### Darwin Initiative Annual Report

Important note:

To be completed with reference to the Reporting Guidance Notes for Project Leaders – it is expected that this report will be about 10 pages in length – Submission deadline 30 April 2008

Project Ref Number	162/15/020
Project Title	Reducing the Impact of Exotic Aquaculture on Chilean
	Aquatic Biodiversity
Country(ies)	UK, Chile, Canada, USA, New Zealand
UK Contract Holder Institution	Swansea University
	(formerly University of Wales Swansea - UWS)
UK Partner Institution(s)	NERC Centre for Ecology & Hydrology, Banchory UK)
	University of Victoria (BC, Canada)
	US Geological Survey (USA)
	Oregon State University (USA)
	Victoria University of Wellington (New Zealand)
Host country Partner Institution(s)	Universidad de Los Lagos (ULA) – Chile
Darwin Grant Value	£193,844
Start/End dates of Project	01 October 2006 / 30 September 2009
Reporting period (1 Apr 200x to 31	01 October 2007 – 31 March 2008
Mar 200y) and annual report number	Report 3
(1,2,3)	
Project Leader Name	Dr. Carlos Garcia de Leaniz
Project website	www.biodiversity.cl
Author(s), date	Carlos Garcia de Leaniz, Gonzalo Gajardo, Kyle Young
	15 May 2008

#### **Darwin Project Information**

#### 1. Project Background

Salmon farming is one of the most buoyant and lucrative business in Chile, capitalizing on a highly valuable export commodity that generates significant revenue. Chile is set to overtake Norway as the world's top salmon producer, and the industry is considered a successful example of the country's commitment to free market, world trade and economic growth. Yet, salmonids are not naturally present in the Southern Hemisphere and constitute a potential threat to indigenous species. Under such a scenario, the Darwin Initiative "Reducing the impact of exotic aquaculture on native aquatic biodiversity" is drawing attention to the potential impacts of salmon farming on Chile's unique aquatic ecosystems, with a view of making the industry more sustainable. The project is based at region X ("Los Lagos"), where most of the salmon farming industry is concentrated and from where it has continued to expand since the 80's. The area (**Annex 3, Photo 1**) is characterized by a complex hydrology and high aquatic biodiversity, with high levels of endemism. Several of the native aquatic species (from a total of 130) are listed as endangered.

The basic problem the project seeks to address is to evaluate and monitor the interaction of exotic salmonids (accidental escapes from hatcheries and net cages, as well as salmonids deliberated stocked for sport fishing) upon native species, in particular fishes. University of Los Lagos, the host-country institution, has its main campus in Osorno (Lake district – **Photo 1**), and another in Puerto Montt (Pacific coast). The University's mandate is to attend local problems with creative solutions, and so this project represents a timely contribution to such endeavour.

#### 2. Project Partnerships

The partnership between the UK lead institution (UWS) and the Chilean host partner (ULA) has continued to be fruitful during the second year of the project (third reporting period, October 2007-March 2008). As in previous occasions, it benefited from fluent and frequent email and telephone contacts, in addition to one visit by the UK leader to Chile during this period.

The partnership was also reciprocal in many respects. For example, active exchange of ideas, working hypotheses and experimental protocols allowed to produce a preliminary database on presence/absence of alien salmonids in previously unsearched rivers of southern Chile (Chiloe Island) with active involvement of relevant stakeholders. Following active discussion between partners, a translocation/competition experiment was carried out for the first time in Chile. This has produced some interesting preliminary results, and a manuscript submitted for peer-reviewed publication, thanks in part to the input from local collaborators and advisors, as well as from the local land owners in Chiloe who provided valuable logistic support.

Standardization and fine-tuning of DNA microsatellite loci for GSI and individual assignment in Atlantic salmon (the main aquaculture species farmed in Chile) were possible thanks to the active involvement of the UK lead institution. Finally, the success of the well attended second workshop at Viña del Mar (see below) demanded an active involvement and interaction of both parties, and was a challenge in terms of gathering the relevant actors both in Chile and elsewhere.

The second thematic project workshop ("Molecular Ecology of Aquatic Invasions") was held at Viña del Mar on 17-19 December 2007 (Annex 3, Photo 2). It benefited from the participation of project partners from UK and Canada, invited speakers from Argentina, as well as Chilean researchers, government officials, stakeholders and students (workshop program and abstracts in www.biodiversity.cl). As in previous occasions, the work of the host project coordinator Dr. Gonzalo Gajardo and the ULA staff were key in securing the active participation of Chilean stakeholders (who were again self-funded), and in obtaining additional funding. The effort done in this respect by officials of Universidad de Los Lagos, and in particular by the Rector of the University (Sr. Aguilar), is greatly appreciated. For example, ULA covered the travel costs of one the two keynote speakers (Prof John Beardmore). As in previous occasions, key Chilean government officials from CONAMA (National Commission for the Environment) participated in this Workshop, as well as a panel of national and international speakers, who included researchers from Argentina, where there is also growing concern about the rapid spread of invasive salmonids. This helped to disseminate the project and resulted in new contacts. Following the second workshop, Dr. Javier Ciancio and Dr. Miguel Pascual from GESA in Argentina are now advising us on the use of stable isotopes for tracing the origin of salmonid escapees from fish farms.

