRGS

Over-activation of the *Drosophila melanogaster hsp83* gene by selenium intoxication.

Running title *Drosophila*

Key words: *hsp83*

Abstract

Drosophila melanogaster

μ

μ

μ

hsp83

hsp83

Introduction

et al *et al*

et al

et al , *et al* ,

et al ,

et al.,

et al

et al.

et al *et al ,* *et al ,* *et al* *et al ,* *et al* *et al*

et al

et al

et al

et al *et al* *et al*

et al *in vitro*

et al *et al*

et al

Drosophila melanogaster

et al ,

et

al ,

Drosophila

83 *70,* *27,* *26,* *-23* *-22*

et al.,

hsp *et al.,* *et al.,*

hsp83 *hsp90*

D. melanogaster *Hsp83*

Materials and Methods

Drosophila stock used and rearing conditions

Drosophila melanogaster

μ

Molecular methods

Hsp83

D. melanogaster

μ

μ

μ

phsp83 *et al* , *D. melanogaster* *Hsp83*

et al ,

Statistical analyses

P

Results

D. melanogaster

D. melanogaster

μ

μ

μ μ

μ μ

μ

μ μ

D. melanogaster

μ

μ μ

μ

μ μ

μ

μ

μ

μ

μ μ

μ

μ

μ μ

Drosophila

hsp83

hsp83

±

μ ±

μ

± ±

Discussion

D. melanogaster.

D. melanogaster

D. melanogaster

μ

D. melanogaster

μ

et al., *et al.,*

μ

et al.

D. melanogaster

D. melanogaster

hsp83

.

et

al

et al *et al*

hsp83

hsp *hsp23*

D. melanogaster

hsp83 *D. melanogaster*

Acknowledgments

References

Drosophila

Drosophila

Drosophila

in vivo

Drosophila hsp22

Drosophila

Drosophila melanogaster

Drosophila melanogaster

Drosophila

hs *in vivo.*

, et al

Table 1 χ *D. melanogaster*

μ

χ

P

×

P

D. melanogaster

Diphenyl diselenide molarity	χ^2	*P*	i	A=0.05 × i/15	Significant (A>*P*)
0.00 versus 500	157.11	0.000000	1	0.00333	Yes
300 versus 500	145.13	0.000000	2	0.00667	Yes
0.00 versus 450	110.90	0.000000	3	0.01000	Yes
350 versus 500	100.53	0.000000	4	0.01333	Yes
400 versus 500	90.76	0.000000	5	0.01667	Yes
300 versus 450	88.62	0.000000	6	0.02000	Yes
0.00 versus 400	59.45	0.000000	7	0.02333	Yes
0.00 versus 350	58.83	0.000000	8	0.02667	Yes
350 versus 450	38.87	0.000000	9	0.03000	Yes
300 versus 0.00	36.91	0.000000	10	0.03333	Yes
400 versus 450	30.86	0.000000	11	0.03667	Yes
450 versus 500	25.82	0.000000	12	0.04000	Yes
300 versus 400	20.20	0.000000	13	0.04333	Yes
300 versus 350	19.66	0.000010	14	0.04667	Yes
350 versus 400	0.24	0.623300	15	0.05000	No

Table 2 χ *D. melanogaster*

μ

χ

P

×

P

D. melanogaster

Diphenyl diselenide molarity					**Significant**
	χ^2	*P*	i	**A=0.05 × i/15**	**(A>*P*)**
300 versus 500	142.00	0,0000	1	0.00333	Yes
0.00 versus 500	129.24	0.0000	2	0.00667	Yes
350 versus 500	97.44	0.0000	3	0.01000	Yes
300 versus 450	87.93	0.0000	4	0.01333	Yes
400 versus 500	84.23	0.0000	5	0.01667	Yes
0.00 versus 450	74.03	0.0000	6	0.02000	Yes
350 versus 450	36.33	0.0000	7	0.02333	Yes
0.00 versus 400	34.64	0.0000	8	0.02667	Yes
0.00 versus 350	33.16	0.0000	9	0.03000	Yes
450 versus 500	31.78	0.0000	10	0.03333	Yes
300 versus 400	28.59	0.0000	11	0.03667	Yes
300 versus 350	27.82	0.0000	12	0.04000	Yes
400 versus 450	23.13	0.0000	13	0.04333	Yes
0.00 versus 300	16.24	0.0001	14	0.04667	Yes
350 versus 400	1.04	0.3077	15	0.05000	No

Table 3 χ *D. melanogaster*

μ

χ *P*

×

P

D. melanogaster

Diphenyl diselenide molarity					**Significant**
	χ^2	*P*	i	**A=0.05 × i/21**	**(A>*P*)**
0.00 versus 800	23.82	0.0000	1	0.0024	Yes
0.00 versus 700	11.71	0.0006	2	0.0048	Yes
600 versus 800	8.91	0.0028	3	0.0071	Yes
0.00 versus 900	8.88	0.0029	4	0.0095	Yes
0.00 versus 1000	6.23	0.0125	5	0.0119	Yes
0.00 versus 500	6.07	0.0138	6	0.0143	Yes
800 versus 1000	4.83	0.0280	7	0.0167	No
0.00 versus 600	3.97	0.0464	8	0.0190	No
500 versus 800	3.44	0.0637	9	0.0214	No
700 versus 800	2.71	0.0998	10	0.0238	No
800 versus 900	2.26	0.1328	11	0.0262	No
600 versus 700	2.07	0.1506	12	0.0286	No
600 versus 900	1.43	0.2313	13	0.0310	No
700 versus 1000	0.52	0.4721	14	0.0333	No
500 versus 600	0.40	0.5248	15	0.0357	No
900 versus 1000	0.37	0.5413	16	0.0381	No
600 versus 1000	0.35	0.5524	17	0.0405	No
500 versus 700	0.34	0.5583	18	0.0429	No
800 versus 900	0.16	0.6895	19	0.0452	No
500 versus 1000	0.02	0.8988	20	0.0476	No
700 versus 900	0.01	0.9413	21	0.0500	No

Figure 1

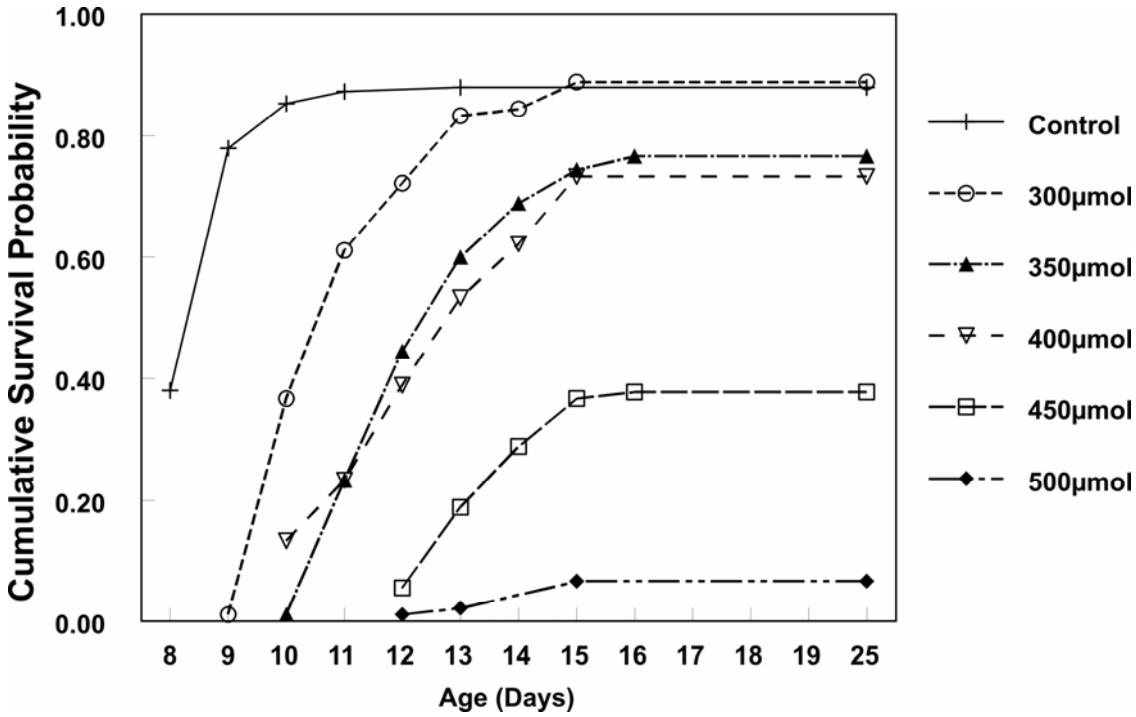


Figure 1 – *Drosophila melanogaster*

Figure 2

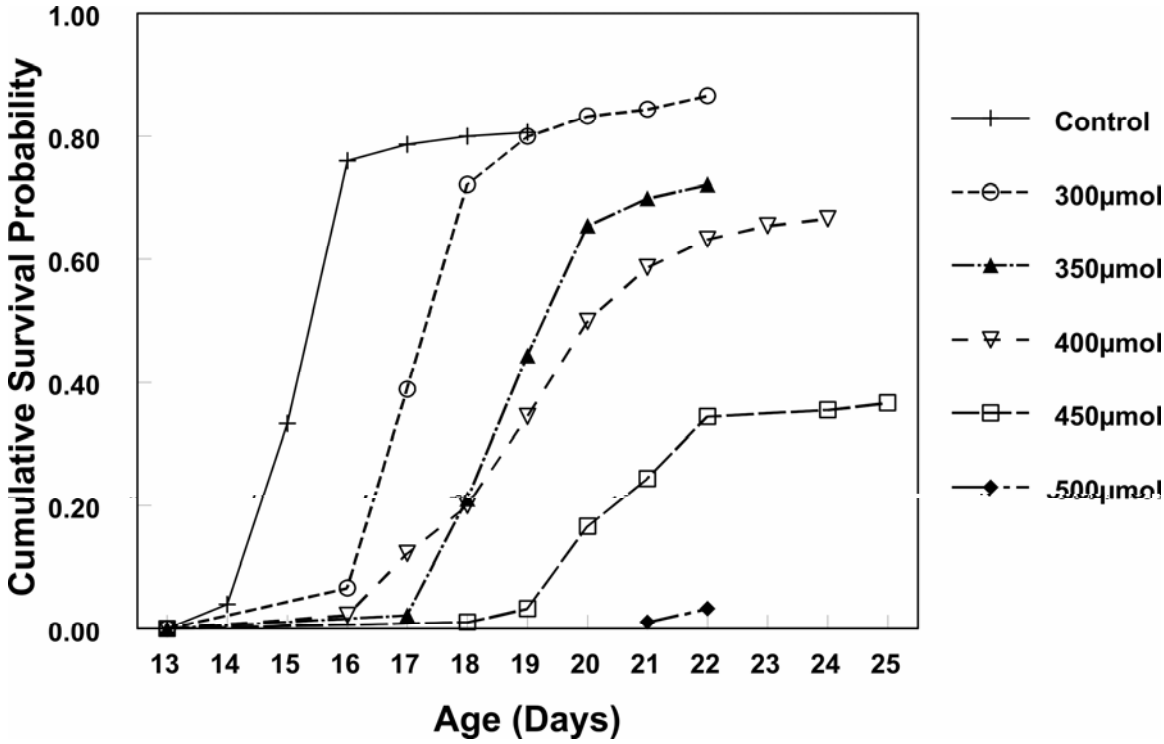


Figure 2 – *Drosophila melanogaster*

Figure 3

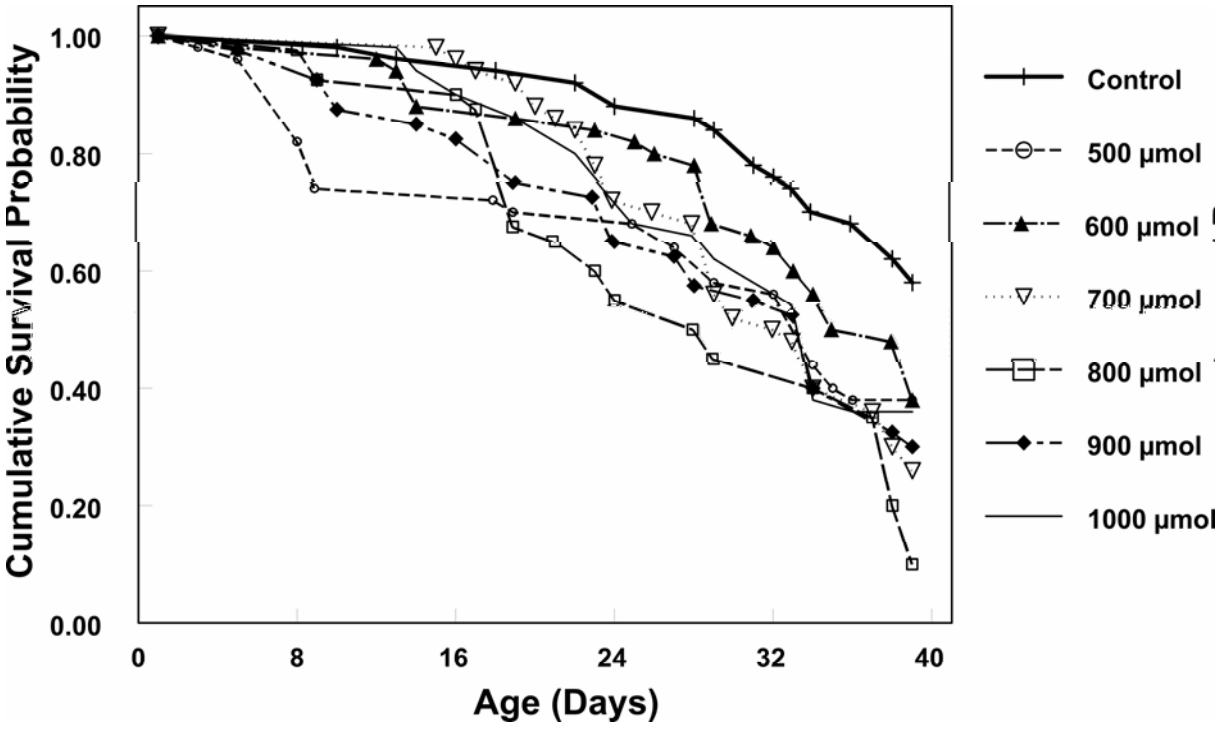


Figure 3 – *Drosophila melanogaster*

Figure 4

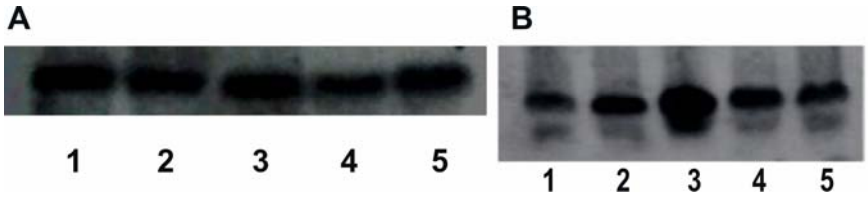


Figure 4 *D. melanogaster*

hsp83

μ

μ

μ

Capítulo III

Parâmetros do desenvolvimento de moscas do subgrupo *willistoni* de *Drosophila* em condição de estresse químico

III.1. Introdução

Drosophila

Drosophila

willistoni

D. willistoni

et al

et al

et al

willistoni *Drosophila*

willistoni *Sophophora,*

et al,

D. willistoni, D. paulistorum, D. equinoxialis, D. tropicalis, D. insularis D. pavlovskiana, D. willistoni willistoni willistoni willistoni quéchua D. tropicalis tropicalis tropicalis tropicalis cubana et al

D. willistoni D. paulistorum D. equinoxialis, D. tropicalis D insularis

willistoni

et al

willistoni Drosophila

Drosophila willistoni

Drosophila

D. willistoni

(

o

et al

D. melanogaster

et al

Drosophila *Drosophila*

D. willistoni

Drosophila

III.1.1. Proteínas de Choque Térmico

Drosophila busckii

Drosophila

hsps

Drosophila

et al

et al

Drosophila

et al

Drosophila melanogaster

- o
- o
- o *et al*
- o
- o

Mycobacterium leprae *M. tuberculosis* *Coxiella burnetti* *Plasmodium falciparum* *Schistosoma mansoni* *Brugia malayii* *Trypasnosoma cruzi* *Leishmania major* *et al*

heat-shock

heat-shock cognate

et al

III.2. Material e métodos

III.2.1. Estoques de *Drosophila*

Drosophila willistoni

D. tropicalis *D. paulistorum* *D. equinoxialis* *D. insularis*

Drosophila willistoni *Sepia* *white.*

et al

III.2.1.1) Indução de estresses químicos

A) *Paraquat*

Drosophila melanogaster, *et al* *et al*

®

B) *Disseleneto de difenila* $(PhSe)_2$

hsp83 *D. melanogaster* *et al.*

III.2.2. Determinação da viabilidade das espécies em diferentes concentrações dos agentes químicos

III.2.2.1. Meios de cultura

Č
Č
Č
Č
Č
Č ®

µ

III.2.3. Desenvolvimento dos ovos de *Drosophila* em meios de cultura

III.2.4. Análise estatística

III.2.5. Análise molecular das expressões dos Genes das Proteínas de Choque térmico e Elemento transponível *P* por PCR em Tempo Real

III.2.5.1 Oligonucleotídeos para a reação de PCR em tempo Real

primers

D. willistoni

D. melanogaster

primers

β

primers

Heat Shock

D. willistoni

D. melanogaster

primers

primers

primers

Oligo *online*

http://biotools.umassmed.edu/bioapps/primer3_www.cgi.

