



SCHOOL OF BIOLOGICAL SCIENCE
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The application of RNA interference to study the biology of the
Neoparamoeba genus

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Thoughts to live by:

"ALL I REALLY NEED TO KNOW I LEARNED IN KINDERGARTEN"

by Robert Fulghum

“Most of what I really need
To know about how to live
And what to do and how to be
I learned in kindergarten.
Wisdom was not at the top
Of the graduate school mountain,
But there in the sandpile at Sunday school.

These are the things I learned:

Share everything.

Play fair.

Don't hit people.

Put things back where you found them.

Clean up your own mess.

Don't take things that aren't yours.

Say you're sorry when you hurt somebody.

Wash your hands before you eat.

Flush.

Warm cookies and cold milk are good for you.

Live a balanced life -

Learn some and think some

And draw and paint and sing and dance

And play and work every day some.

Take a nap every afternoon.

When you go out into the world,

Watch out for traffic,

Hold hands and stick together.....”

TABLE OF CONTENTS:

SUMMARY	i
DECLARATION	iii
ACKNOWLEDGEMENTS	iv
STATEMENT OF THE CONTRIBUTIONS OF JOINTLY AUTHORED PAPERS	ix
LIST OF FIGURES	ix
LIST OF TABLES	xii

CHAPTER 1: General Introduction 1

1.1. Amoebic gill disease	1
1.2. AGD treatment and monitoring	2
1.3. Tasmanian Atlantic salmon industry vs. AGD	4
1.4. Attempts to control AGD.....	5
1.5. Using RNAi to study gene function and validate new drug targets against AGD	6
1.6. Aim of the study and thesis outline	9

CHAPTER 2: Literature Review 11

2.1. Introduction.....	12
2.1. RNA interference	13
2.3. RNAi as a natural antiviral defence mechanism in vertebrates and invertebrates.....	17
2.4. RNAi applied to aquaculture	19
2.4.1. RNAi as an antiviral tool in fish.....	20
2.4.1.1. RNAi-mediated viral immunity in fish	22

2.4.1.2. Gene silencing mediated by <i>in vitro</i> transcribed and chemically synthesized siRNAs	24
2.4.1.3. Gene silencing mediated by vector-based RNAi constructs	26
2.4.1.4. Gene silencing mediated by other RNAi triggers	27
2.4.1.5. <i>In vitro</i> delivery of RNAi into fish.....	28
2.4.1.6. The future of RNAi to control viral diseases in fish	30
2.4.2. RNAi as an anti-parasitic tool in fish	31
2.4.3. RNAi as an antiviral tool in shrimp.....	33
2.4.3.1. Stimulation of innate immunity and antiviral silencing by dsRNA	35
2.4.3.2. Different factors influencing RNAi silencing in shrimp	38
2.4.3.3. Improving protection by simultaneous knockdown of shrimp and virus specific genes.....	39
2.4.3.4. The ability of RNAi to induce preventive and therapeutic effect in shrimp.....	40
2.4.3.5. Seeking oral delivery of RNAi-based technology in shrimp	40
2.4.3.6. The future of RNAi to control viral diseases in shrimp	41
2.4.4. RNAi technology applied to other crustaceans	42
2.4.5. The early stages of RNAi application to shellfish aquaculture	43
2.5. RNAi limitations and further perspectives	45
2.5.1. General limitations	45
2.5.2. RNAi limitations directly applied to aquaculture.....	50
2.6. Conclusions.....	52

CHAPTER 3: Experimental - 1..... 54

3.1. Introduction.....	56
3.2. Material and Methods	58
3.2.1. Dicer and Argonaute candidates.....	58
3.2.2. Amoeba culture conditions.....	59
3.2.3. RNA isolation and reverse transcription	59
3.2.4. Preparation of DNA template for dsRNA synthesis	60
3.2.5. <i>In vitro</i> transcribed dsRNA	61
3.2.6. Endoribonuclease-prepared siRNA pool.....	61
3.2.7. Validation of RNAi-trigger uptake.....	62