Following the second workshop in December, Dr. Young began advising Paula Moreno, the aquaculture coordinator for the World Wildlife Fund Chile (<u>www.wwf.cl</u>). Though time commitments to the Darwin Initiative prevented Dr. Young from agreeing to a request to author a WWF report on the impacts of escaped salmon in Chile, he contributed an outline for this report and contributed an invited review of the WWF's recent publication on the same topic from a global perspective. During the last six months Dr. Young established working relationships with two international organizations involved in protecting Chilean freshwater biodiversity, hosted a world authority on the ecology of aquaculture-sea lice interactions, initiated a collaborative research project with a doctoral student to study the impact of aquaculture on fish communities in Chilean lakes, and secured a contribution from a Chilean entrepreneur in form of access to private land adjacent to a stream ideal for conducting enclosure experiments. Dr. Young has also advised Aaron Sanger, a U.S. based attorney leading the campaign by International Rivers (<u>www.internationalrivers.org</u>) against hydroelectric development in the Baker and Pascua River basins of southern Patagonia. Mr. Sanger contacted Dr. Young to ask him and Darwin Initiative volunteers to conduct biological surveys of the fish communities in the threatened basins. Logistic challenges and time constraints prevented the Darwin team from

conducting these surveys, but Dr. Young provided a summary of appropriate field methods and sampling designs based on his experience working on large rivers in North America. In March Dr. Young hosted Dr. Martin Krkosek (<u>www.math.ualberta.ca/~mkrkosek</u>), a mathematical biologist whose research on the role of exotic salmonid aquaculture as a parasite vector in British Columbia, Canada, has received international attention. The two planned collaborative projects in Chile, particularly to investigate the potential for sea lice to shift hosts from exotic salmonids to native species exploited by artisinal fisheries.

In January Dr. Young met with Ivan Arismendi, a Chilean PhD student at Oregon State University, one of the project's partner institutions. Before beginning his degree, Mr. Arismendi worked for three years on various projects related to the impacts of aquaculture in southern Chile. We have agreed to collaborate on a project during December 2008 to sample the fish communities of four lakes with net pen smolt facilities. The fish communities of these lakes have been sampled in the past, providing a reference for the community composition data we collect. The planned sampling will extend previous work by including stomach content analysis of escaped salmonids, allowing us to link patterns of exotic salmonid predation to changes in native fish communities.

Other activities that are helping to strengthen our partnership is the recent meeting between the host coordinator Dr. Gajardo and Dr. Marleni Ramirez, representative of Biodiversity International (BI) during a Latin American Congress of Genetics in Perú. They discussed the possibility of organizing a workshop sponsored by BI and the Darwin Initiative for the next Congress scheduled to be held in Chile by 2010, the year of Biodiversity. Dr Gajardo has recently been elected President of the Latin American Association of Genetics, and since the Board of this Association will be organizing the 2010 Congress in Chile, we reckon this will be a perfect opportunity to disseminate the results of the Darwin Initiative in front of a large audience, and for advertising the launching of the MSc Program in Sustainable Aquaculture at ULA.

#### **Other Collaborations**

Dr. Eric Verspoor (Head of Conservation and Restoration Group, FRS Freshwater Laboratory, Scotland) (<u>http://www.frs-scotland.gov.uk/FRS.Web/Delivery/display\_standalone\_with\_menu.aspx?contentid=568</u>) agreed to become our second external project advisor and to provide a keynote lecture during the Second International Darwin Workshop. He also helped, during the last day of the workshop, with the training of Chilean students on Parentage assignment and Genetic Stock Identification (GSI), lead by

Dr. Sofia Consuegra.

Important links were also forged with national and international groups during the Second Workshop (Annex 3, Photo 2). For example, Dr. Kathleen Whitlock from the University of Valparaiso participated in the workshop with some of her Chilean PG students, and this helped to develop a joint MSc project under the Darwin Initiative between Swansea University, U. Valparaiso and U. Lagos. After suitable training at Swansea, an MSc student will travel to Dr. Whitlock's laboratory at U. Valparaiso early this summer to examine anti-predatory behaviour in endemic Chilean galaxiids. Additional funding for this project will be provided by Swansea University through the European Social Fund, and U. Valparaiso (through Dr. Whitlock).

Contacts have also been maintained with Günter Försterra and Dr. Vreni Häussermann from Fundacion Huinay (Chile), with whom we also developed student placements, and are currently advertising their work in our webpage. During this period, meetings have also been held with WWF-Chile, as well as with other NGO's (sport and professional fishermen), government agencies (CONAMA, SubPesca), and representatives of the salmon farming industry, including INTESAL (the salmon association), Marine Harvest, and salmones Multi-Export. Collaborations were also developed with Dr. Marcela Astorga (Universidad Austral, Chile) and Dr. Ricardo Galleguillos (U. Concdepcion, Chile) who agreed to participate and present their research in our workshop. Collaborations developed through the Darwin Initiative between UWS-ULA and the Falklands contacts have been extremely fruitful. A previous Darwin Scoping Award to examine the effect of introduced salmonids on native galaxiid fishes in the Falklands (extending from the current Darwin Initiative) lead to the development of a full Darwin application to be submitted on the next call for proposals. A MSc thesis (Ben Perry) on the impact of invasive salmonids on native galaxiids was jointly supervised by UWS, ULA and Falklands & Islands Development Corporation staff last year, and resulted in top qualification (Distinction). The summary and conclusions of this thesis have been uploaded into the project webpage, and are presented in the last progress report. New plans are now being made to develop a similar project this summer (June-Aug 2008). Also contacts developed during the Darwin Initiative has resulted in students from the Falklands Islands applying for a place in the MSc in Aquaculture & the Environment offered by Swansea University.