III.2.6. Extração de RNA total

III.2.7. Análise eletroforética dos RNAs

III.2.8. Síntese de DNA complementar

µ

µ

Taq

polimerase ° ° °

III.2.9. Quantificação das expressões gênicas por *Real Time PCR*

µ *Mix*

µ *primer*

SYBR green *Platinum Taq DNA polimerase*

µ

III.3. Resultados sobre o desenvolvimento do subgrupo *willistoni* de *Drosophila*

III.3.1. Efeito de concentrações variadas de disseleneto de difenila sobre os diferentes estágios de desenvolvimento das espécies do subgrupo *willistoni*

Drosophila *willistoni*

D. willistoni

D. willistoni *Sepia* *white*
Sepia *white*
background
D.
willistoni *Sepia* *white*

D. equinoxialis
D. willistoni

D. equinoxialis
D. insularis, D. paulistorum *D.*
tropicalis
willistoni *Drosophila*

Drosophila

μ

equinoxialis, D. insularis, D. paulistorum D. tropicalis

willistoni Drosophila

Sepia white

willistoni

et al D. melanogaster

willistoni

Drosophila melanogaster

melanogaster-willistoni

Tabela 1 – *D. willistoni*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
500µmol				

Tabela 2 – *D. willistoni*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
500µmol				

Tabela 3 – *D. willistoni*

Tratamentos	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**
5mM					
10mM					
20mM					
30mM					
40mM					

Tabela 4 – *D. willistoni*

Tratamentos	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**
5mM					
10mM					
20mM					
30mM					
40mM					

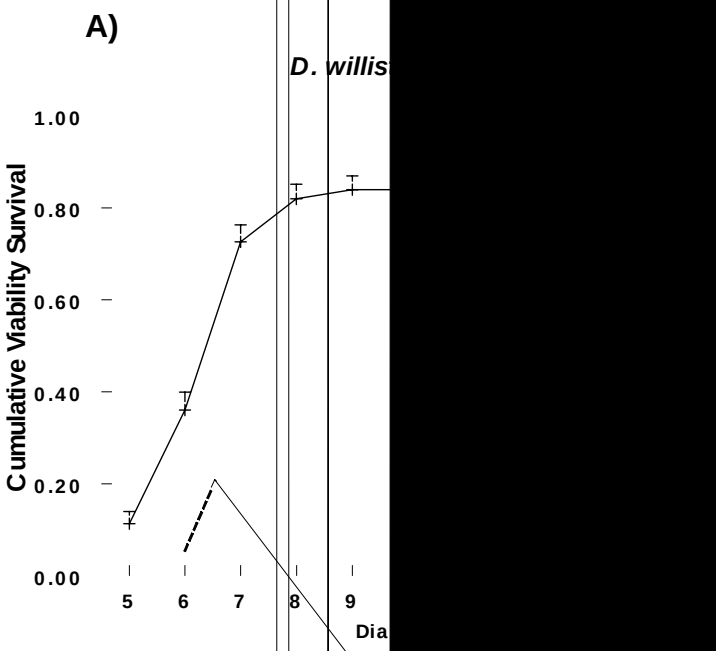

A)
D. willis
1.00
0.80
0.60
0.40
0.20
0.00
Cumulative Viability Survival
5
6
7
8
9
Dia

Figura 3 –

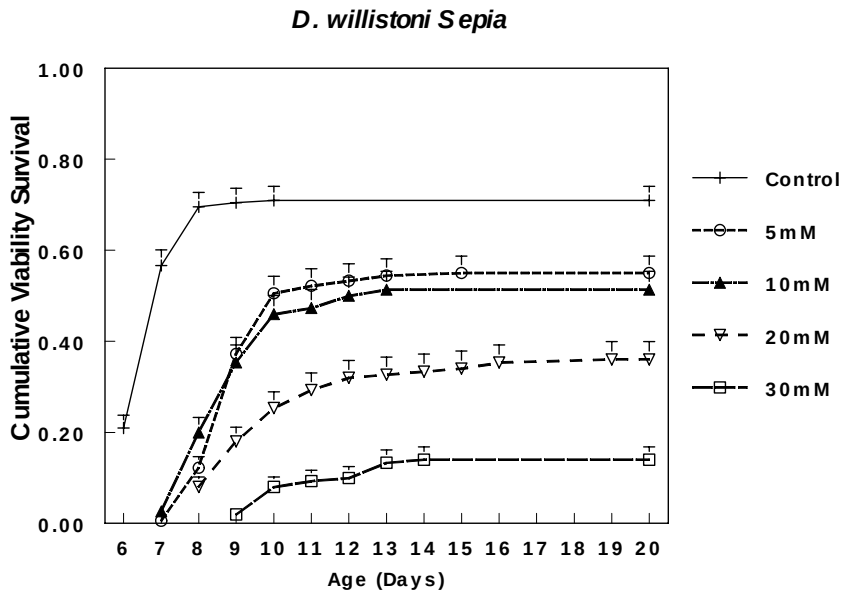
Tabela 5 – *Sepia* *D. willistoni*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
450µmol				

Tabela 6 – *Sepia* *D. willistoni*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
450µmol				

A)



B)

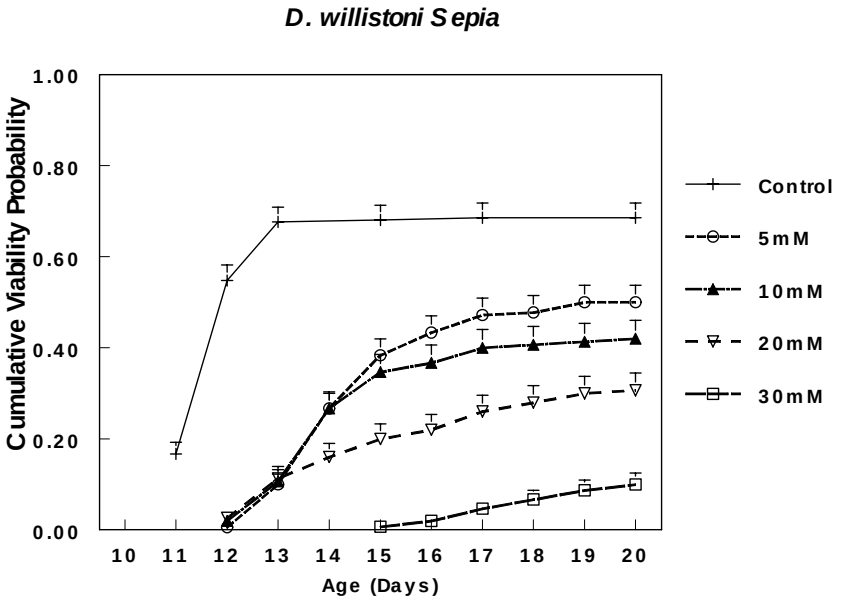
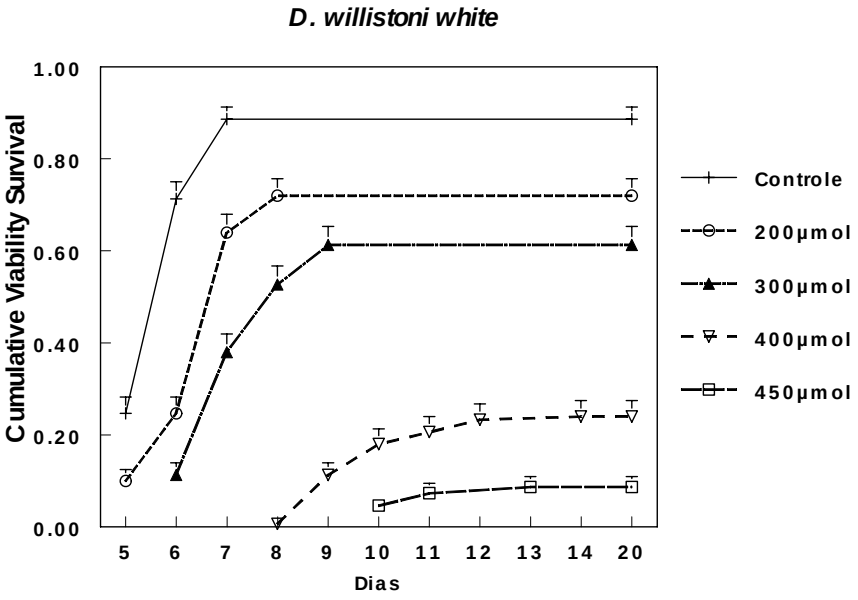


Figura 4 – *Sepia* *D. willistoni*

A) **B)**

A)



B)

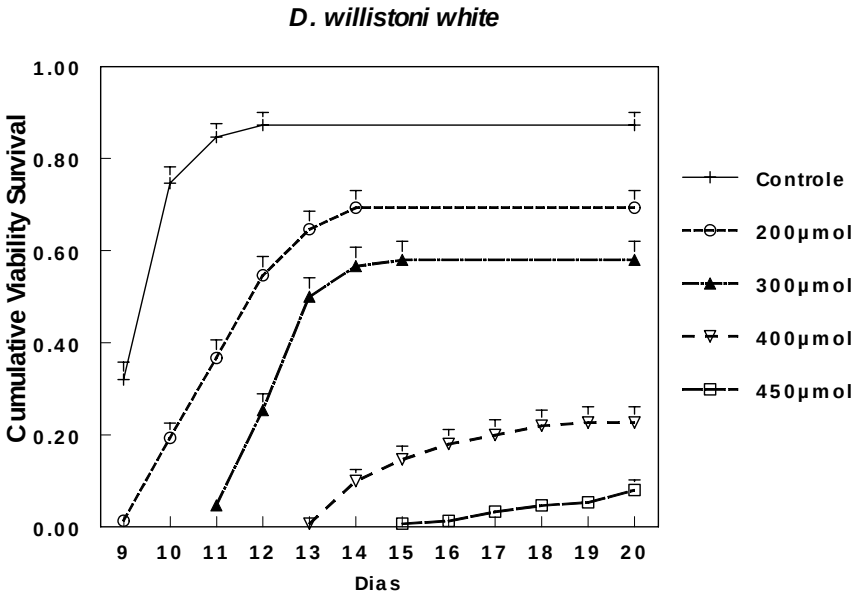


Figura 5 – *white* *D. willistoni*

A) **B)**

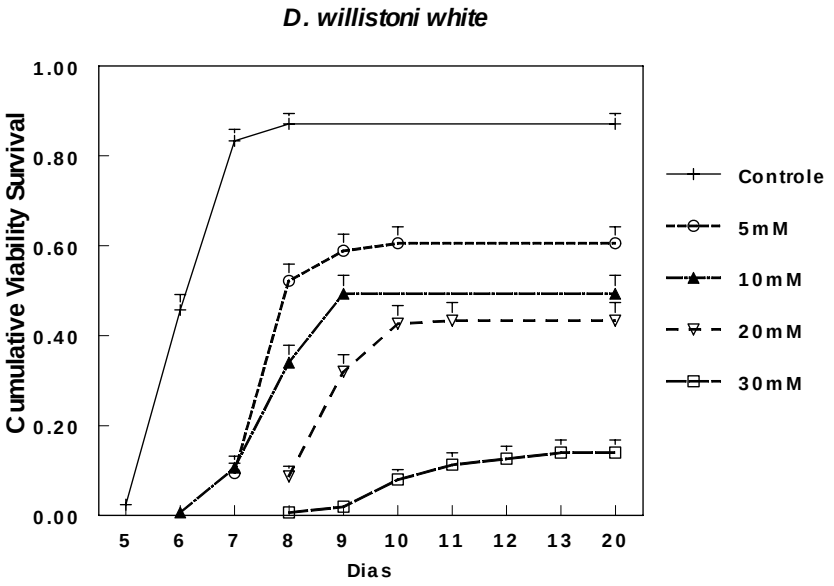
Tabela 9 – *white* *D. willistoni*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
500µmol				

Tabela 10 – *white* *D. willistoni*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
500µmol				

A)



B)

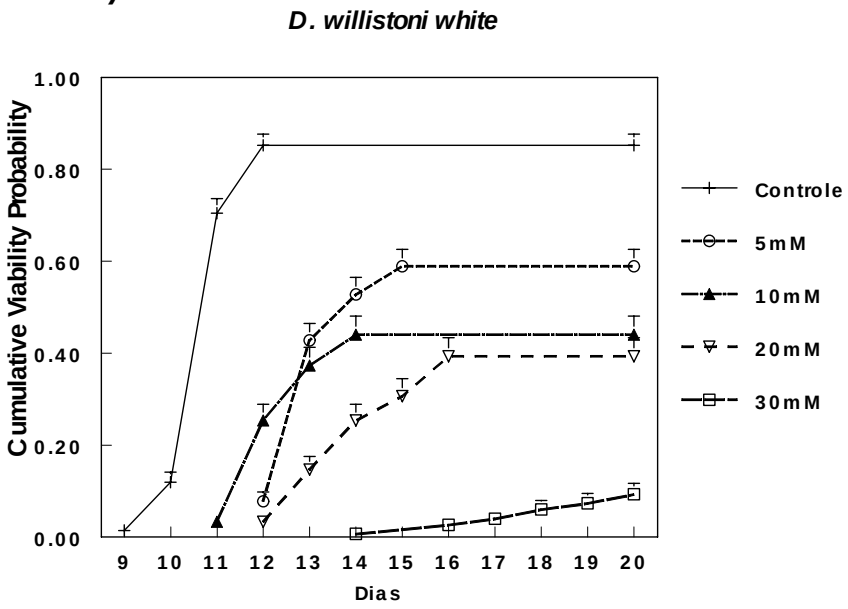


Figura 6 – *white* *D. willistoni*

A) **B)**

Tabela 11 – *white* *D. willistoni*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**
5mM				
10mM				
20mM				
30mM				

