

CONSORTIUM ON LAW AND VALUES
AWARD REPORT FOR THE 2015-2016 ACADEMIC SCHOOL YEAR

**“Antibiotic Resistance and Aquaculture in Chile:
Bridging the Gap Between Science and Policy”**

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Brief project summary

Antibiotic Resistance (AR) is a grand challenge in global health. The emergence and spread of AR is very complex. It is not only influenced by the selective pressures that antibiotics and other compounds (such as disinfectants) exert on bacteria, but also by how we manage human and agricultural waste. The effluents of anthropogenic point sources discharge antibiotic residues, Antibiotic Resistant Bacteria (ARB), and Antibiotic Resistance Genes (ARG) to the aquatic ecosystem. Watersheds play an important role in the dissemination of AR, but this is still poorly understood. A critical barrier to advance the knowledge on this issue has been the reliance on culture-dependent methods when less than 1% of environmental bacteria are culturable. To avoid this limitation, we have used culture-independent approaches and thus focused our research on ARG.

We have worked on the Valdivian watershed in the Region de los Rios in southern Chile to characterize and quantify dissemination pathways of ARG from wastewater treatment plants (WWTP) and freshwater aquaculture sites into this watershed. We originally proposed to work in the Rio Bueno watershed with the salmon industry, but due to unforeseen challenges within their industry, they could not collaborate with us in our research. We, however, were able to establish a new collaboration with another sector of the aquaculture industry in Chile (trout industry), and expand a collaboration with the Department of Health of the Region to assess the risk of dissemination of AR from wastewater treatment plants as well.

In October 2015, Irene Bueno, Consortium Scholar, and her advisor, Dr. Randy Singer, attended a meeting held by the Chilean Trout industry, the Government, and Academia in Valparaiso, Chile. The purpose of this meeting was to discuss the implementation of new regulations for the aquaculture industry, and it was a great opportunity to establish the collaboration between the University of Minnesota and the trout company from the south of Chile. This company, the biggest trout company in the country, owns 5 farms, each of them located in different rivers throughout the Region, and all of them drain into the common Valdivian watershed. During the same trip to Chile meetings were also held with the Department of Health of Region de los Rios (SEREMI de Salud), and with professors from both the Universidad Austral (Valdivia) and Universidad de Concepcion (Concepcion, Chile) with the goal of expanding the antibiotic resistance project in the Valdivian watershed.

Thanks to these collaborations, a total of 51 river sediment samples were taken upstream and downstream from 3 wastewater treatment plants and the 5 trout farms from this at one time point. Additionally, bird fecal samples were collected from one of the wastewater treatment plants. Birds are thought to be disseminators of ARB and ARG, and they are exposed to wastewater at the wastewater treatment plants, where they can acquire ARB and ARG and thus spread them. All the sampling locations were georeferenced and incorporated into a map (ArcGIS 10.2.2), and these same locations will be sampled again in future sampling campaigns (Figure 1).

Additionally, antibiotic use data from the 5 trout farms was obtained through a survey tool designed by Irene Bueno with input from the team from the University of Minnesota.

At the laboratories in Chile (Universidad Austral and Universidad de Concepcion), DNA was extracted from both the sediment and the bird fecal samples. At this point the DNA is stored in Chile

and it will be shipped to the University of Minnesota in the upcoming months to quantify a variety of ARG in the samples using high-throughput methods.

A mathematical model is also being developed to predict the risk of ARG dissemination from the point sources (wastewater treatment plants and trout farms) into the watershed. The data obtained from the laboratory analysis will inform the mathematical model.

Financial summary

- Trip to Chile (October 2015): \$1,735. Irene Bueno travelled to Chile in October 2015 to attend a meeting with the trout industry in Valparaiso, and then to attend meetings in Valdivia with the remaining stakeholders and to plan the field work. This amount included airfare, lodging and meals for 2 weeks in Valdivia, and transportation by bus between the North and the South of Chile. Specifically:
 - \$1,261: Flight US-Chile round trip October 2015 (receipt attached).
 - \$ 330: Lodging in Valdivia (Aparthotel Rio Cruces, <http://www.aparthotelriocruces.cl/>) for 2 weeks, receipt not available.
 - \$ 99: Meals (receipts not available).
 - \$ 75: Bus (Turbus; <https://www.turbus.cl/wtbus/indexCompra.jsf>) from Valparaiso to Valdivia, and from Valdivia to Santiago (No receipt available).
- Microbiology analysis at Universidad de Concepcion (\$4,410). Invoice attached directed to Dr. Randall Singer (\$4,380), and receipt from MoneyGram attached (cost of shipping the money from the U.S. to Chile via MoneyGram was \$30.00).
- Microbiology analysis at Universidad Austral, Valdivia (\$855). Invoice attached (542 Chilean Pesos).

Results

Publications (planned, in press, or published)

- We plan to publish the work completed thanks to the Consortium grant in a peer-reviewed journal, and most likely it will be divided into 2-3 publications. They will also be part of Dr. Bueno's PhD Dissertation.

Presentations (planned or completed)

- We plan to present this work in 2017 at 2 different international conferences (to be determined), and to the stakeholders involved in the project, both in the United States and in Chile.

Grant proposals (planned or submitted)

- Thesis Research Travel Grant 2016 (awarded)
- Anderson Fellowship January 2016 (awarded)
- Gates Foundation May 2016 (pending decision)
- Grand Challenges UMN June 2016 (pending decision)
- Institute in the Environment, UMN, May 2016 (awarded)

Future project plans

This work is part of Irene Bueno's PhD dissertation. She is a VMED PhD Candidate at the College of Veterinary Medicine, and she expects to complete her research by May 2018.

The current plan to continue the research started thanks to the Consortium funds is to repeat the same sampling and analysis strategy at the trout farms and wastewater treatment plants in the Fall 2016, and throughout 2017 to capture seasonal changes, using other funds.

The work completed thanks to the Consortium funds has already led to new collaborations and expansion of the work. Specifically, our team is collaborating with Professor Satoshi Ishii (University of Minnesota) to implement innovative molecular methods to quantify antibiotic resistance genes associated with anthropogenic point sources in environmental samples.

For the bird fecal study at one of the wastewater treatment plants, we plan to place GPS devices on some of the birds to assess their dispersal and thus the dissemination of ARG.

Also, the trout farm company in Chile has showed interest in having our team develop other mathematical models to assess the risk of pathogen dissemination from their farms into the watershed.

Long-term, the plan is to continue characterizing other point sources in this watershed to implement an antibiotic resistance monitoring program in the Valdivian watershed in Chile.

Figure 1. Map of Region de los Rios showing the 5 trout farms and 3 wastewater treatment plants (these were the point sources considered for the study, and the sediment samples were collected both upstream and downstream from each point source).