Cristian Correa, a Chilean PhD student who participated in the First Darwin Workshop and who applied with our support for a Darwin Fellowship, has recently submitted two research proposals to study the effect of exotic salmonids in Patagonia, with full support of our Darwin Initiative. He has also agreed to merge our databases on the distribution of native and exotic freshwater fishes in Chile, and to develop the GIS interface with support from the Darwin Initiative. Contacts were also made with Don Staniford, from 'Pure Salmon Campaign' (<u>http://www.puresalmon.org/</u>) and this has resulted in a fruitful exchange of information on the extent of farmed salmon escapes and their potential impacts upon native biodiversity in Chile and elsewhere.

During our sampling work on the island of Chiloe we found an unnamed second order river of the size and channel morphology perfect for conducting stream enclosure experiments. A bridge crosses the river approximately 300 metres above high tide with the river below accessible only through private land. Through neighbours Dr. Young was able contact the owner, Abraham Guevara, the general director of a Santiago based transport company (<u>www.lokaltrafik.cl</u>), who purchased the land as an investment five years earlier. After receiving permission, the Darwin field team set up a permanent camp adjacent to the river and conducted a six week enclosure experiment while their survey work continued (**see 3.1**). At the end of the field season Dr. Young was able to meet Mr. Guevara in person to thank him for his contribution and secure a similar commitment for the coming field season.

#### 3. Project progress

The key activity milestone for year 2 was capacity training and the development of methods for assessing the impact of exotic salmonids on native Chilean biodiversity.

#### 3.1 Progress in carrying out project activities

Activity 1. Research & monitoring of exotic and naturalized fish species, in relation to Output 1 (Assessment of abundance, disyrubution, and impact of exotic and naturalized salmonids). We had four primary goals for the summer 2007-08 field season: (1) establish a network of sites suitable for long-term monitoring, (2) quantify interactions between exotic salmonids and native fish, (3) collect samples from aquaculture facilities for genetic analysis, (4) identify sites for long-term intensive investigation.

#### (1) Monitoring program

We focused our field work on Chiloe Island for a number of reasons. This east side of the island has a well developed road network which provides access to dozens of small streams entering directly into the ocean. The area has one of the highest concentrations of net pen aquaculture in Chile. The importance of tourism and aquaculture to the island's economy increase the visibility and local relevance of the Darwin Initiative.

Including sites from previous field seasons we have now surveyed the fish communities of 216 sites in 114 watersheds. Every site is matched to an individual latitude and longitude for building the GIS atlas of Chilean freshwater fish. In addition to presence-absence and relative abundance of native and

non-native species, every site has a description of physical habitat, anthropogenic disturbance, and riparian type. As the data base grows this information will allow comparative analysis of the relationship between geographical, physical and biological variables across Chilean rivers. Of the over 200 sites that will be part of the GIS fish atlas more than 80 have the location, access, physical attributes and fish communities to be candidates for the long term monitoring program. We continue to add and remove sites from this list as we survey more rivers. At every site we record the number of individuals (by size class) of every species. For the majority of fish we record length (and for fewer weight). For a subset of focal salmonids and rare natives we collect scale and adipose samples. For aquaculture escapes (and select native species when population size make it ethical), we collect muscle and stomach samples. **Table 1** provides a summary of fish for which we have collected individual information, a small fraction of the total number of fish represented in our data base (whole fish are collected for museum collections in accordance with our Chilean sampling permit).

#### (2) Interactions between exotic salmonids and native fish.

We have focused our research on interactions between widely invasive rainbow trout, *Oncorhynchus mykiss*, and zebra trout, *Aplochiton zebra*, an ecologically similar native galaxiid that is locally abundant in small coastal streams of Chiloe. During this field season we conducted four studies.

*Synoptic surveys*-We are presently analyzing data from a subset of sites in the fish atlas/monitoring data base (58 streams on the east side of Chiloe) to study the invasion biology of rainbow and brown trout and their impact on zebra trout. Preliminary analyses reveal three important patterns. Rainbow trout have invaded more streams than brown trout. The native zebra trout is less likely to be present in streams with brown trout than in those with rainbow trout. In the northern quarter of the island there are still watersheds with 'pristine' native fish communities, not invaded by exotic salmonids. We intend to submit a paper based on these results within the next 6 months.

Species	# Fish	Whole	Genetic	Stomach	Muscle	Egg	Scale	
	processed	Fish	sample	sample	sample	sample	sample	
NATIVE SPECIES								
	50	2	15	1	1	0		
Aplochiton taeniatus	50	3	45	1	1	0	na	
Aplochiton zebra	746	5	279	48	48	0	na	
Brachygalaxias bullocki	204	50	57	0	6	0	na	
Galaxias maculatus	675	20	97	44	60	0	na	
Galaxias platei	422	15	122	156	137	0	na	
Geotria australis	7	1	1	0	0	0	na	
Percichthys trucha	2	0	2	1	1	0	1	
Trichomycterus areolatus	46	2	5	0	5	0	na	
EXOTIC SPECIES								
Salmo trutta (wild)	962	5	47	19	29	0	29	
Salmo salar (escapee)	146*		146	91	138	0	138	
Oncorhynchus mykiss (w)	1711	11	634	334	350	0	437	
Oncorhynchus mykiss (e)	40	0	40	40	40	1	40	
Oncorhynchus kisutch (e)	6		6	6	6	3	6	

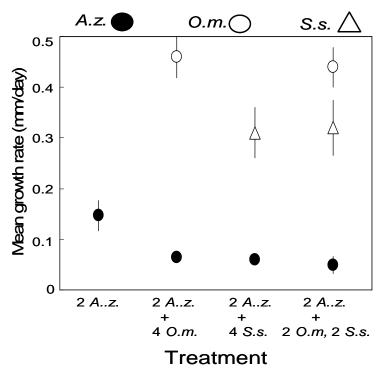
**Table 1.** Summary of individual fish samples collected to date.