Tabela 12 – *white* *D. willistoni*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**
5mM				
10mM				
20mM				
30mM				

A)

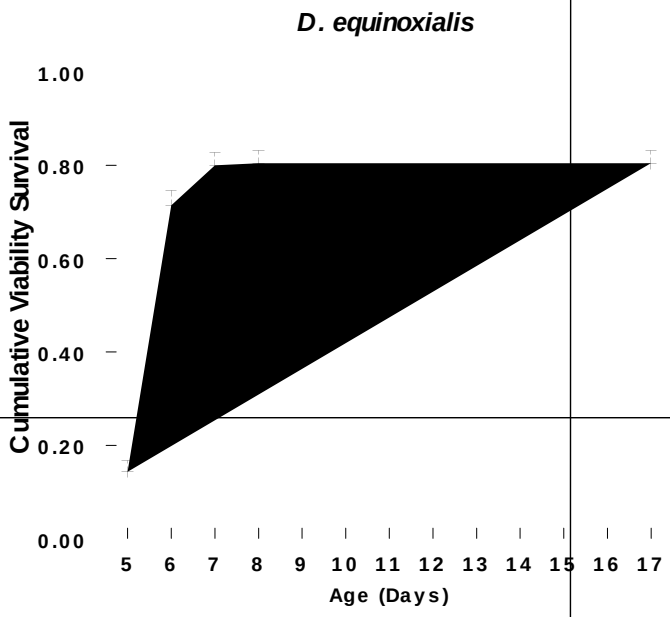

D. equinoxialis
Cumulative Viability Survival
1.00
0.80
0.60
0.40
0.20
0.00
5 6 7 8 9 10 11 12 13 14 15 16 17
Age (Days)

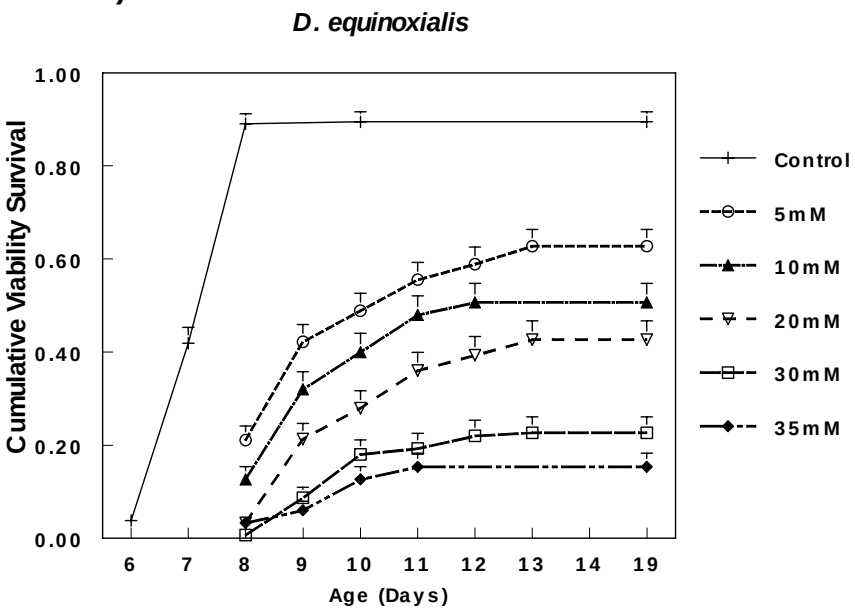
Tabela 13 – *D. equinoxialis*

Tratamento	**Controle**	**100µmol**	**200µmol**	**300 µmol**	**400 µmol**	**500 µmol**
100µmol						
200µmol						
300 µmol						
400 µmol						
500µmol						
600µmol						

Tabela 14 – *D. equinoxialis*

Tratamento	**Controle**	**100µmol**	**200µmol**	**300 µmol**	**400 µmol**	**500 µmol**
100µmol						
200µmol						
300 µmol						
400 µmol						
500µmol	**1**		**0**		**9**	**,**
600µmol						

A)



B)

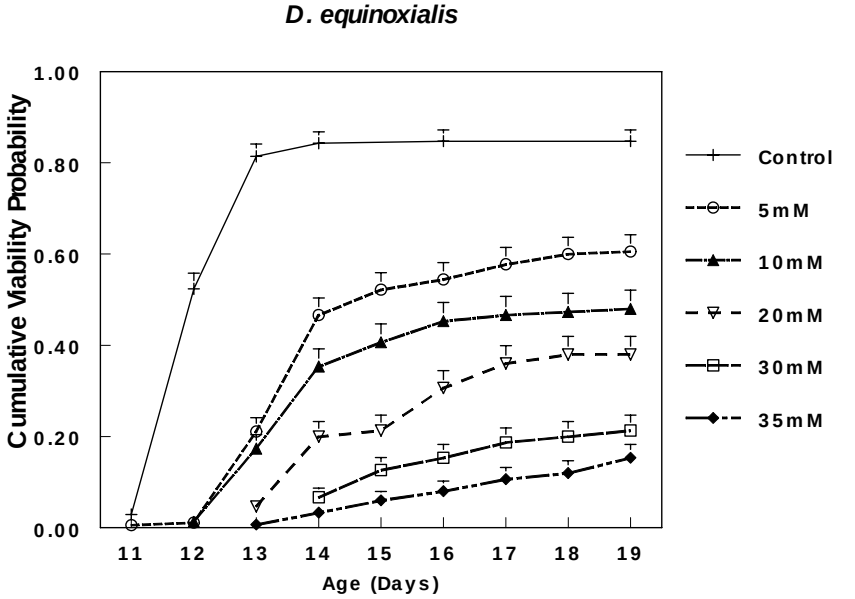


Figura 8 – *D. equinoxialis*

A) **B)**

Tabela 15 – *D. equinoxialis*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**
5mM					
10mM					
20mM					
30mM					
35mM					

Tabela 16 – *D. equinoxialis*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**
5mM					
10mM					
20mM					
30mM					
35mM					

A)

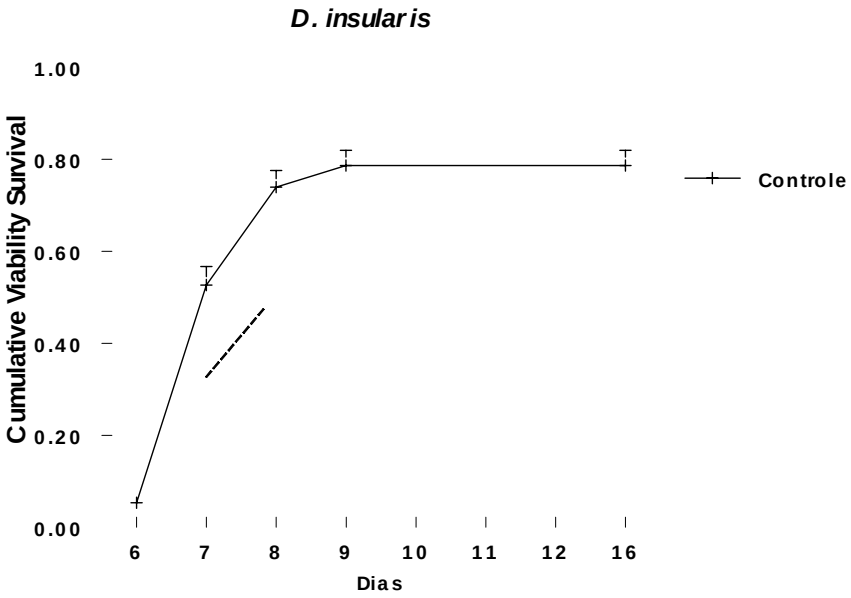

D. insularis
Cumulative Viability Survival
1.00
0.80
0.60
0.40
0.20
0.00
6
7
8
9
10
11
12
16
Dias
Controle

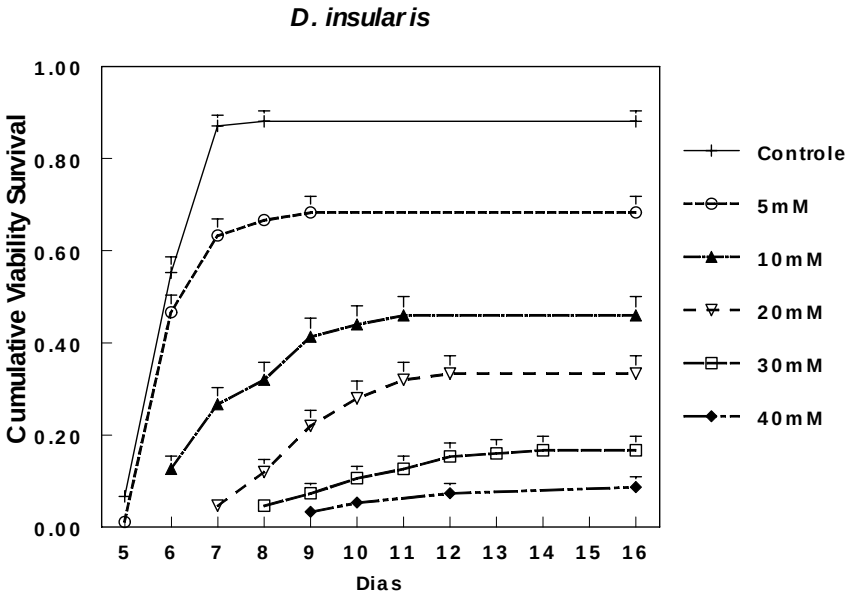
Tabela 17 – *D. insularis*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
500µmol				

Tabela 18 – *D. insularis*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
500µmol				

A)



B)

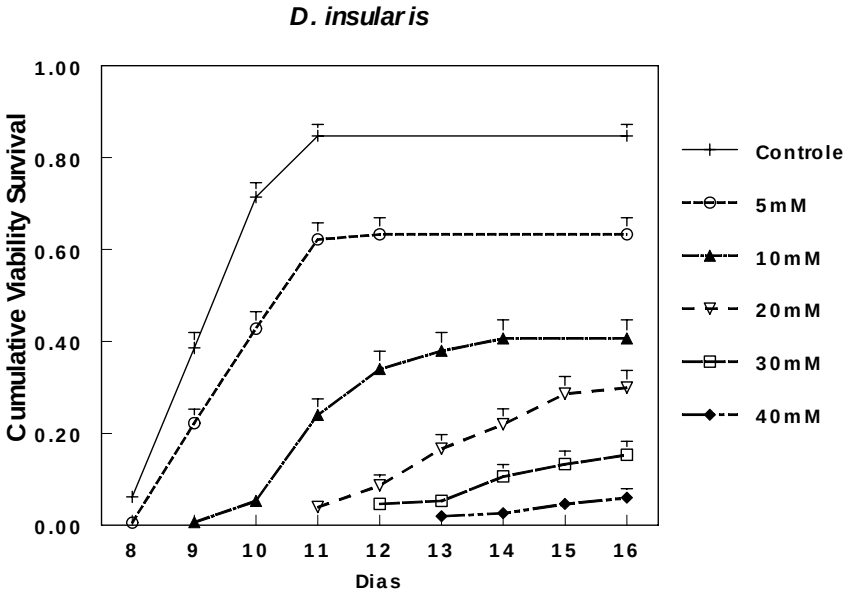


Figura 10 – *D. insularis*

A) **B)**

Tabela 19 – *D. insularis*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**
5mM					
10mM					
20mM					
30mM					
40mM					

Tabela 20 – *D. insularis*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**
5mM					
10mM					
20mM					
30mM					
40mM					

A)

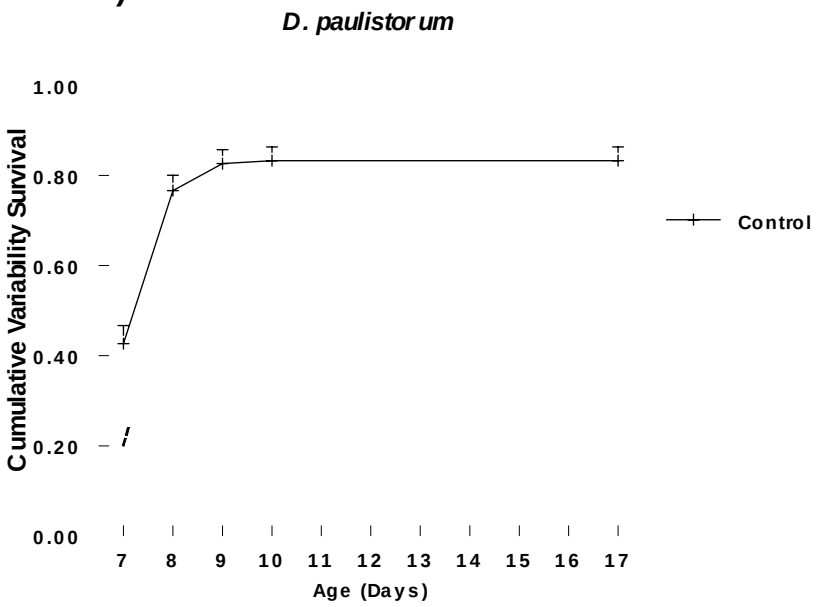

D. paulistorum
1.00
0.80
0.60
0.40
0.20
0.00
Cumulative Variability Survival
7
8
9
10
11
12
13
14
15
16
17
Age (Days)
Control

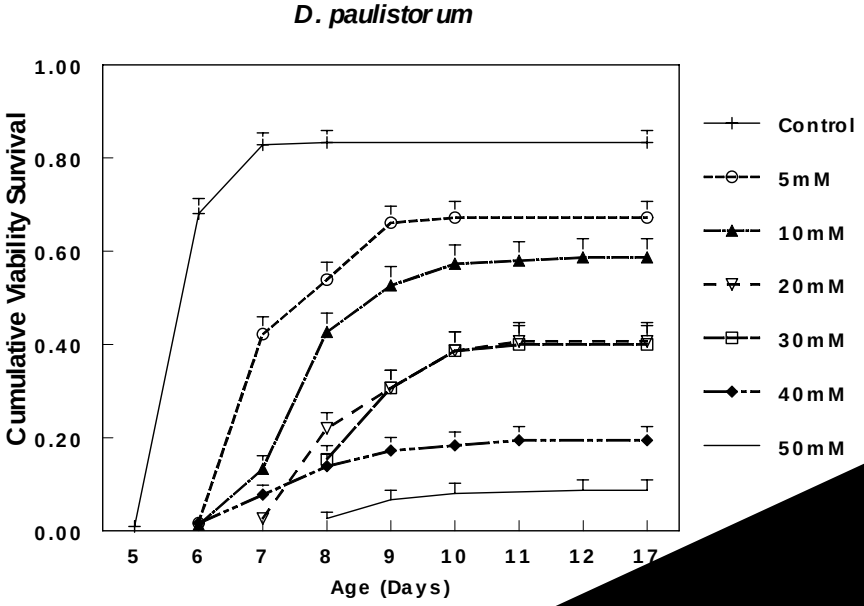
Tabela 21 – *D. paulistorum*

Tratamento	**Controle**	**300µmol**	**400 µmol**
300µmol			
400 µmol			
500µmol			

Tabela 22 – *D. paulistorum*

Tratamento	**Controle**	**300µmol**	**400 µmol**
300µmol			
400 µmol			
500µmol			

A)


D. paulistorum
Cumulative Viability Survival
1.00
0.80
0.60
0.40
0.20
0.00
5
6
7
8
9
10
11
12
17
Age (Days)
Control
5mM
10mM
20mM
30mM
40mM
50mM

