Detection of morpholino molecules transfer to *Atlantic Salmon* ovulated eggs and fertilized eggs

Xinlin Zhao*[a,c], Ten-Tsao Wong*[b], Theódór Kristjánsson[a,c], Ragnar Jóhannsson[a]

[a] Hafrannsóknastofnun, Marine and Freshwater Research Institute (HAFRO), Hafnarfjörður, Iceland
[b] Department of Marine Biotechnology, Institute of Marine and Environmental Technology, University of Maryland Baltimore County, Baltimore, USA
[c] Faculty of Life and Environmental Science, University of Iceland, Reykjavik, Iceland

xinlin.zhao@hafogvatn.is

Introduction

Morpholino oligomers (MOs) offer promising effectiveness in developmental biology research involving gene knockdown and have a wide range of applications as specific and effective treatments for genetic disorders.

An immersion-based method has been applied to transport modified MO delivery systems into unfertilized salmon eggs.

Since Vivo-MOs are incompatible with common fluorescence labelling, a ZP9 molecular transporter was designed to enable the fluorescence labelling of MOs, thereby enhancing the immersion technology.

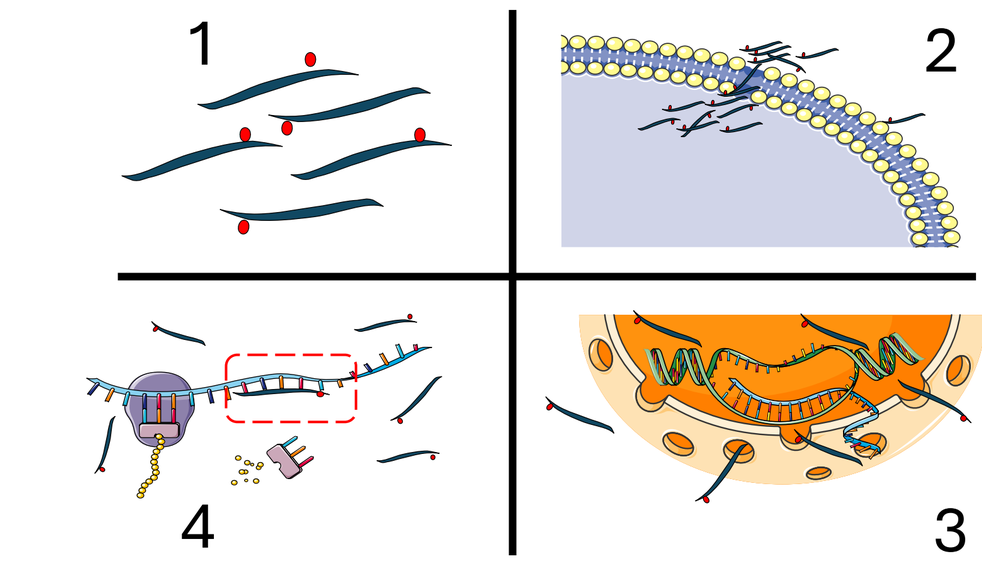

Figure 1. Mechanism of morpholino delivery systems in gene knockdown. (1) morpholino molecules conjugate with the ZP9 delivery system, and the MO-ZP9 is labeled with the fluorescent tag lissamine. (2) Lis-MO-ZP9 penetrates the eggs through the micropores in the chorion. (3) Lis-MO-ZP9 enters the nucleus and inhibits the spliceosome assembles (4) In the cytoplasm, Lis-MO-ZP9 binds to the target mRNA and blocks the translation.