\* includes juvenile escapes collected in rivers below smolt hatcheries.

*Niche overlap 1, habitat use-* Understanding the potential impacts of exotic salmonid aquaculture requires first understanding how exotic salmonids affect native species. One type of evidence for interspecific competition is if the presence of one species changes habitat use in another. We conducted a study to test for niche shifts, and by implication interspecific competition, in rainbow trout and zebra trout. We quantified patterns of habitat use in three streams: one with allopatric zebra trout, one with allopatric rainbow trout, and one with the two species in sympatry. These data are being analyzed.

*Niche overlap 2, isotope analysis*-Ecologically similar species may compete through interference competition for habitat and/or exploitation competition for consumable resources. Based on the same first principles as the work above we are comparing trophic niche overlap and evidence of niche shifts in rainbow trout and zebra trout using isotopic analysis of muscle tissue. We collected muscle samples from rainbow and zebra trout in three streams: one with allopatric zebra trout, one with allopatric rainbow trout, and one with the two species in sympatry. The samples are being processed in Swansea.

*Experimental test of invader impact-* Rainbow trout is a widespread invader in southern Chile. Atlantic salmon is the most important species in aquaculture. One of the most likely scenarios by which exotic aquaculture might impact native species is the colonization by Atlantic salmon of streams already invaded by rainbow trout. The two species have similar patterns of habitat utilization during stream rearing phase. We used these two species to conduct a stream enclosure experiment to test whether the identity and/or diversity of exotic salmonids affected their competitive impact on native zebra trout during the juvenile stage. We found rainbow trout and Atlantic salmon had a similar competitive impact on zebra trout and that the two species together did not change this impact (**Figure 1**). Consistent with other studies, our results suggest the two exotics are ecologically similar in the context of juvenile competition. This manuscript has been submitted for publication.



**Figure 1.** Growth rates  $(\pm SE)$  of the endemic galaxid *Aplochiton zebra* and two exotic salmonid species (Atlantic salmon and rainbows trout) in the four experimental treatments. Each point is the mean of the cage means of individually marked fish. *A.z.= Aplochiton zebra, O.m. = Oncorhynchus mykiss, S.s. = Salmo salar.* (Young et al., submitted)

#### (3) Samples from aquaculture facilities for genetic analysis.

During the field season we were able to collect tissue samples for genetic analysis from four aquaculture facilities, two each for Atlantic salmon and rainbow trout. Salmones Multiexport (www.multiexport.cl), one of the main Chilean salmon farms and collaborator in this project, has pledged to facilitate the sampling of their marine and freshwater facilitate.

#### (4) Sites for long-term intensive investigation.

During our synoptic surveys we discovered what appears to be an active invasion front on the island of Chiloe. There is a 100 km long sympatric gradient between rainbow and zebra trout, which contains allopatric rainbow in the south, allopatric zebra trout in the north and full range of sympatric relative abundances between the two zones of allopatry. These small coastal streams provide independent replicated samples with which to study different stages of the invasion process at the population level. Their size makes it possible to remove exotic trout from a subset of streams after an initial monitoring period and record the response of zebra trout populations. Combined with annual monitoring of over two-dozen populations along the invasion front, this ecosystem scale manipulative experiment will provide powerful insights into this system in particular and salmonid invasions in general. Recently we have found large numbers of 'naturalized' juvenile Atlantic salmon in a stream near Puerto Montt, which are unlikely to represent hatchery fish. (Annex 3, Photo 3). If confirmed, this finding would constitute the first evidence of natural reproduction of the species in Chile following the rapid development of salmon farming in the 1980's.

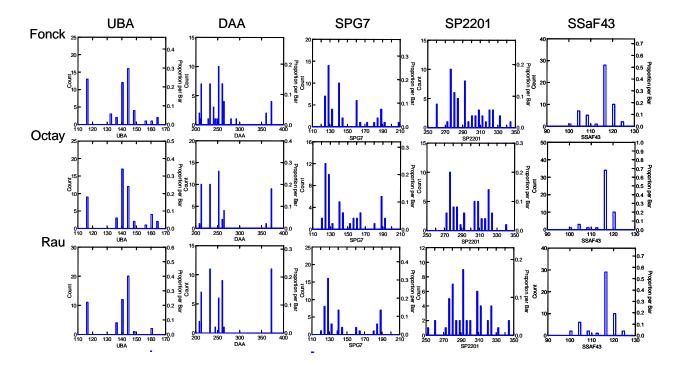
Activity 2. Capacity building and training in assessing impact of exotic invasions in relation to **Output 2** (Development of monitoring and impact assessment programme with trained personnel). The activity milestones for year 2 were to continue with training and capacity building, and to organize the second Darwin training workshop. As in year 1, progress on this activity was generally very satisfactory. The two research positions are now in place (Dr. Kyle Young- Evolutionary Ecology of Salmonid Invasions; Dr. Carlos Flores - Molecular Methods), and the ULA Genetics & Aquaculture Laboratory is fully operational (see previous progress report). Development of GIS database continues to progress and transfer and initial optimization of molecular protocols are complete for Atlantic salmon, and nearly complete for rainbow trout. A network of sampling stations for monitoring the impact of exotic salmonids was identified, and relevant sampling permission were obtained from Government. Testing of the toolkit for molecular stock identification is well under way, and set of multiplexes for both A. salmon and R. trout were optimized (Tables 2-3). So far we have processed around 974 exotic individuals (343 Atlantic salmon, 548 Rainbow trout, 83 brown trout) at various stages. A panel of thirteen microsatellites were transferred from UK partners to ULA, and these proved successful for Atlantic salmon. Eighty two Atlantic salmon from three locations have been genotyped for multiplex C (5 microsatellites; Figure 2).