B)

D. pauli

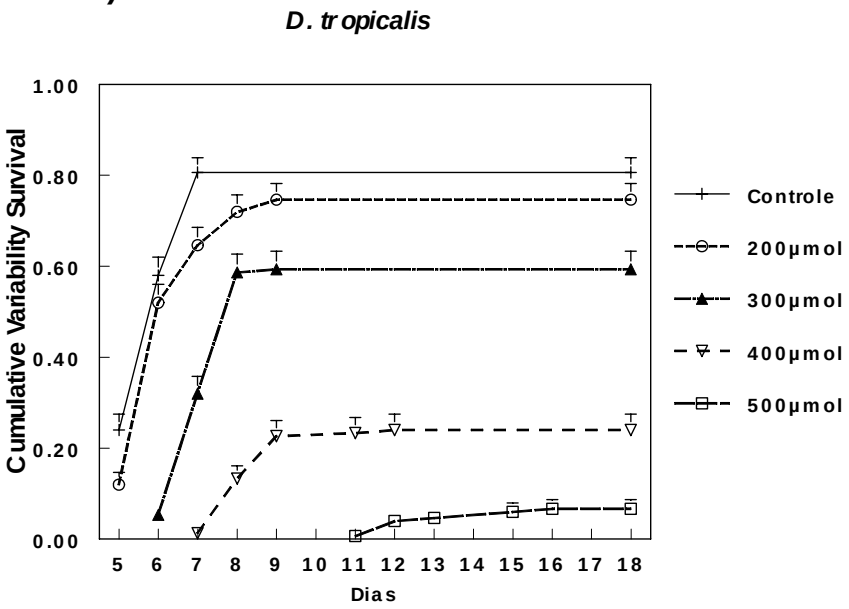
Tabela 23 – *D. paulistorum*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**	**40mM**
5mM						
10mM						
20mM						
30mM						
40mM						
50mM						

Tabela 24 – *D. paulistorum*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**	**40mM**
5mM						
10mM						
20mM						
30mM						
40mM						
50mM						

A)



B)

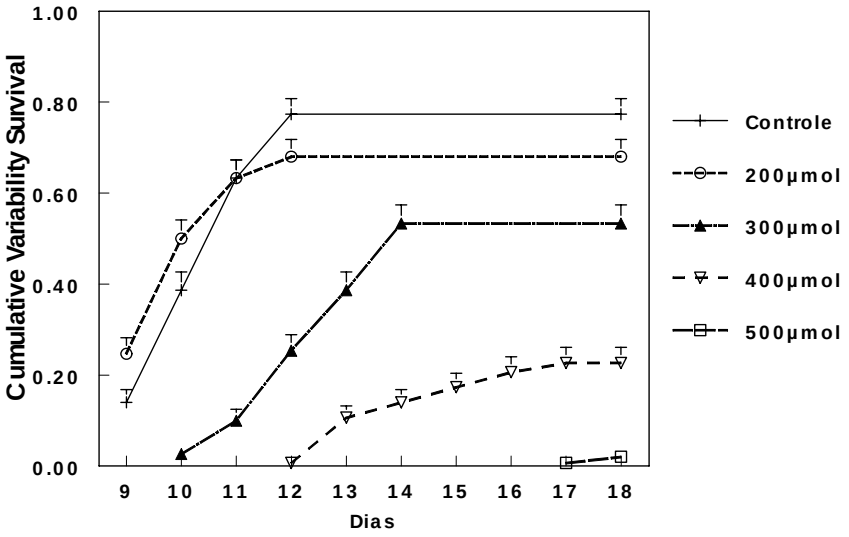


Figura 13 – *D. tropicalis*

A) **B)**

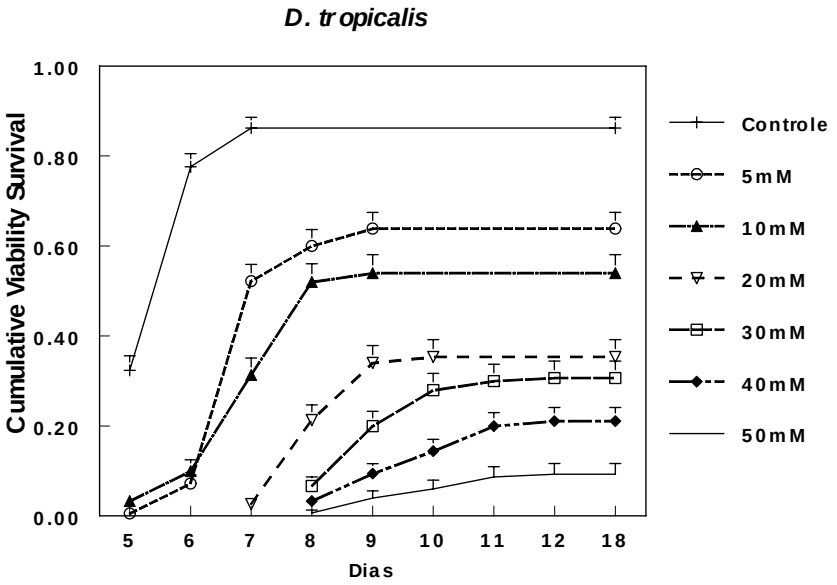
Tabela 25 – *D. tropicalis*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
500µmol				

Tabela 26 – *D. tropicalis*

Tratamentos	**Controle**	**200µmol**	**300µmol**	**400 µmol**
200µmol				
300µmol				
400 µmol				
500µmol				

A)



B)

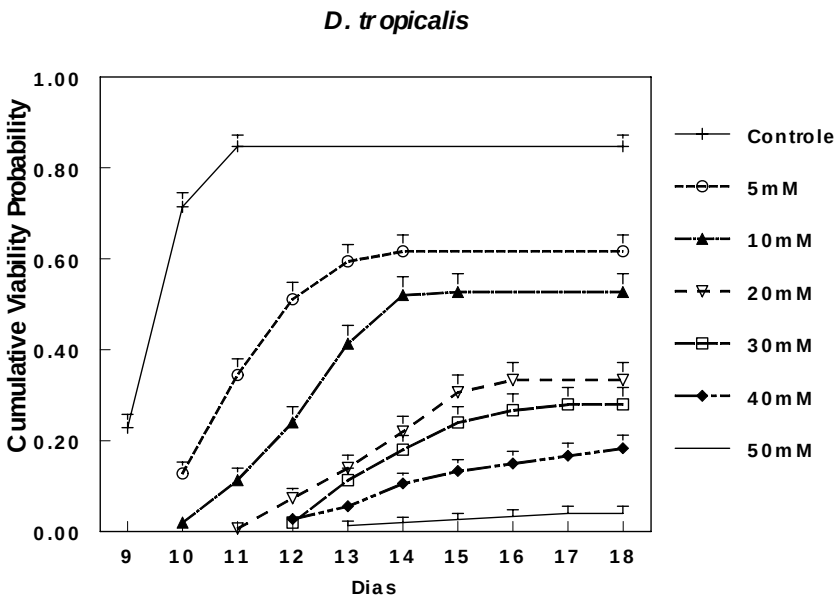


Figura 14 – *D. tropicalis*

A) **B)**

Tabela 27 – *D. tropicalis*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**	**40mM**
5mM						
10mM						
20mM						
30mM						
40mM						
50mM						

Tabela 28 – *D. tropicalis*

Tratamento	**Controle**	**5mM**	**10mM**	**20mM**	**30mM**	**40mM**
5mM						
10mM						
20mM						
30mM						
40mM						
50mM						

III.4. Resultados sobre a expressão de Hsp70 e Hsp83 nos mutantes *Sepia* e *white* de *Drosophila willistoni*

III.4.1. Estudo da expressão gênica dos genes de hsp70 e hsp83 em mutantes *Sepia* e *white* de *D. willistoni*

hsp70 *hsp83*

β

Sepia

D. willistoni β

Real Time PCR,

β

Heat

Shock

primers

β

primers

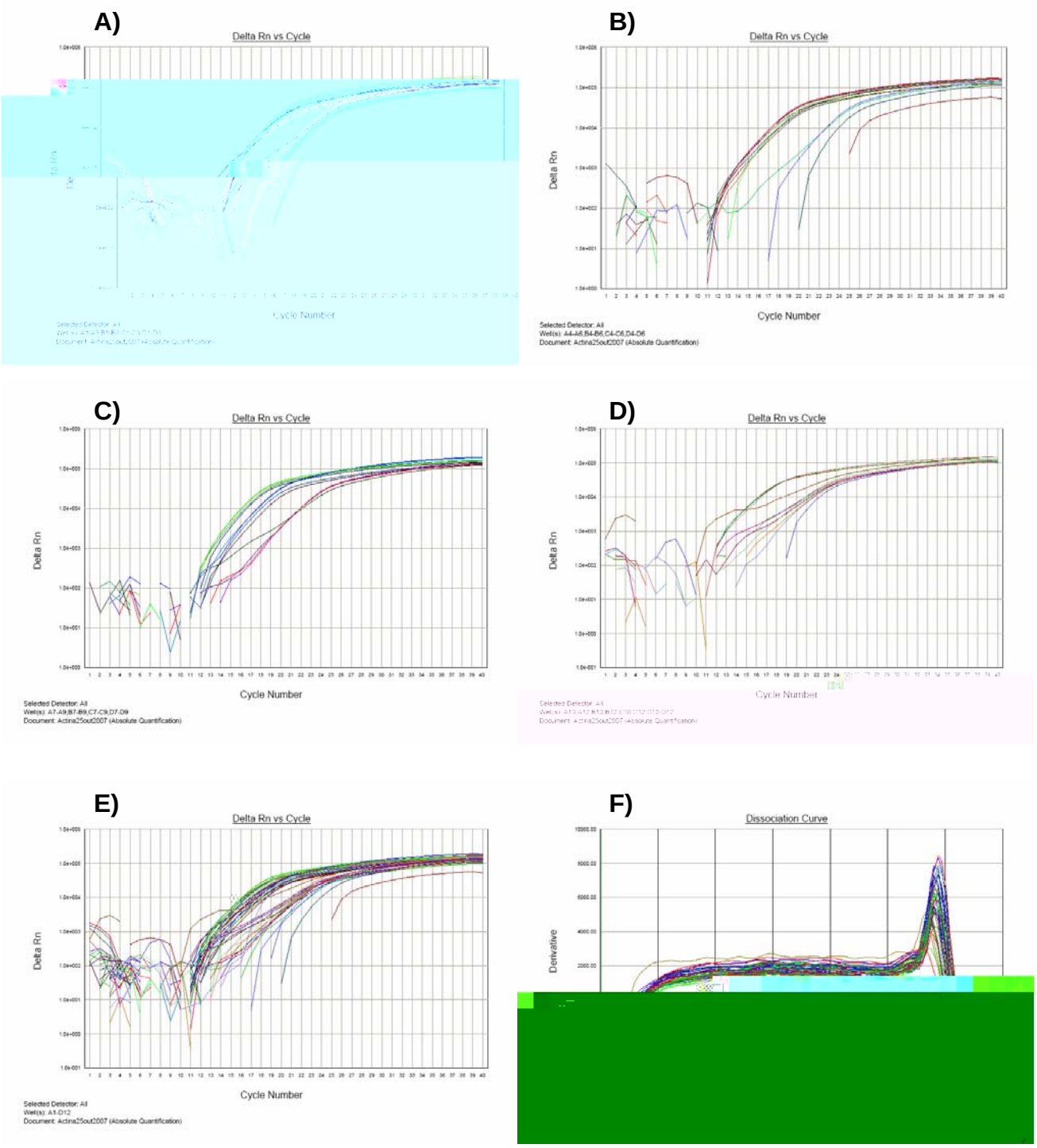


Figura 1) *Real Time PCR* **β-Actina** *D. willistoni Sepia* **A)** **B)** **C)** **D)** *Heat Shock* **E)** **F)**

β *white*

D. willistoni

β

β

primers

β

primers

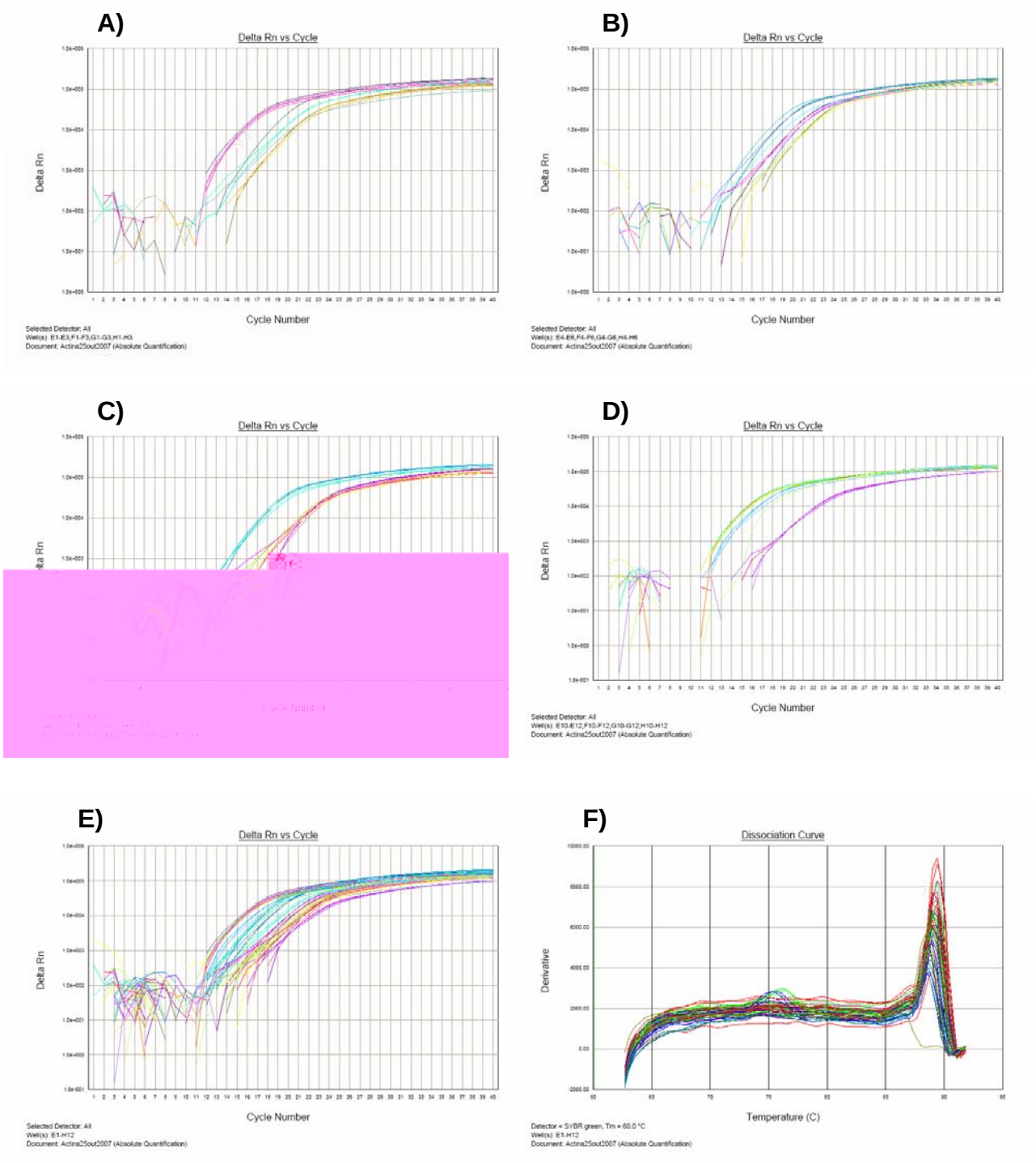


Figura 2) *Real Time PCR* **β-Actina** *D. willistoni white* **A)** **B)** **C)** **D)** *Heat Shock* **E)** **F)**