3.2.8. Gene silencing experiments.....	63
3.2.9. Total RNA extraction and semi quantitative RT-PCR.....	63
3.2.10. Quantitative RT-PCR	64
3.2.11. Up-regulation of Dicer and Argonaute in response to RNAi duplexes	65
3.2.12. RNase III activity assay.....	66
3.3. Results.....	66
3.3.1. Dicer and Argonaute homologues	66
3.3.2. dsRNA and esiRNA validation	71
3.3.3. RNA-duplex up-take	72
3.3.4. Dicer and Argonaute up-regulation.....	73
3.3.5. Gene silencing by <i>in vitro</i> transcribed dsRNA.....	74
3.3.6. Gene silencing by esiRNA pool	75
3.3.7. RNase III activity	78
3.4. Discussion.....	79
CHAPTER 4: Experimental - 2.....	85
4.1. Introduction.....	87
4.2. Material and Methods	89
4.2.1. <i>Neoparamoeba pemaquidensis</i> culture conditions	89
4.2.2. RNA extraction and reverse transcription	89
4.2.3. Synthesis of bacterially expressed dsRNA.....	90
4.2.4. Bacterial IPTG induction.....	91
4.2.5. dsRNA purification from bacteria.....	91
4.2.6. Delivery of bacterial dsRNA via soaking.....	92
4.2.7. Total RNA extraction and RT-PCR	93
4.2.8. Quantitative real time RT-PCR.....	93
4.2.9. Statistical analysis	94
4.2.10. Microscopy.....	95
4.3. Results.....	95
4.3.1. Verification of dsRNA integrity.....	95
4.3.2. dsRNA delivery by soaking and its effect on β -actin mRNA expression levels.....	96

4.3.3. dsRNA delivery by soaking and its effect on EF1 α mRNA expression levels	99
4.3.4. Daily administration of dsRNA does not improve effectiveness of targeted genes knockdown	99
4.3.5. Suppression of β -actin mRNA levels induces unexpected phenotypic changes in <i>N. pemaquidensis</i>	102
4.4. Discussion	103

CHAPTER 5: Experimental - 3 110

5.1. Introduction.....	112
5.2. Material and Methods	114
5.2.1. <i>Neoparamoeba pemaquidensis</i> culture conditions	114
5.2.2. RNA isolation and reverse transcription	115
5.2.3. Synthesis of bacterially expressed dsRNA.....	115
5.2.4. dsRNA validation	116
5.2.5. Delivery of dsRNA-expressing bacteria via feeding.....	117
5.2.6. Total RNA extraction and RT-PCR	117
5.2.7. Quantitative real time RT-PCR	118
5.2.8. Statistical analysis	119
5.2.9. Validation of dsRNA expressing bacteria ingestion	119
5.3. Results.....	120
5.3.1. Verification of dsRNA integrity	120
5.3.2. Target genes downregulation by ingestion of bacterial dsRNA.....	121
5.3.3. Confirmation of bacteria ingestion.....	123
5.4. Discussion.....	124

CHAPTER 6: Experimental - 4 129

6.1. Validation of <i>N. perurans</i> <i>in vitro</i> culture.....	129
6.2. Identification of RNAi-associated genes in the <i>N. perurans</i> transcriptome database.....	130

6.3. Using the <i>Entamoeba histolytica</i> U6 promoter to drive the expression of short hairpin RNAs in <i>N. perurans</i>	136
6.3.1. Preparation of the <i>Entamoeba histolytica</i> U6 promoter	138
6.3.2. Plasmid-based shRNA expression vector	138
6.3.3. Transfection and knockdown validation by fluorescent microscopy	140
6.4. Conclusions.....	142

CHAPTER 7: General Discussion..... 143

7.1. Identification of putative proteins encoding conserved domains of Dicer and Ago	143
7.2. Functional evidence for the presence of active RNAi machinery in <i>Neoparamoeba</i> spp.	146
7.3. Final conclusions and future directions	149

REFERENCES..... 151

APPENDIX..... 172

SUMMARY:

RNA interference (RNAi) is a natural regulatory mechanism of most eukaryotic cells that uses small double-stranded RNA (dsRNA) molecules as triggers to direct homology-dependent control of gene activity. This technique has emerged as a powerful tool for rapid analysis of gene function in non-model organisms and has the potential to identify candidate targets for intervention against diseases of economic importance to aquaculture.