Experiment design

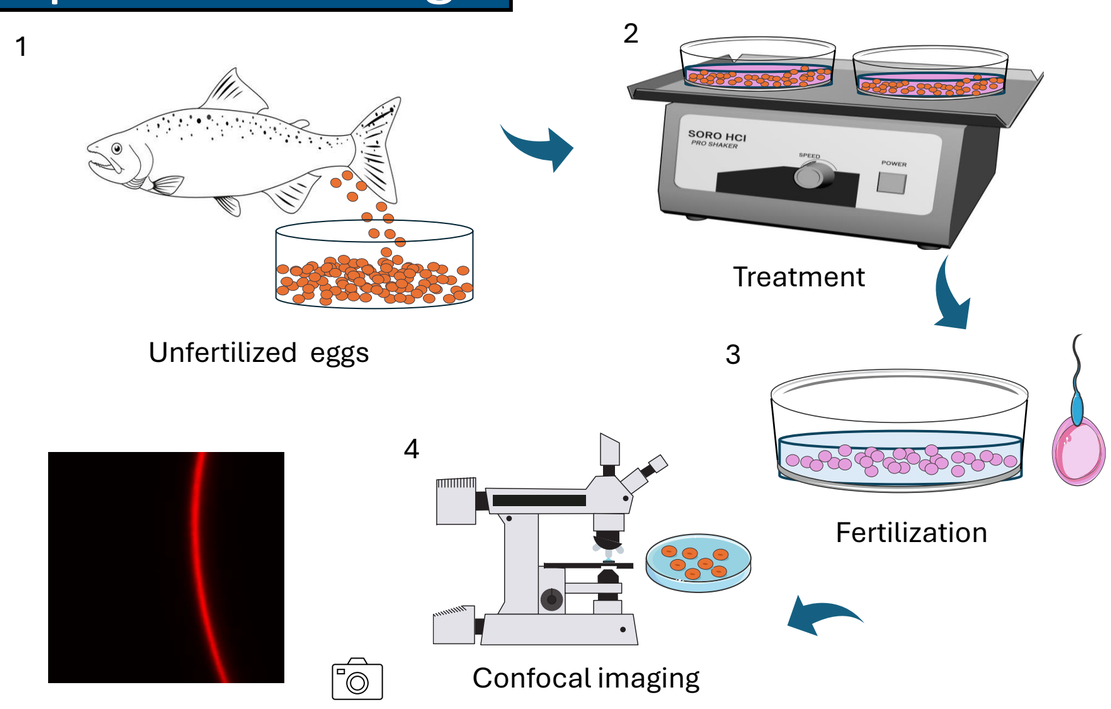

Figure 2. Flowchart of the immersion treatment and sampling process involved in this study. (1) Unfertilized eggs were provided by Benchmark genetics, collected from mature females. (2) The ovulated eggs were treated in an immersion medium containing Lis-MO-ZP9. (3) After treatment, eggs were fertilized either by normal sperm or by Lis-MO-ZP9-treated sperm. (4) Samples were taken after fertilization and analyzed using a confocal microscope to trace the MO uptake.

Results

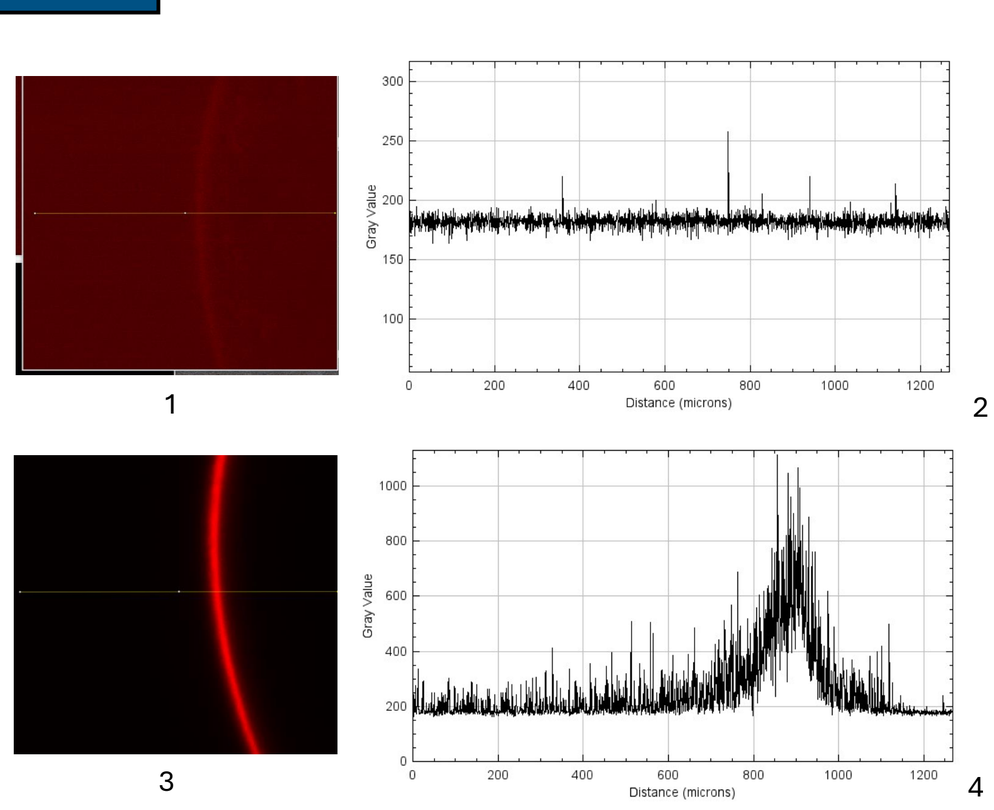

Figure 3. Confocal imaging (1 &2) and the line fluorescence intensity profiles plotted (3&4). The excitation wavelength for lissamine was 575.0nm. Control eggs (1&2) did not exhibit any fluorescence. In Lis-MO-ZP9-treated eggs, a high intensity of lissamine was localized in the chorion, and fluorescence signals were also detected inside the eggs.