Sepia *D. willistoni*

Real Time PCR,

primers

Real Time PCR

white *D. willistoni*

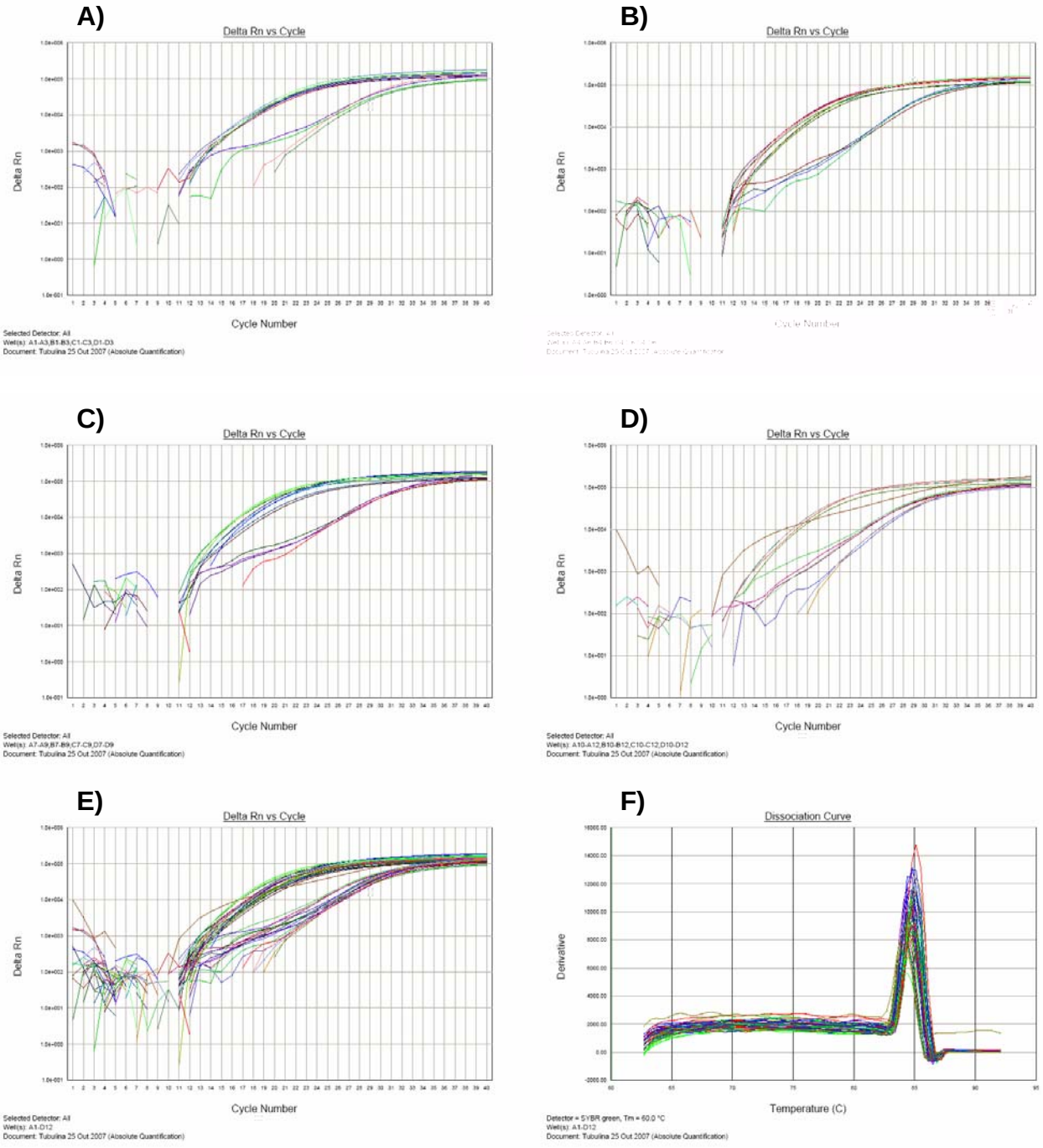


Figura 3) *Real Time PCR* **Tubulina** *D. willistoni Sepia* **A) B) C) D)** *Heat Shock* **E) F)**

hsp70 *Sepia* *D. willistoni* *Real Time PCR,*

hsp70

hsp70

hsp70

primers

hsp70 *white* *D. willistoni*

hsp70

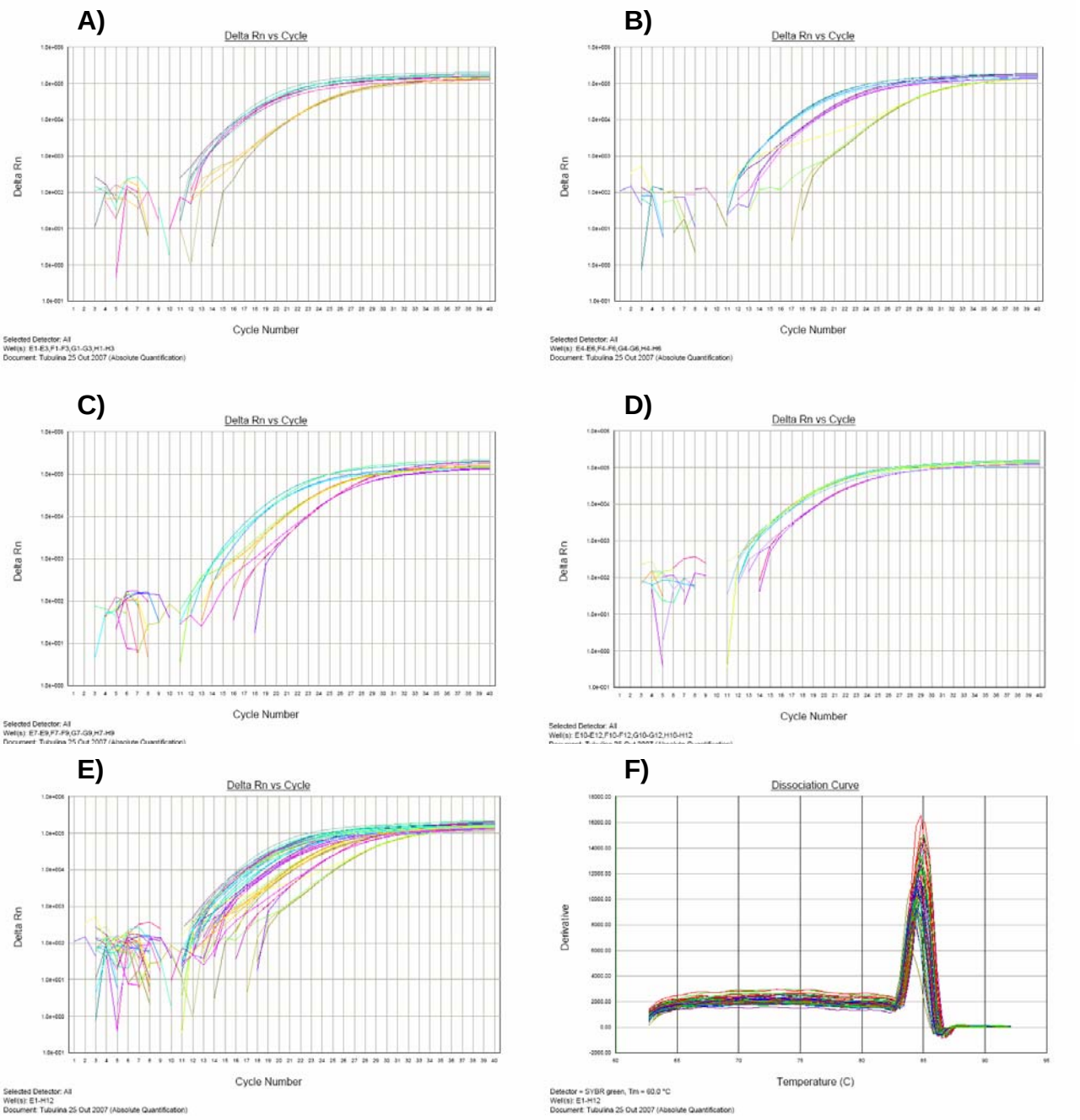


Figura 4) *Real Time PCR* **Tubulina** *D. willistoni white* **A)** **B)** **C)** **D)** *Heat Shock* **E)** **F)**

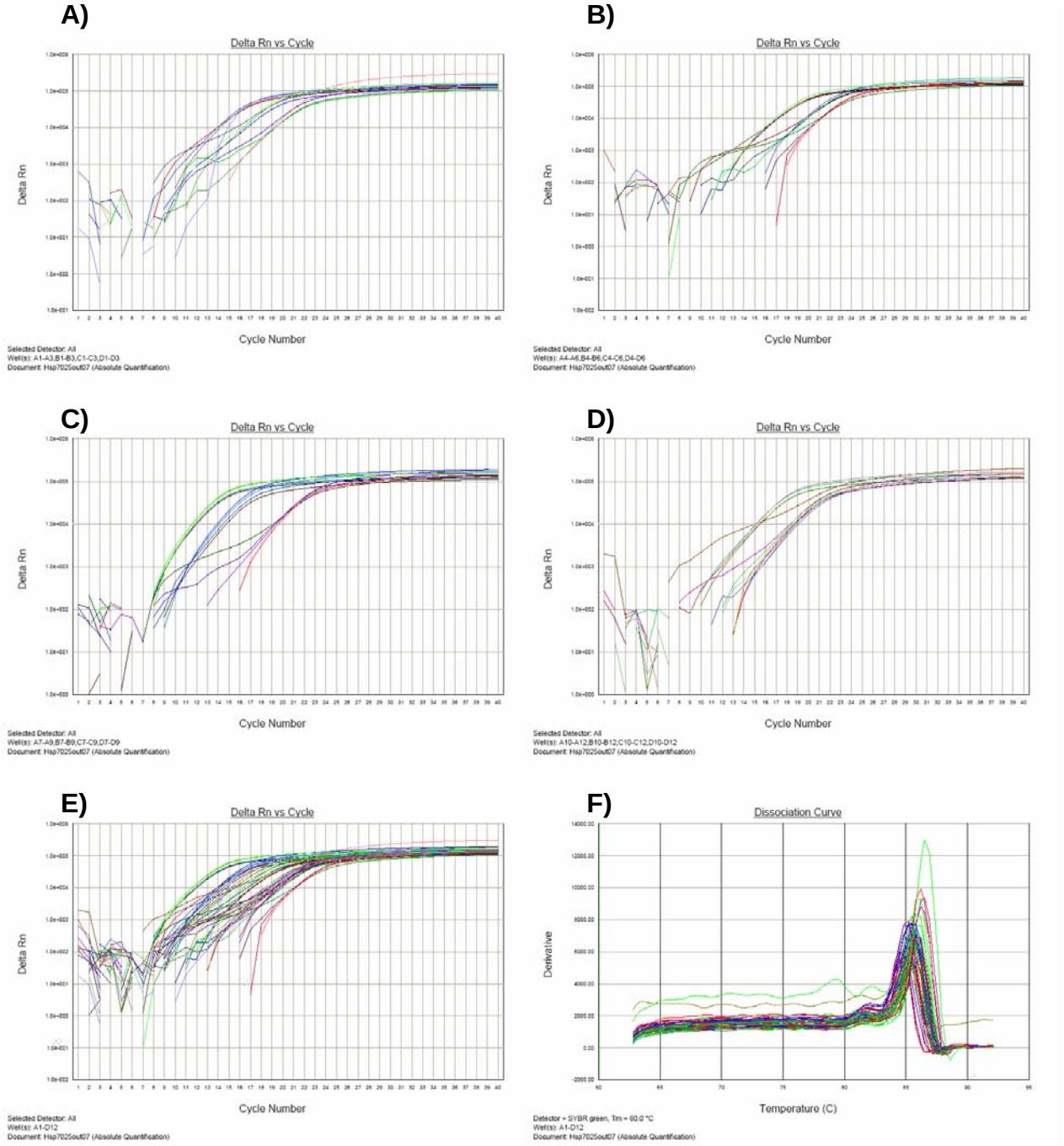


Figura 5) *Real Time PCR* ***hsp70*** *D. willistoni Sepia* **A)** **B)** **C)** **D)** *Heat Shock* **E)** **F)**

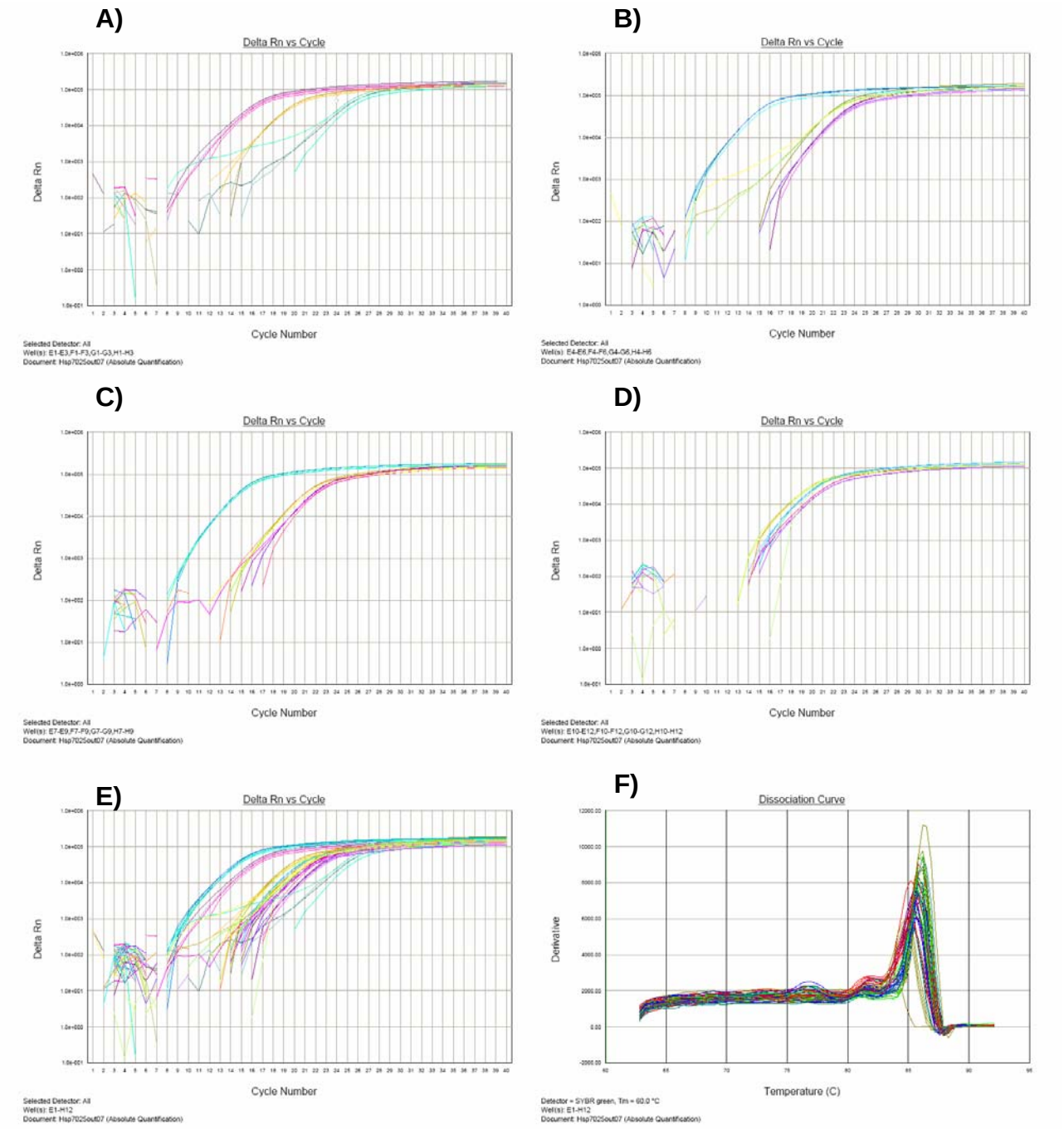


Figura 6) *Real Time PCR* ***hsp70*** *D. willistoni white* **A)** **B)** **C)** **D)** *Heat Shock* **E)** **F)**