Locus name	No. base pairs	Motif	Allele Size (pb)	Max. no. alleles	Reference
Multiplex A					
Ssosl 438	2	(TG)	117-151	16	Slettan et al (1995).
Ssosl 85	2	(GT)	177-226	19	Slettan et al (1995).
Ssa 171	4	(TGTA)14	189-319	32	O'Reilly et al. (1996)
Sssp 2210	4	(GTTA)11	100-189	15	Paterson et al. (2004)
Ssa D144b	4	(TAGA)	104-302	35	King et al. (2005)
Multiplex B					
SSsp2215	4	(GATA)11	110-142	14	Paterson et al. (2004)
Ssa 202	4	(CTCA)17	192-334	18	O'Reilly et al (1996)
SP1605	4	(GATA)11	171-280	14	Paterson et al. (2004
Ssa 197	4	(GTGA)15	126-283	30	O'Reilly et al (1996)
Multiplex C					
DAA	10		300-360	14	Deyto et al (2007)
SPG7	4	(GTTA)18	104-218	24	Paterson et al. (2004)
SsaF43	2	(CA)	99-135	13	Sánchez et al. (1996)
SP2201	4	(GATA)34	200-375	21	Paterson et al. (2004)
UBA	2		120-180	30	Deyto et al (2007)

Table 2. Microsatellite prime	ers used for screenin	g Atlantic salmon	populations
-------------------------------	-----------------------	-------------------	-------------

No.	Motif	Allele	Max.	Reference
base		Size	no.	
pairs		(pb)	alleles	
4	(CATA)12	112 1700	10	Decreased at $a1 (2002)$
-				Rexroad et al. (2002)
	· /		-	Rexroad et al. (2002)
				Rexroad et al. (2002)
4		134-174	6	Rexroad et al. (2002)
4	(TATC)38	247-357	15	Rexroad et al. (2002)
4	(AGAC)23 (GATA)16	131-200	12	Rexroad et al. (2002)
				Rexroad et al. (2002)
2	(TC)26	269-303	10	Rexroad et al. (2002)
4	(TCTA)13	237-291	13	Rexroad et al. (2002)
4	(TCTA)14	141-191	12	Rexroad et al. (2002)
4	(TATC)7	186-223	9	Rexroad et al. (2002)
3	(ATC)8	222-275	4	Rexroad et al. (2002)
				Rexroad et al. (2002)
4	(GATA)26	201-304	20	Rexroad et al. (2002)
4		166-227	15	Rexroad et al. (2002)
4		190-325	26	Rexroad et al. (2002)
4	(TATC)17	151-258	16	Rexroad et al. (2002)
	base pairs 4 4 2 4 4 4 4 4 2 4 4 4 3 3	base pairs 4 (GATA)12 4 (GATA)9 2 (AG)22 4 (GTGA)13 4 (TATC)38 4 (AGAC)23 (GATA)16 2 (TC)26 4 (TCTA)13 4 (TCTA)13 4 (TCTA)14 4 (TATC)7 3 (ATC)8 4 (GATA)26 4 (GATA)15 4 (TATC)38	base pairs         Size (pb)           4         (GATA)12         113-1700           4         (GATA)9         225-262           2         (AG)22         237-291           4         (GTGA)13         134-174           4         (TATC)38         247-357           4         (AGAC)23 (GATA)16         131-200           2         (TC)26         269-303           4         (TCTA)13         237-291           4         (TCTA)14         141-191           4         (TATC)7         186-223           3         (ATC)8         222-275           4         (GATA)15         166-227           4         (TATC)38         190-325	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Table 3. Microsatellite primers used for screening rainbow trout populations



**Figure 2.** Allele frequencies of Atlantic salmon from three locations (Fonck, Octay, Rau) screened for five nuclear markers (*UBA*, *DAA*, *SPG7*, *SP2201*, and *SSaF43*).

The second training workshop took place during 17, 18 and 19 of December 2007 at Viña del Mar, with the participation of 38 registered people who paid a symbolic fee (total attendance: 50 people on average) These included tudents (undergraduates, Master, PhD), several stakeholders, and partners from UWS, ULA, and U. Victoria, as well as an External Project Advisor (Dr. Eric Verspoor). The workshop (programme and abstracts can be found in the project webpage, <u>www.biodiversity.cl</u>) ended with a hands-on training session on the use genetics software for genetic stock identification and parental assignment which was very well received by students as well as some staff from Universidad de Valparaiso who also attended.

After the departure of Jane Macdonald and Ben Perry in the summer of 2007, Dr. Young provided training to two French volunteers (Alexandre Terreau of the Polar Institute and Anne-Flore Thailly of University of Orsay) who helped with the field work.