With regards to amoebic gill disease (AGD) of farmed Atlantic salmon, RNAi could become an invaluable research instrument to unravel the role of proteins involved in amoeba attachment and pathogenicity, as well as to validate important treatment targets by investigating the effect of specific gene knockdown on amoeba survival and physiology. Additionally, RNAi technology could greatly assist in the elucidation of possible factors associated with the loss of virulence in certain species from the *Neoparamoeba* genus.

However, before RNAi technology can be employed in *Neoparamoeba*, it is important to consider whether members of this genus possess the required set of proteins involved in the RNAi pathway. As a result, the main purpose of the present study was to use functional and comparative genomics approaches to investigate whether functional RNAi machinery has been retained or lost in species from the *Neoparamoeba* genus. As the *in vitro* culture of the causative agent of AGD (*Neoparamoeba perurans*) has been successfully achieved only recently, most of the gene regulation assays were performed using the closely-related *Neoparamoeba pemaquidensis*, which is readily amenable to culture.

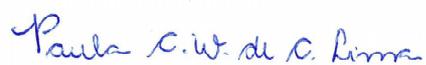
Using a *N. perurans* and *N. pemaquidensis* transcriptome database we were able to identify putative proteins containing conserved domains of RNAi-related

genes, such as Dicer and Argonaute. For *N. pemaquidensis*, the candidates' involvement in the RNAi pathway was validated by assessing their levels of expression followed the administration of dsRNA and small interference RNA (siRNA), respectively. The presence of an active Dicer in both species was also corroborated by utilizing an RNase III assay, which showed complete degradation of dsRNA following incubation in amoeba lysate. Further evidence for the presence of an active RNAi machinery was also supported by gene silencing experiments, where *N. pemaquidensis* specific genes (β -actin and EF1 α) were successfully downregulated by the administration of RNAi-trigger molecules. However, knockdown efficiency was dependent on dose, administration frequency, target gene, delivery method and RNAi molecule. Additionally, trophozoites soaked with bacterially expressed dsRNA targeting β -actin unexpectedly transformed into a cyst-like stage, which has not been previously reported in this species. Unfortunately, the attempts to employ the *Entamoeba histolytica* U6 promoter to confirm the existence of a functional RNAi pathway in *N. perurans* haven't succeeded yet.

The results altogether provide strong evidence for the presence of functional RNAi machinery in *Neoparamoeba* spp. Despite being promising, these findings are still preliminary and the reality of applying RNAi technology to develop new treatment strategies against AGD still needs further effort. Therefore, more work needs to be undertaken in order to fully elucidate the RNAi mechanisms in *Neoparamoeba perurans*.

DECLARATION:

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.



Paula Cristina Walger de Camargo Lima

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Literature Review:

CHAPTER 2

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Authors contribution: Lima, P.C. surveyed the literature and composed the manuscript; Harris, J.O. and Cook M. contributed reviewing the manuscript structure and content.

Experimental Chapters:

CHAPTER 3

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CHAPTER 4

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CHAPTER 5

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Authors contribution: Lima, P.C. assisted with experimental design, conducted the research experiments, analysed/interpreted the data and composed the manuscripts;

Botwright, N.A. provided assistance with laboratory and analytical tasks and reviewing the manuscripts; Harris, J.O. made contribution to the drafting and revising the manuscript; Cook M. participated on assay development and results interpretation, as well as revising the manuscripts structure and content.

The following authors agree that the Statement of the contributions of jointly authored papers accurately describes their contribution to research manuscripts 1, 2, 3, and 4 and give consent to their inclusion in this thesis.