Heat Shock

primers

hsp83 *Sepia* *D. willistoni* *Real Time PCR,*
hsp83

hsp83

Real Time PCR

Heat Shock

hsp83

hsp83 *white* *D. willistoni* *Real Time PCR,*

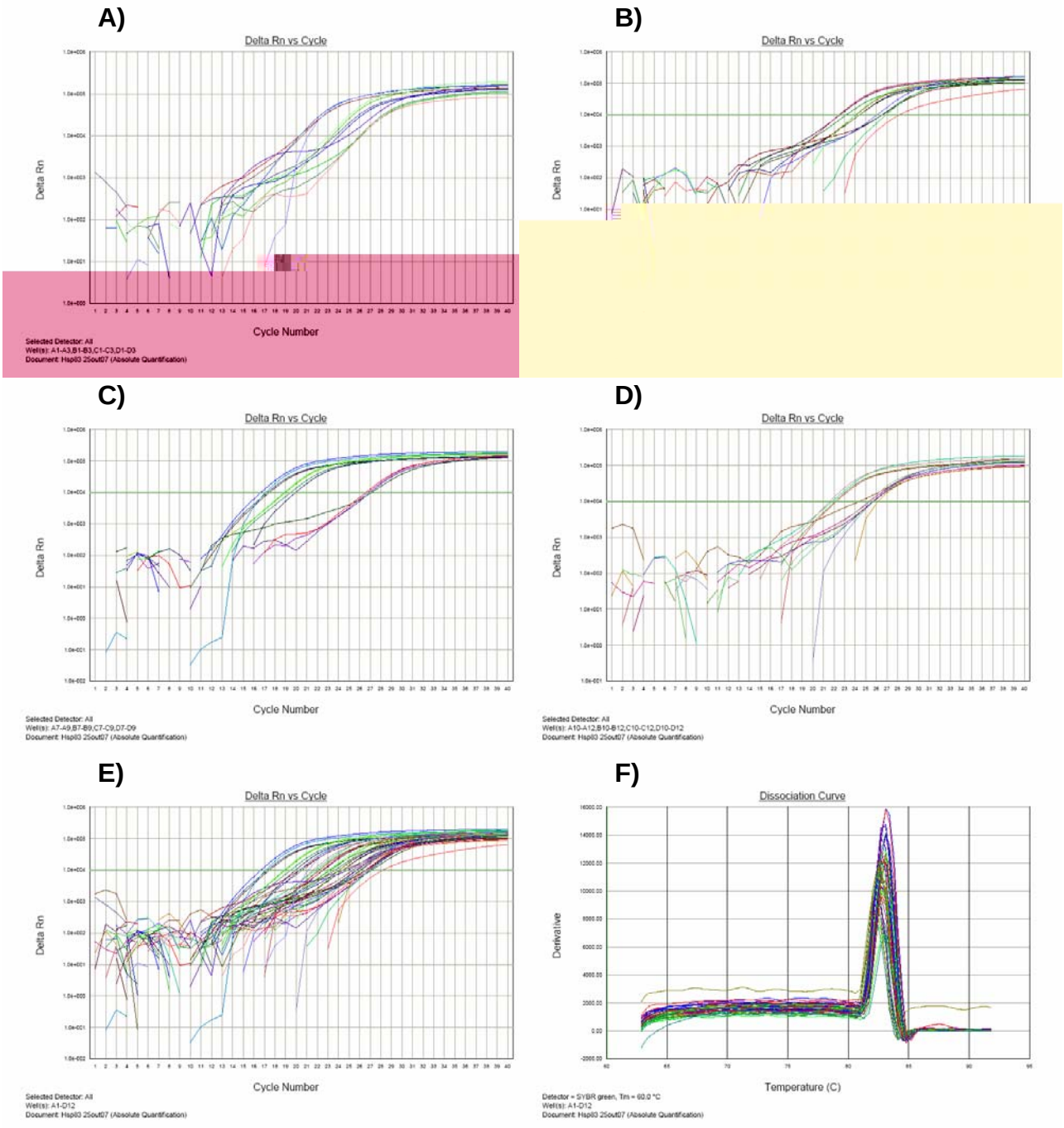


Figura 7) *Real Time PCR* ***hsp83*** *D. willistoni Sepia* **A)** **B)** **C)** **D)** *Heat Shock* **E)** **F)**

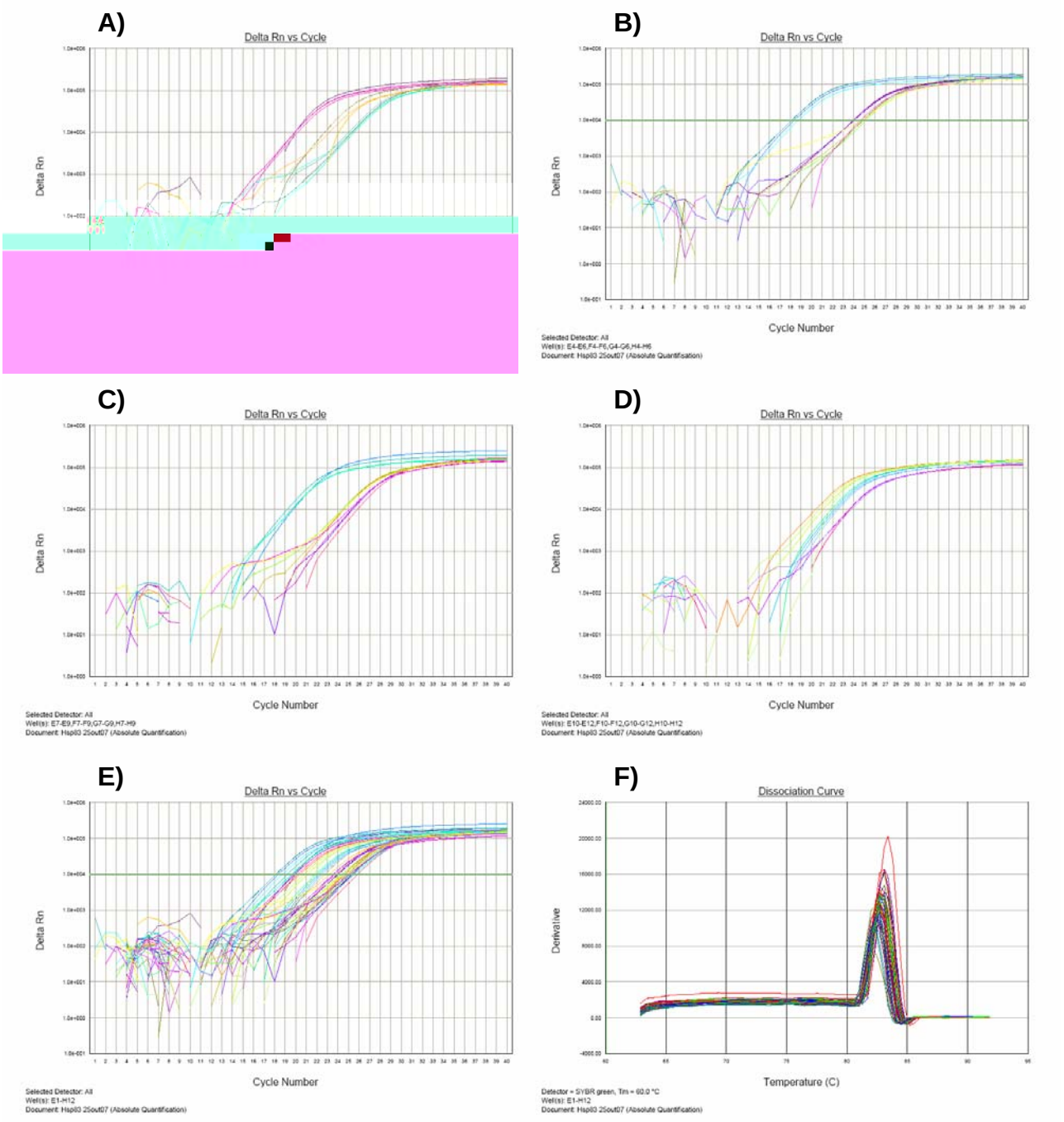


Figura 8) *Real Time PCR* ***hsp83*** *D. willistoni white* **A)** **B)** **C)** **D)** *Heat Shock* **E)** **F)**

hsp83

primers *hsp83*

primers

P

Sepia *white* *D. willistoni*
primers

hsp70

Sepi

heat shock
hsp70 *white*

heat shock

hsp70

β *Sepia,*

heat shock
white

heat shock.

hsp70

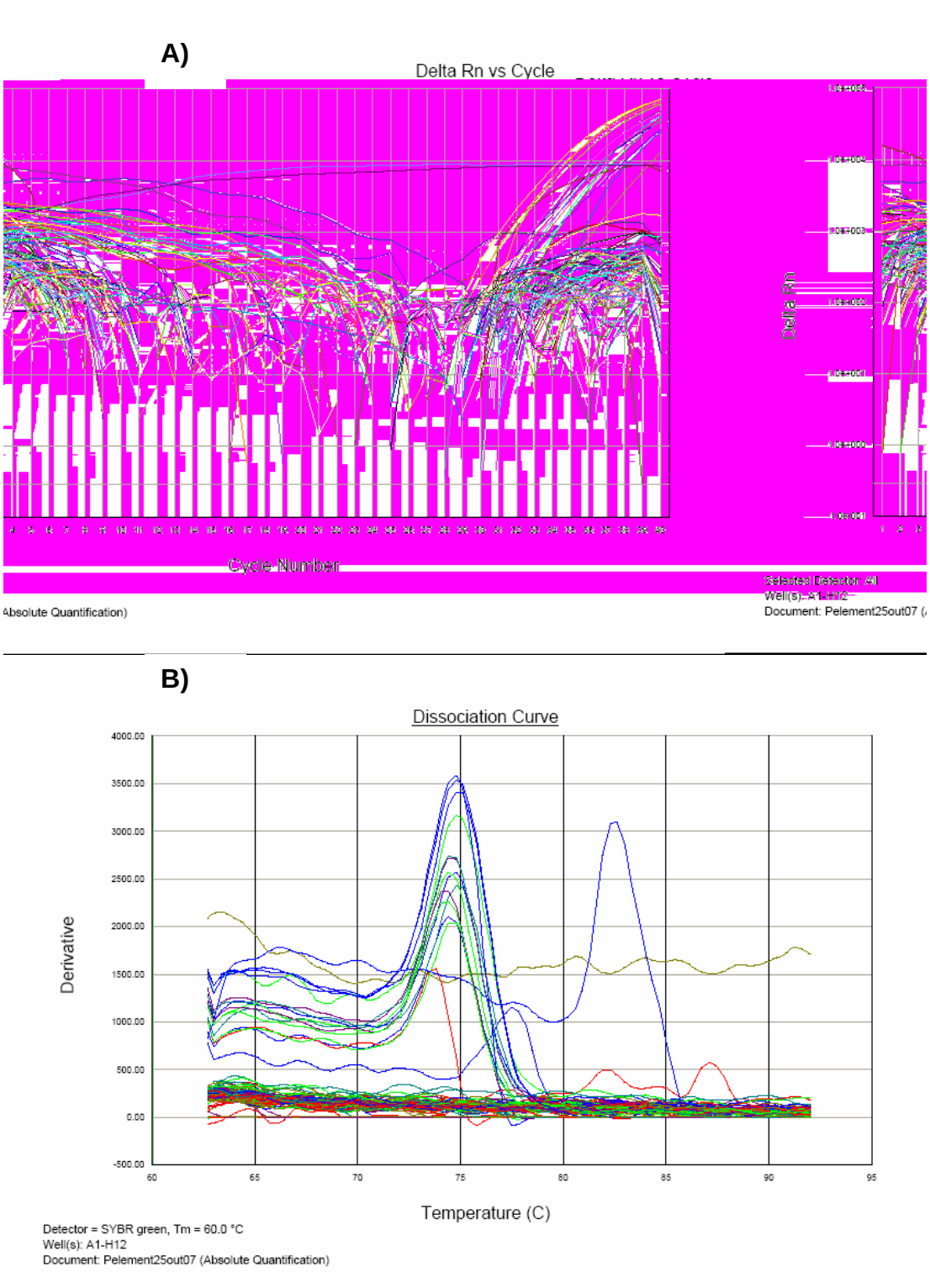
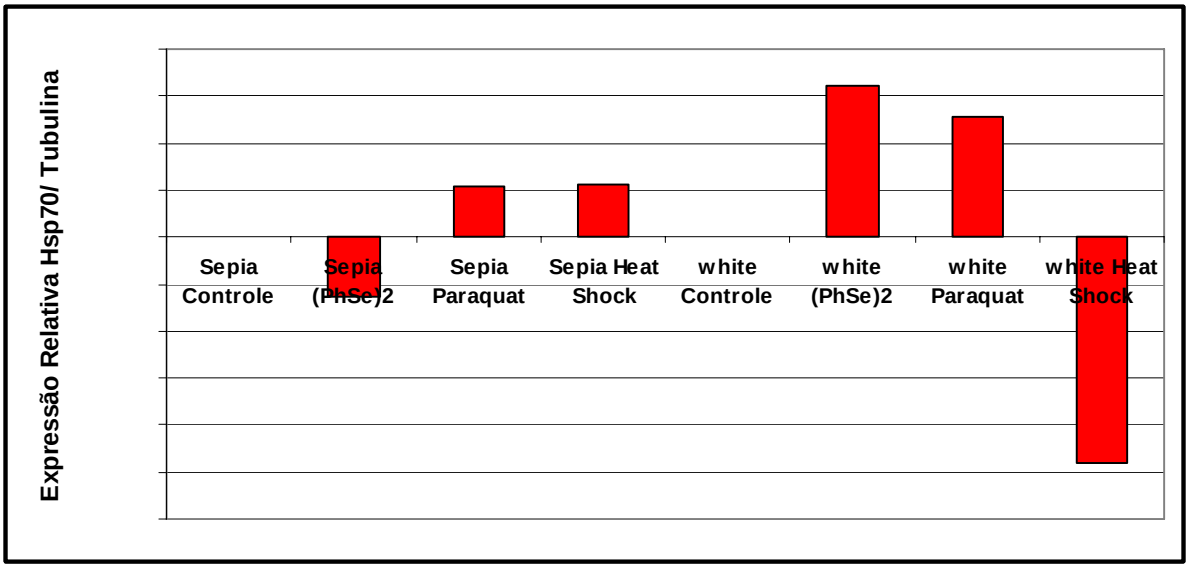


Figura 9) *Real Time PCR* *P*

A)

Sepia *white* **B)**

A)



B)