As in previous occasions, subsistence, accommodation and local transport were met by ULA and the Darwin Initiative. On February 2008 the two French volunteers left and were replaced by Jessica Stephenson (University of Oxford) who was trained on fish identification, sampling, stream habitat assessment, experimental design, and data analysis while helping with the field work. Gabriel Orellana, a Chilean student from Universidad Austral (Puerto Montt) on his final UG year joined our project recently and is conducting his undergraduate dissertation under Dr. Young's supervision. Gabriel's thesis will focus on the freshwater ecology of adult escapees from marine net pens. While the marine ecology of escaped salmon has been studied in Chile and British Columbia, there are presently no data on the freshwater ecology and potential impacts on native fish of escapees that enter rivers. Gabriel's research will address this outstanding knowledge gap using two approaches. We will repeatedly survey the lower reaches of a handful of area streams where we have found escapees during previous winter surveys. Using stomach content analysis Gabriel will quantify the frequency with which adult escapes feed on freshwater prey and test whether species identity and body size affect the propensity to feed and type prey items consumed. By sampling the stomachs of wild exotic salmonids and native species, Gabriel will also determine trophic niche overlap between escapees and other fish, providing a proxy for potential impact through exploitation competition for consumable resources. The second part of Gabriel's thesis will be an experimental investigation of escapee predation on a common native species, Galaxias maculatus. Using 'cattle tank' enclosures and established experimental procedures we will test how species and size affect the likelihood that escapees prey on G. maculatus. We will also manipulate artificial 'cover' levels to test how habitat quality affects predation rates. As cover levels are typically reduced by human disturbance (e.g. logging, farming, urbanization), this experiment will test how two types of anthropogenic disturbance may interact to affect freshwater biodiversity in Chile.

At the Osorno laboratory, the local host coordinator Dr. Gajardo, provided practical training on molecular protocols to new Chilean students: Nicole Püschel, student of Environmental Biology, from University of Chile (Santiago), Carolina Moraleda, student of Veterinarian Medicine from University Mayor (Santiago), Jorge Castro, undergraduate student of aquaculture from University Los Lagos (Osorno). Soledad Cortez, a PhD student at University Austral, Valdivia, approved her qualification and is now ready to start her thesis in the framework of the project with the title: "Effect of geographic isolation and the migratory strategy on the genetic structure of resident and diadromous *Galaxias maculatus* in the Rio Valdivia basin". She carried out a first sampling survey during 29-30 March 2008 and collected 109 individuals from 8 locations. Dr. Elie Poulin, from Universidad de Chile and member of her examination panel, who also works on the same Galaxids species, offered Soledad the primers already standardized in his laboratory. This is also a relevant contact for the project.

Héctor Venegas, an undergraduate student at Department of Aquaculture, Universidad de Los Lagos is doing his undergraduate thesis in the laboratory funded by a Fondef-Conicyt (Fund for Entrepreneur Development, National Comission for Scientific and Technological Research). This project is aimed at helping the sport fishing industry by characterizing rainbow and brown trout of four rivers of region X. This Fondef project has provided £10,000 worth of support to the Darwin project and allowed us to get trout samples from rivers not initially considered in our surveys. The primers utilized (6) were

already selected in the Fondef project, and so are considered a useful by-product to the Darwin project. Héctor Venegas also provided rainbow trout scales from rivers and lakes of region X sampled in 1982. Good quality DNA was obtained from a few initial scales, and this may allow us to compare contemporary and historical genetic diversity of rainbow trout, just when the salmon farming industry started. Francisca Hatton, a Master student at University of Chile (Santiago), is collaborating with the project by gathering key statistical data on the extent and frequency of salmon farm escapes (data not available in the web pages of governmental offices), sport fishing licences, etc.

Details of these and other training activities provided by each partner are given in **Annex 4**. Work in the next period will involve further training of personnel and recruitment of additional volunteers/students. Opportunities for student placements and volunteer are advertised in our webpage, in EvolDir, and also in flyers distributed in several Chilean Institutions and Universities.

Activity 3. Education programme and dissemination of results in relation to Output 4 (Educational events and media coverage for local people, fish farmers, and rest of stakeholders). The activity milestones for years 2 and 3 were to develop the joint MSc *curriculum* in Sustainable Aquaculture, the presentation of results at international conferences, and to maintain and update the project website with access to results and material from the workshops. Progress on this activity during the reporting period can also be considered satisfactory, though development of the MSc curriculum is taking longer than anticipated (though launch is not due till Sep 2009). The second workshop was well attended and the feedback received was in general very positive. The Darwin project continued to be publicized at conferences and seminars and featured in several media events (see previous report). Our project website (in English and Spanish) is frequently visited and is regularly updated and linked to all partners, stakeholders and sponsors. As in a previous occasion dissemination material (flyers, fact sheets) was also produced and distributed during the second workshop and also at conferences and seminars. A powerpoint slide show outlining the background, aims and methodology of project was developed and presented at several Aquaculture seminars.

In order to expand our database on the distribution of escapees and naturalized exotics, a flyer was produced and is being distributed to sport and artisan fishermen by e-mail, an also as a poster (Annex 3, Photo 4).

One manuscript was prepared and recently sent for publication to *Biological Invasions* (K.A. Young et al. Exotic salmonid diversity does not affect their competitive impact on a native galaxid) and two more are being reviewed by colleagues. Over the last 6 months we gave 12 presentations at local, national, and international conferences and/or seminars in relation to the project (full details in **Annex 4**):

G. Gajardo (ULA, Chile)

11/12/2007. MBSH-07 International Symposium. Viña del Mar, Chile.

17/12/2007. 2<sup>nd</sup> Darwin Workshop Molecular Ecol. Aquatic Invasions, Viña del Mar, Chile. C. Flores (ULA, Chile).

8/11/2007. XL Annual meeting Genetics Society of Chile. Tomé, Concepción. Chile. K.A. Young (ULA, Chile)

17/12/2007. 2<sup>nd</sup> Darwin Workshop Molecular Ecol. Aquatic Invasions, Viña del Mar, Chile. C. Garcia de Leaniz (UWS, UK)

17/12/2007. 2<sup>nd</sup> Darwin Workshop Molecular Ecol. Aquatic Invasions, Viña del Mar, Chile. 22/02/2008. SALSEA-MERGE Conference, Paris, France (keynote talk).