Results

Table 1. List of immersion media and treatment conditions

Group	Immersion medium	Lis-MO-ZP9 (μM)	Immersion time (Hours)		
	Batch A				
A1	IMG	6 μM	24		
A2	IMG	12 μM	24		
A3	IMG +5%KOSR	12 μM	24		
A4	IMG+5% KOSR	12 μM	36		
A-C	IMG	-	24		
	Batch B			ssdnd-MO-Vivo (μM)	
B1	IMG	2 μM	24	-	
B2	IMG	2 μM	24	6 μM	
B3	IMG	2 μM	24	12 μM	
B4	IMG	2 μM	36	18 μM	
B5	IMG+5%KOSR	2 μM	24	18 μM	
B-C	IMG	-	24	-	
	Batch C			MO-ZP9 treated sperm *	Lis-MO-ZP9 after fertilization (μM)
C1	IMI+5% KOSR	12 μM	36	-	-
C2	IMI+5% KOSR	12 μM	36	-	5μM, 4 h
C3	IMI+5% KOSR	12 μM	36	40μM,2h	-
C4	IMI+5% KOSR	12 μM	36	40μM,2h	5μM, 4 h
C5	OVF	-	-	40μM,2h	-
C-6	OVF	-	-	-	10μM, 4 h
C-C	OVF	-	-	-	-

Additional Lis-MO-ZP9 was applied in the medium of groups C2, C4, and C6 after fertilization. Samples from these groups were analyzed 4 hours post-fertilization to assess the effects of the treatment.

* Fresh milt was pre-treated in an extender medium containing 40μM Lis-MO-ZP9. This treatment was conducted in a cool room for a duration of 2 hours before fertilization.

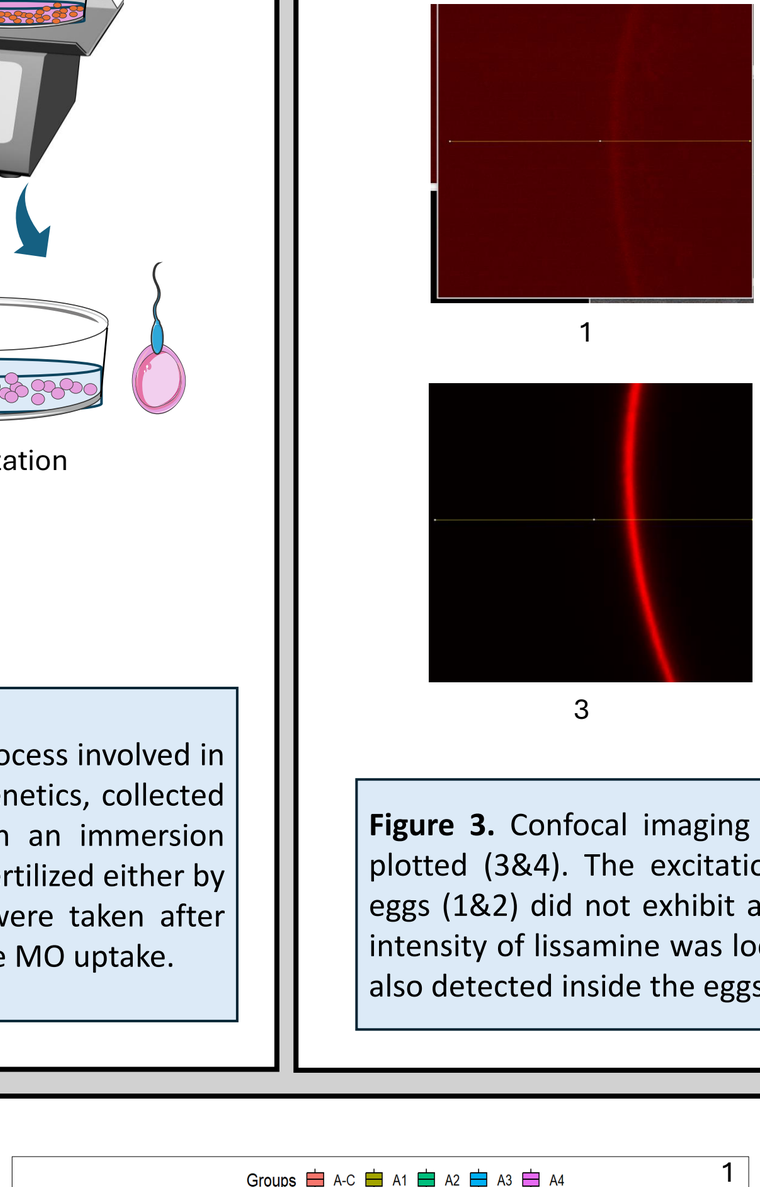

Figure 4. Relative fluorescence signal intensity inside eggs among treatment groups. (1) Knockout serum replacement (KOSR) in the immersion medium enhanced MO uptake. (2) Vivo-MOs in the medium improved cell penetration, thereby increasing MO uptake. (3) Lis-MO-ZP9 could enter the eggs, with additional MO occurring in the medium post-fertilization.

Discussion

The research aims to enhance the delivery of MOs into Atlantic Salmon eggs through immersion, with the ultimate goal of knocking down the Dead-end genes and producing sterile fish. This study traced the MO uptake under various treatment conditions, and the results demonstrated that certain experimental factors significantly enhanced MO uptake, which will contribute to optimizing the immersion sterilization methodology.

Acknowledgment

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