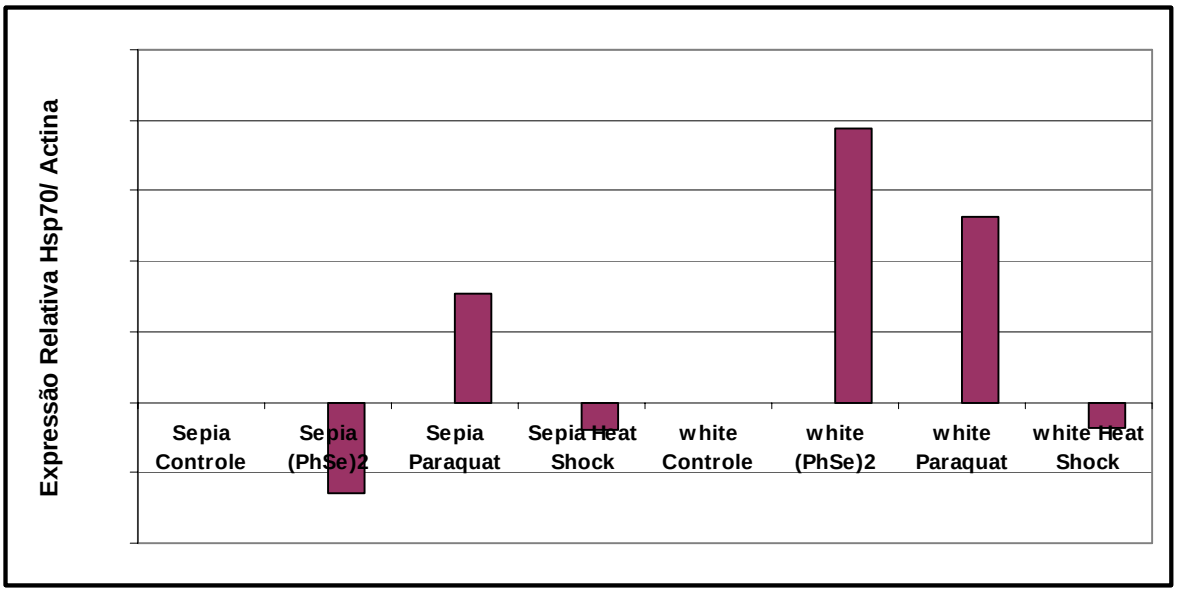


Figura 10 – ***hsp70*** **A)** **tubulina** **B)** **β- actina**

Tabela 1 - *hsp70*

		***Hsp70*/tubulina**	***Hsp70*/β-actina**
Sepia	**Controle**		
	$(PhSe)_2$		
	Paraquat		
	Heat Shock		
white	**Controle**		
	$(PhSe)_2$		
	Paraquat		
	Heat Shock		

*****

hsp83

β

Sepia

heat shock

white

heat shock

β

Sepia *heat shock*

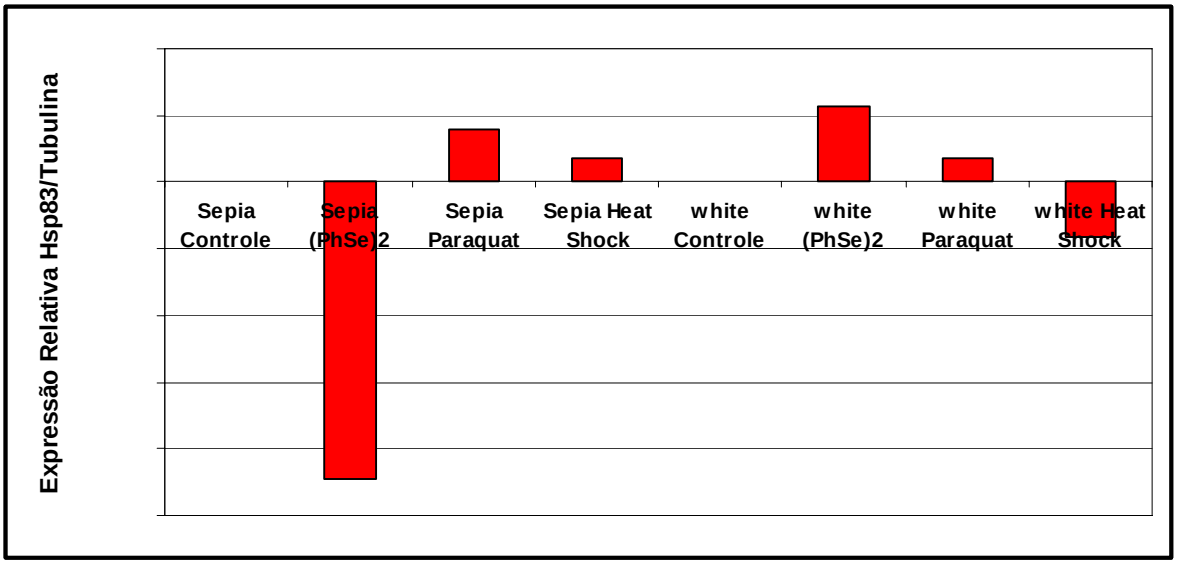
hsp83

white

heat shock

hsp70

A)



B)

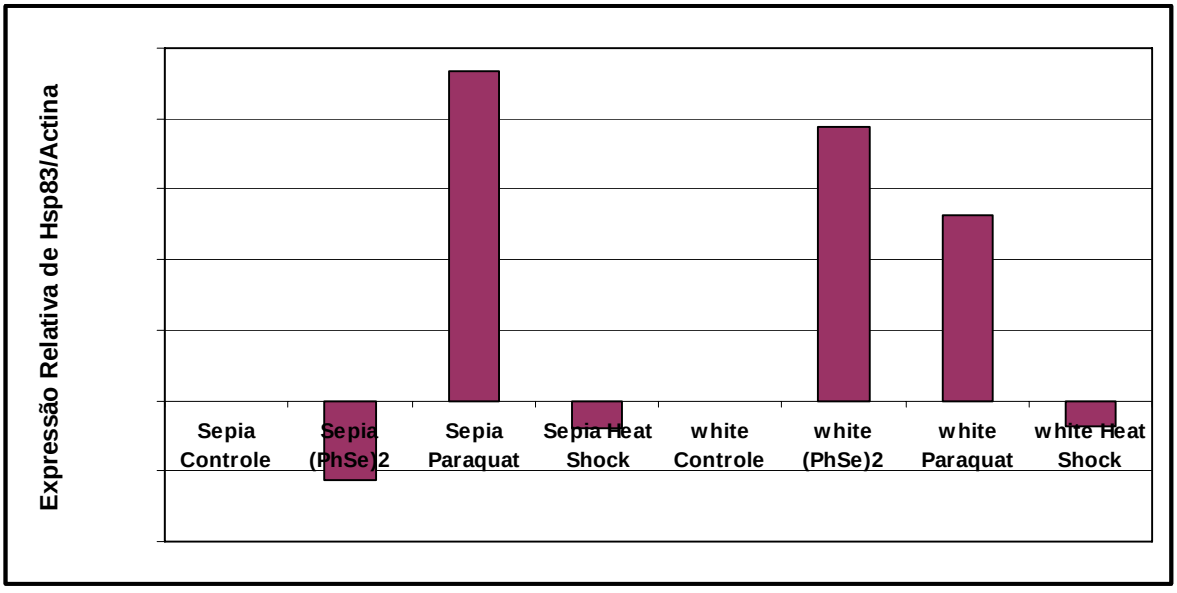


Figura 11 - ***hsp83*** **A)**

tubulina B)

β- actina

Tabela 2 - *hsp83*

		***Hsp83*/tubulina**	***Hsp83*/β-actina**
Sepia	**Controle**		
	$(PhSe)_2$		
	Paraquat		
	Heat Shock		
white	**Controle**		
	$(PhSe)_2$		
	Paraquat		
	Heat Shock		

*****

Análise da expressão do elemento *P* em tecido gonadal dos mutantes de *D. willistoni Sepia* e *white*.

P

β

P

β

Sepia *D. willistoni* β

primers

β

primers

β

white *D. willistoni* β

β

β

primers β

primers

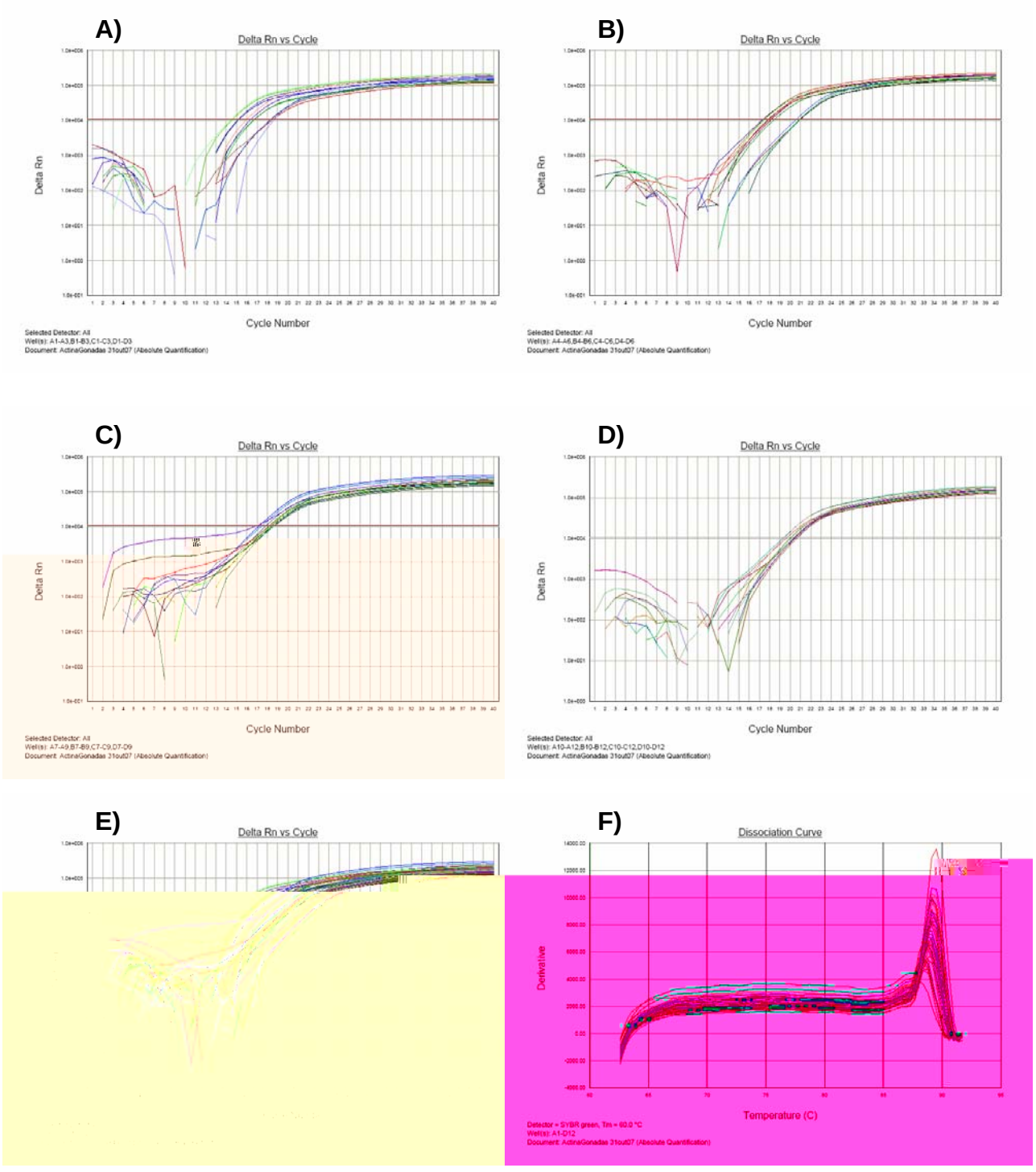


Figura 12 - *Real Time PCR* **β-Actina** *D. willistoni Sepia* **A) B) C) D)** *Heat Shock* **E) F)**

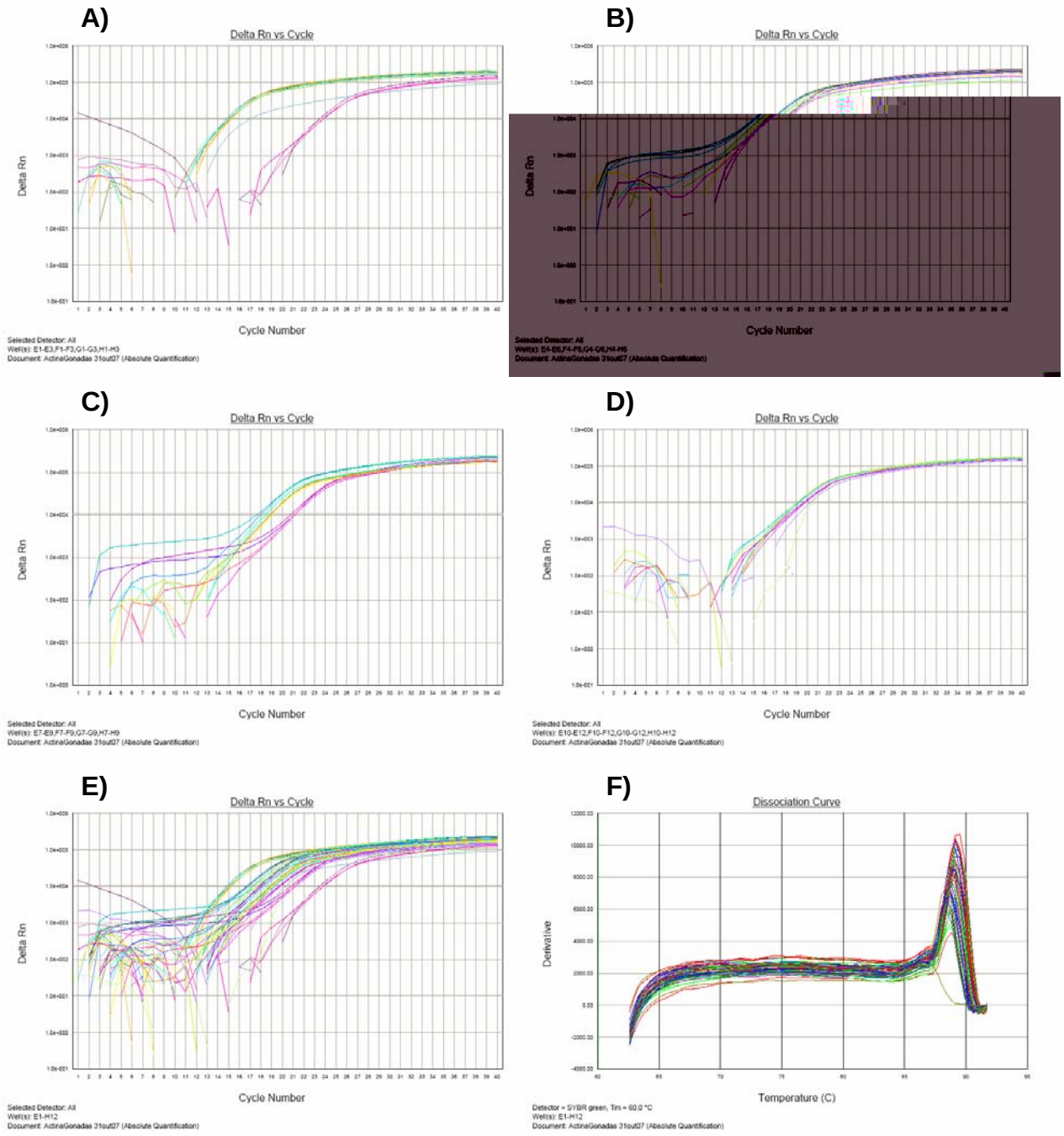


Figura 13 - *Real Time PCR* **β-Actina** *D. willistoni white* **A) B) C) D)** *Heat Shock* **E) F)**