S. Consuegra (UWS, UK)

17/12/2007. 2<sup>nd</sup> Darwin Workshop Molecular Ecol. Aquatic Invasions, Viña del Mar, Chile. 22/02/2008. SALSEA-MERGE Conference, Paris, France

J. Volpe (U. Victoria, Canada)

10/10/2007. OSU Fisheries and Wildlife Seminar Series, Oregon USA.

17/12/2007. 2<sup>nd</sup> Darwin Workshop Molecular Ecol. Aquatic Invasions, Viña del Mar, Chile. J.A. Beardmore (UWS, UK)

17/12/2007. 2<sup>nd</sup> Darwin Workshop Molecular Ecol. Aquatic Invasions, Viña del Mar, Chile. E. Verspoor (FRS, UK)

17/12/2007. 2<sup>nd</sup> Darwin Workshop Molecular Ecol. Aquatic Invasions, Viña del Mar, Chile.

Two MSc thesis supervised by the UK-leader were produced in relation to the project (see previous report): Ben J.Perry. "Effects of non-native salmonids on native fish fauna in Chile and the Falkland Islands" (awarded mark = 81%, *Distinction*) and Anne Rees. "Determinants of establishment success among non-native fish" (awarded mark = 64%). These were presented at a one-day Symposium on October 2007 and their summaries and conclusions posted in the project webpage.

#### 3.2 Progress towards Project Outputs

Overall progress towards achieving project outputs can be considered satisfactory. Valuable links, based on mutual trust, respect and the need for rigorous scientific data, have been forged with the salmon industry and other key stakeholders, including NGO's and Government. It is hoped that continuing dialogue with stakeholders will help to develop (and ensure necessary endorsement of) MAP and CBP towards the end of the project. The main challenges and difficulties lie in the logistics of the project, which in Chile are particularly complicated, and the extreme difficulty of sampling remote aquatic ecosystems, particularly in autumn and winter. More recently, problems caused by Aysen earthquake, the ISA virus, and difficulties with the export market have made the Chilean salmon industry more cautious

#### 3.3 Standard Output Measures

#### **Training output measures**

Training output measures include the attendance by 10 Chilean students (5 undergraduate and 5 postgraduate) to the second project workshop (total attendance c. 50 people each day). Students were issued with official certificates of attendance, jointly signed by the UK leader on behalf of University of Wales Swansea, and the local host coordinator on behalf of Universidad de Los Lagos.

Project partners also provided advice and training to RA's during and after the workshop. Dr. Young trained two project volunteers during the second field season, and is currently training two more (including an undergraduate student). Training at the Osorno laboratory included 6 people: four undergraduates and two post-graduate sudents (including Soledad Cortez, a PhD candidate from Universidad Austral in Valdivia). Other training output measures include an information leaflet highlighting the project objectives and main features, a powerpoint presentation describing the project, a project website in English and Spanish, as well as protocols for field sampling, DNA extraction, mtDNA RFLP analysis, and microsatellite amplification.

#### **Research output measures**

Research output measures include 18 days spent in Chile working on the project by partners from UK & Canada during the last six months the project (including Project External Advisor – Dr. Eric Verspoor). In addition we estimate we have spent over 900 hrs (excluding time spent by paid staff) in the project, including time spent on manuscripts, one submitted (Young et al) and two in preparation. We are currently making arrangements to present the first results of our project at the forthcoming Conference on 'Managing Alien Species for Sustainable Development of Aquaculture and Fisheries' to be held on 5-7 November 2008 at Florence (Italy) <a href="https://www.dbag.unifi.it/maliaf/">http://www.dbag.unifi.it/maliaf/</a>

#### **Dissemination output measures**

Dissemination output measures include the organization of the second training workshop (Vina del Mar, 17-19 December 2007), 12 oral presentations at national and international seminars/conferences, We also hosted a one-day (8 october 2007) visit by school children to the Laboratory of Genetics & Aquaculture (Osorno), and presented a poster at the Annual Meeting of the Chilean Genetics Society (November 3-7 2007). A delegation from Conicyt (National Commission for Science and Technology)

and the European Union visited the ULA-Osorno laboratory equipped by the Darwin Initiative, and learnt on the objectives and progress of the project.

#### **Physical output measures**

Physical output measures include the following: cash contribution by ULA to cover expenses for the second workshop (£2,000) and a total of £12,032 for ticket Prof. Beardmore, accommodation for Drs. M. Pascual and J. Ciancio ( $2^{nd}$  workshop), Courier service, and contribution from other projects (FONDEF, project funding thesis of UG student Héctor Venegas). Contributions in kind also include an estimated £3,500 paid directly by partners and stakeholders to attend the second workshop and over £23,000 in time invested by partners on the project (**Table 4**).