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LIST OF FIGURES:

CHAPTER 1

Figure 1.1. Gross pathology and histopathology associated with Atlantic salmon AGD.....	2
Figure 1.2. Gills arch from AGD-infected Atlantic salmon and the corresponding gill score.....	4

CHAPTER 2

Figure 2.1. A simplified model for the endogenous RNAi pathway	14
--	----

CHAPTER 3

Figure 3.1. Schematic representation of the domain architectures found in eukaryotic RNAi-related proteins.	57
Figure 3.2. Candidates sharing significant level of homology with conserved motifs found in Dicer	68
Figure 3.3. Candidate sharing high levels of homology with proteins from the Ago subfamily	69
Figure 3.4. Candidate sharing high levels of homology with proteins from the Piwi subfamily	70
Figure 3.5. Candidates partially covering the Piwi domain	71
Figure 3.6. Validation of dsRNA and esiRNA synthesis by gel electrophoresis ..	72
Figure 3.7. Validation of RNAi duplexes up-take by the amoeba.....	73
Figure 3.8. Up-regulation of Dicer (<i>NpDEXDc</i>) and Ago (<i>NpAGO-2</i>) candidates following the administration of dsRNA and esiRNA, respectively.....	74
Figure 3.9. qRT-PCR analysis of β -actin and EF1 α relative expression levels in amoebae treated with <i>in vitro</i> transcribed dsRNA via immersion	76

Figure 3.10. qRT-PCR analysis of β -actin and EF1 α relative expression levels in amoebae treated with esiRNAs via immersion and transfection	77
Figure 3.11. Agarose gel demonstrating the RNase III activity assay.....	78

CHAPTER 4

Figure 4.1. Agarose gel demonstrating dsRNA construct integrity.....	96
Figure 4.2. Silencing of <i>N. pemaquidensis</i> β -actin gene expression by bacterially expressed dsRNA.....	98
Figure 4.3. Silencing of <i>N. pemaquidensis</i> EF1 α gene expression by bacterially expressed dsRNA.....	100
Figure 4.4. Inhibition of <i>N. pemaquidensis</i> β -actin and EF1 α by daily administration of bacterially expressed dsRNA (20 μ g/mL).....	101
Figure 4.5. Phenotypic changes of dsRNA-treated amoeba	102
Figure 4.6. Cyst-like amoeba under 100x magnification.....	103

CHAPTER 5

Figure 5.1. Agarose gel electrophoresis of dsRNA purified from the <i>E. coli</i> HT115(DE3)	121
Figure 5.2. Validation of bacterially expressed dsRNA ingestion by fluorescent microscopy.....	122
Figure 5.3. Gene knockdown in <i>N. pemaquidensis</i> orally administrated with bacteria expressing dsRNA designed against β -actin and EF1 α	123

CHAPTER 6

Figure 6.1. Validation of cultured trophozoites identity by PCR analysis using <i>N. perurans</i> and <i>N. pemaquidensis</i> specific primers	130
Figure 6.2. Candidate sharing significant level of homology with DEAD-like helicase (DEXDc) of Dicer.....	132

Figure 6.3. Candidate sharing high levels of homology with proteins from the Ago subfamily	133
Figure 6.4. Candidate sharing high levels of homology with proteins from the Ago subfamily	134
Figure 6.5. Agarose gel demonstrating the RNAse III activity assay.....	135
Figure 6.6. Diagram demonstrating the one-step PCR method employed to produce shRNA expression vectors.....	140
Figure 6.7. Validation of EGFP expression by fluorescence microscopy (40x magnification).....	141

LIST OF TABLES:

CHAPTER 1

Table 1.1. Gross gill score system used by farmers to assess the severity of infection.....	3
Table 1.2. RNAi-associated genes and functional studies undertaken in amoeba species	8

CHAPTER 2

Table 2.1. Summary of RNAi studies applied to fish viruses.....	23
Table 2.2. The applications of RNAi to investigate the interactions between shrimp and viruses	36

CHAPTER 3

Table 3.1. Primer sequences used for dsRNA <i>in vitro</i> transcription (ds) and qRT-PCR (q)	60
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CHAPTER 4

Table 4.1. Primer sequences used for construction of dsRNA-expression vectors (ds) and qRT-PCR (q).....	90
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CHAPTER 6

Table 6.1. Primer sequences used for construction of <i>EhU6p</i> -driven shRNAs....	139
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