Sepia *D. willistoni*

white *D. willistoni*

P

D. willistoni Sepia white

primers

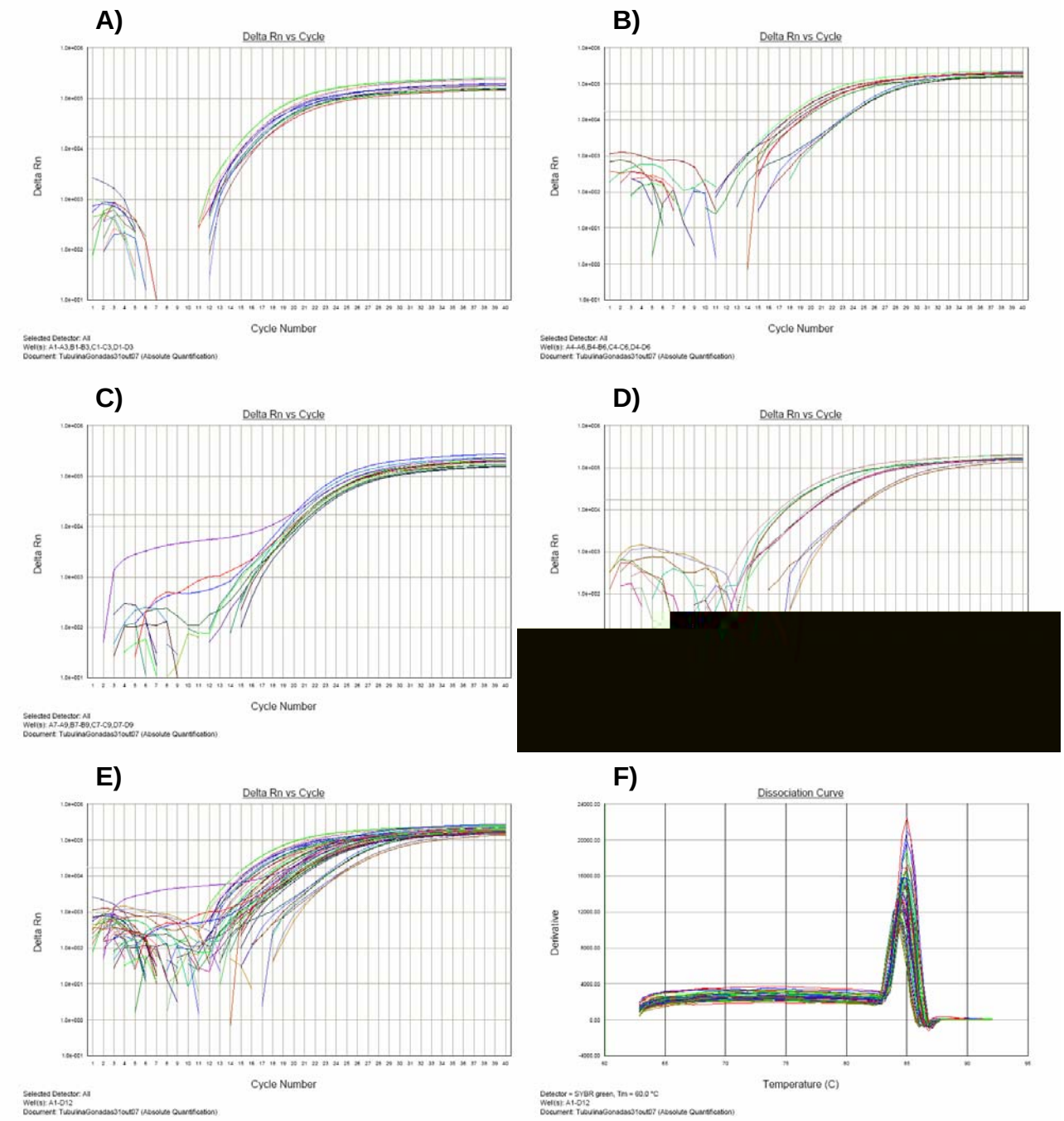


Figura 14 - *Real Time PCR* **Tubulina** *D. willistoni Sepia* **A) B) C) D)** *Heat Shock* **E) F)**

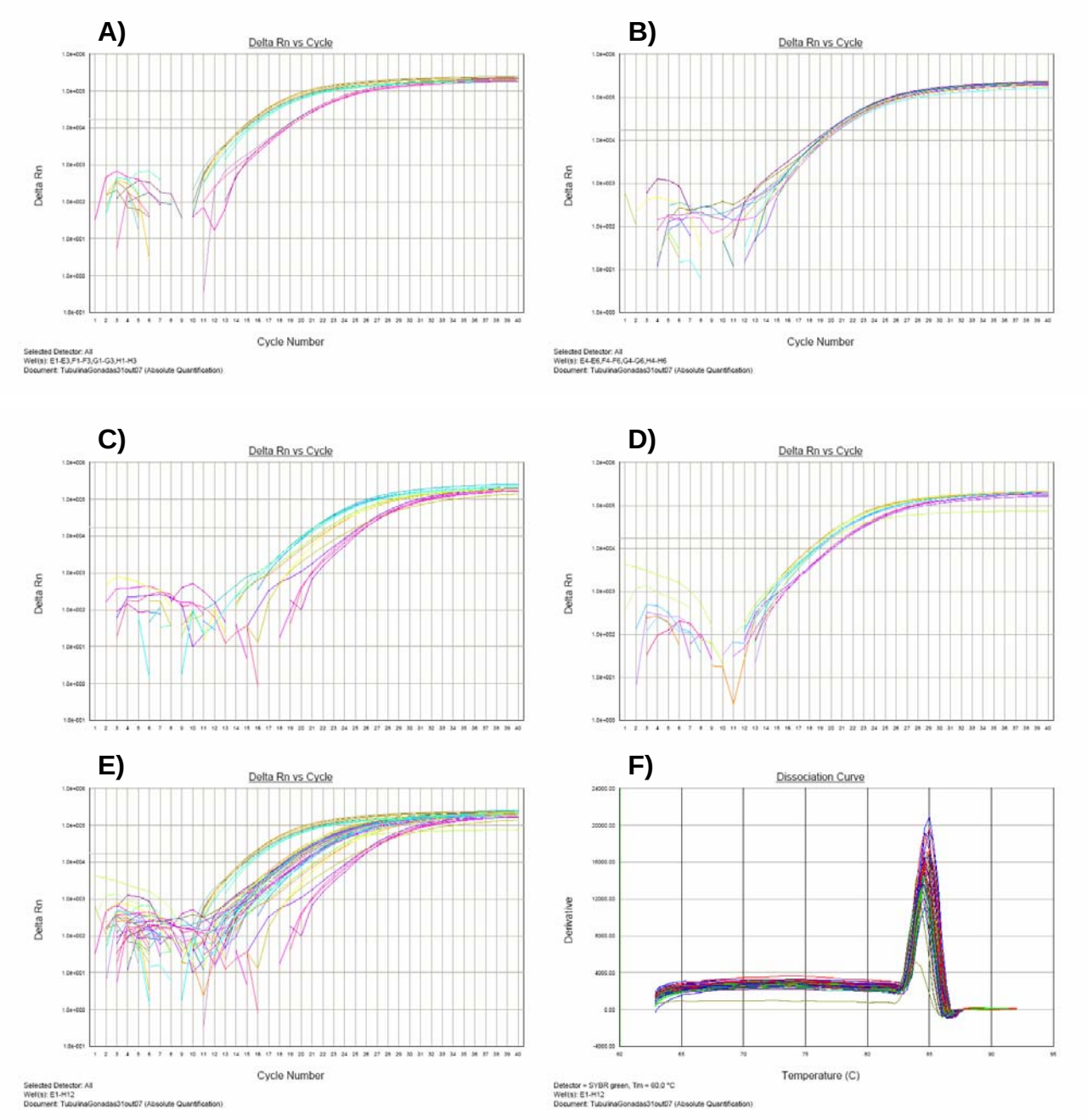


Figura 15 - *Real Time PCR* **Tubulina** *D. willistoni white* **A) B) C) D)** *Heat Shock* **E) F)**

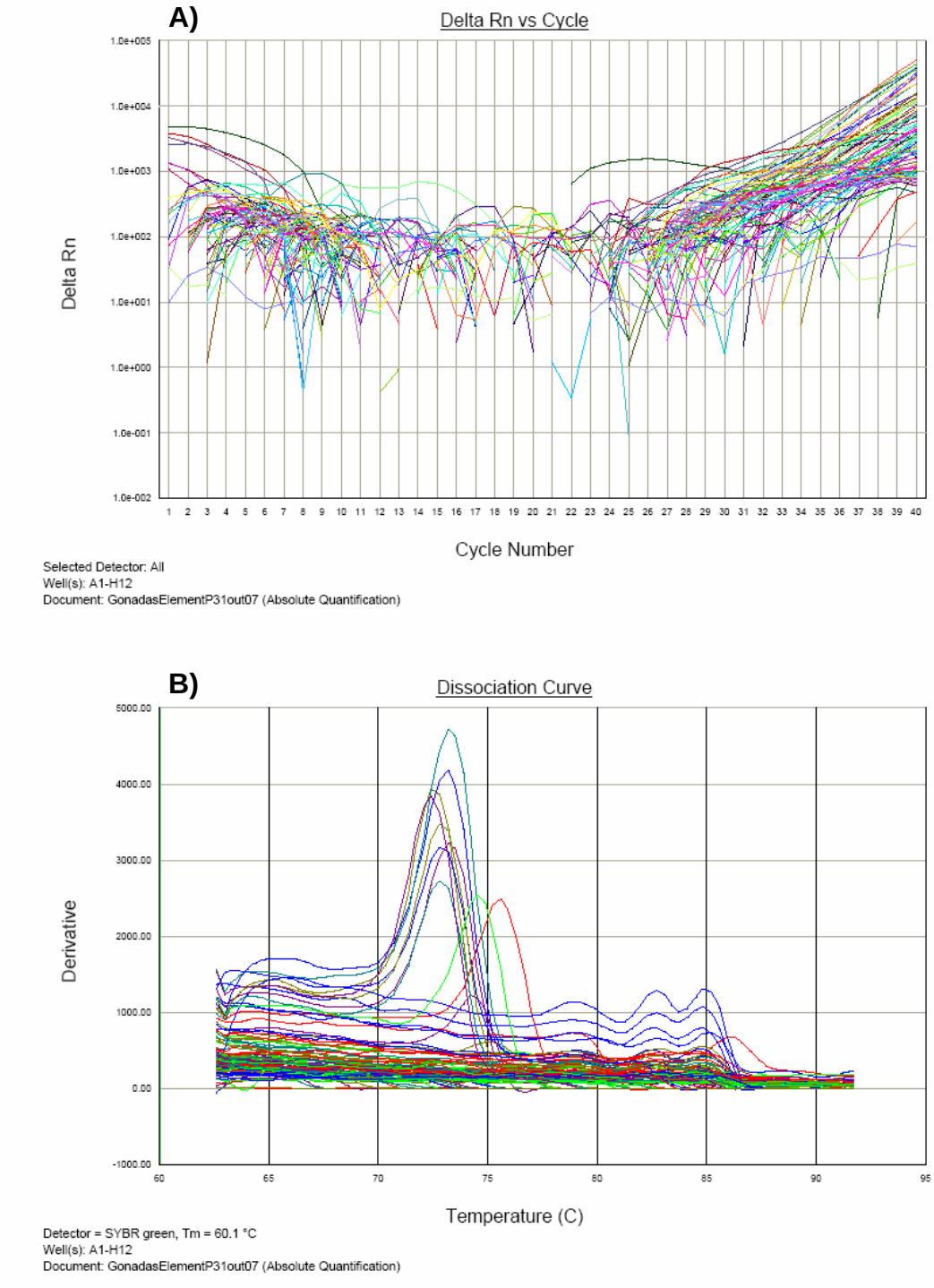


Figura 16 - *Real Time PCR* *P*

A) **B)**

III.5. Discussão, Conclusões e Perspectivas

Drosophila melanogaster *willistoni*

willistoni *D. willistoni*
D. melanogaster

melanogaster-willistoni,

Drosophila
D. melanogaster *Drosophila*
D. willistoni

D. willistoni

D. willistoni

D. willistoni

D. melanogaster *willistoni* *D. melanogaster*

μ

willistoni

D. willistoni

Sepia *white*

D. equinoxialis

D. insularis

D. insularis *D. equinoxialis*

et al

willistoni

D. paulistorum *D. tropicalis*

Sepia *white* *D. willistoni*

D.willistoni

PCR

hsp70 *hsp83,*

, β

heat schock

et al

D. melanogaster

hsp83 *Northern blot*

willistoni

Northern blot *hsp70* *hsp83*

.

PCR Real time

Sepia *white* *D. willistoni*

Heat shock

hsp70 *hsp83*

et al.,

Real Time PCR

et al

housekeeping

P

PCR

primers

primers

IV. Referências bibliográficas

Drosophila melanogaster

patufet

Drosophila melanogaster

Escherichia coli

Euglena gracilis.

Drosophila

Drosophila melanogaster

Drosophila

Drosophila *D. melanogaster*

Drosophila melanogaster

Drosophila hydei

Drosophila

Drosophila willistoni

In situ

willistoni

Drosophila

willistoni *Drosophila*

Drosophila

Drosophila simulans

Drosophila

willistoni *In*

SSC1,

Saccharomyces cerevisiae,

SSC1, *S cerevisiae*

Drosophila melanogaster

Drosophila melanogaster *Genet Sel Evol*

Drosophila willistoni. *no prelo*

Drosophila melanogaster

Drosophila

Drosophila

Drosophila willistoni

Drosophila

mtp70,

Abstract.

Escherichia coli

In

Drosophila

Drosophila

mariner *Drosophila melanogaster*

Drosophila mercatorum

mariner

Drosophila simulans

Drosophila willistoni

Drosophila melanogaster hsp83

No prelo)

Tnt1

Drosophila

melanogaster Genetics

E. Coli

mel(1999) Tra

Drosophila melanogaster *D. buzzattii.*

Drosophila melanogaster

Drosophila

Drosophila melanogaster

Drosophila willistoni

Drosophila melanogaster

Drosophila melanogaster

Escherichia coli

Drosophila melanogaster

Drosophila melanogaster

S cerevisiae

Drosophila melanogaster

Drosophila

Drosophila melanogaster

Drosophila

Drosophila

Drosophila

Drosophila willistoni.

in situ

willistoni *Drosophila*

Drosophila.

Drosophila

KAR2,

Drosophila

Drosophila

Drosophila willistoni *Drosophila paulistorum*

Drosophila melanogaster

Drosophila. :

412 Drosophila simulans

Drosophila

Livros Grátis

(http://www.livrosgratis.com.br)

Milhares de Livros para Download:

Baixar livros de Literatura
Baixar livros de Literatura de Cordel
Baixar livros de Literatura Infantil
Baixar livros de Matemática
Baixar livros de Medicina
Baixar livros de Medicina Veterinária
Baixar livros de Meio Ambiente
Baixar livros de Meteorologia
Baixar Monografias e TCC
Baixar livros Multidisciplinar
Baixar livros de Música
Baixar livros de Psicologia
Baixar livros de Química
Baixar livros de Saúde Coletiva
Baixar livros de Serviço Social
Baixar livros de Sociologia
Baixar livros de Teologia
Baixar livros de Trabalho
Baixar livros de Turismo