Code No.	Description	Year 2
		(Oct-Mar)
Training of	utputs	
2A	UG Chilean students attending 2 <sup>nd</sup> workshop and other training	9
4B,D	Training	53 weeks
4C	PG Chilean students attending 2 <sup>nd</sup> workshop and other training	7
	PG Non-Chilean students receiving training	3
5	RA's training	2
6A,B	Attendance of 2 <sup>nd</sup> workshop and seminar	50
7	Education/training material produced: information leaflet, PowerPoint	6
	slideshow, microsatellite protocols, website, flyers on exotic salmonids	
Research of	outputs	
8	Time spent in Chile by partners (days); UK partners*: 15	18
	Canada partner: 3	
11A,B	Peer-reviewed MS submitted	1
	Peer-reviewed MS in preparation	2
Dissemina	tion outputs	
14A	Project training workshop	1
14B	Presentation conferences/seminars	12
15A,B	Press coverage in Chile	2
16A	Newsletter	1
17B	Project website, regularly updated and linked to all partners	1
19C	Radio features in Chile	2
20	Equipment	£410
23	ULA cash contribution to 2nd workshop	£1,919
	Courier Service	£469
	Contributions from other projects (e.g. FONDEF)	£9,634
	Paid by partners & stakeholders to participate in 2nd workshop	£3,500
	Time spent in project (all partners)	£30,938
	Total	£46,460

Table 4. Project Standard Output Measures (same codes as per last half-yearly report)

#### Table 5. Publications

Type *	Detail	Publishers	Available from	Cost
(eg journals,	(title, author, year)	(name,	(eg contact address,	£
manual, CDs)		city)	website)	
MSc thesis	BJ. Perry. 2007. Effects of non-	Swansea	Summary and	
	native salmonids on native fish fauna	University	conclusions in project	
	in Chile and the Falkland Islands	January	webpage.	
		2008	www.biodiversity.cl	
MSc thesis	A. Rees. 2007. Determinants of	Swansea	Summary and	
	establishment success among non-	University	conclusions in project	
	native fish	January	webpage.	
		2008	www.biodiversity.cl	
Journal	K.A. Young et al. Exotic salmonid	Biological		
	diversity does not affect their	Invasions		
	competitive impact on a native	(submitted)		
	galaxid			

#### 3.4 Progress towards the project purpose and outcomes

Overall progress towards achieving the project's ultimate goal (To build, in collaboration with government, industry and other stakeholders, the capacity for assessing, monitoring, and reducing the impact of the accidental or deliberate introduction of exotic fish species on Chilean aquatic biodiversity) is still a long way off on our second year into the project but we believe that the necessary steps are being taken. Despite recent problems with the Chilean salmon industry (which served to highlight the lack of sustainability in some farming practices) the basic assumptions hold true and the project indicators seem adequate.

# 3.5 Progress towards impact on biodiversity, sustainable use or equitable sharing of biodiversity benefits

We believe that the most lasting legacy of our project will be the quality of our scientific results regarding the impact of salmonids upon native aquatic biodiversity, the training of students, and the development and endorsement of a Management Action Plan and Code of Best Practices in relation to salmonid farming in Chile.

The project is far from achieving this generic goal, but is going in the right direction to help change attitudes that allocate high priority to immediate economic returns, without considering medium and long term goals that are needed for sustainability. The project has helped to permeate this vision to stakeholders (see statement after first workshop: (www.biodiversity.cl/workshop) and students, who see the project filling an empty niche in Chile. It is worth pointing out that in the 2007 workshops important officers of governmental bodies, such as CONAMA (Natl. Commission for the Environment), responsible for implementing the national programme on biodiversity, and SUBPESCA (Undersecretariat for Fisheries), have continued to express to the UK and Chilean partners their full support and endorsement of the project.

#### 4. Monitoring, evaluation and lessons

We still see the main challenges and difficulties of the project in the logistics, which in Chile are particularly complicated, and the extreme difficulty of sampling remote aquatic ecosystems, particularly in autumn and winter. The acquisition of a 4WD vehicle by ULA and a lease agreement with the project has greatly facilitated the field work, increasing our capacity for more extensive sampling and surveying. In addition, the skills gained during the first year, and the help of additional volunteers and personnel have contributed to make our work more efficient.

As for the previous reporting period, research progress in some aspects of our project was slower than we had hoped. Although much progress has been achieved after the last workshop, the application of the molecular toolkit is taking longer than we anticipated, and a more efficient analysis of samples is required. Likewise, the analysis of stable isotopes has continued to suffer delays though we are drafting an agreement with the Natural Isotopes Laboratory at the Millenium Centre (Swansea University) to process our samples this summer. On the other hand, the help of highly motivated and skilled volunteers has continued to be particularly fruitful, and valuable results were obtained in 2 MSc theses, which were awarded good marks.

Progress in disseminating the project through oral presentations and other activities has continued to be successful, and two papers have already been submitted and two are in preparation. We have continued to attract highly motivated volunteers from overseas (France, UK) this is greatly aided by arrangements with ULA to cover accommodation and living expenses. After initial delays, we have finally succeeded in recruiting Chilean volunteers to work in the field and there is a growing number of Chilean students heloping us in several aspects o the work following a more proactive advertising campaign at Universities and other teaching institutions.

Finally, we have continued to react to opportunities using the Darwin Initiative as leverage for securing additional funding and support for the project, and to encourage joint supervision of research students working on the project with colleagues in Chile and elsewhere.

#### 5. Actions taken in response to previous reviews (if applicable)

We believe we have taken the necessary actions in response to previous comments made by the reviewer, namely: (we expanded on these in section 4 above, as suggested)

- 1. We have conducted a critical and thorough assessment of progress to date
- 2. The project is fully staffed since April 2007 when the second RA (Carlos Flores) was appointed.
- 3. Volunteers and equipment are also in place for the field and laboratory work

#### 6. Other comments on progress not covered elsewhere

No further comments seem necessary

#### 7. Sustainability

Scarcity of suitable baseline data was highlighted by all stakeholders as one of the most important threats to achieving sustainability in the Chilean salmonid industry. Thus, the success of our project will largely depend on the quality of our data on extent and impact of exotic salmonids upon native species, principally fishes. This, we believe, will in turn generate trust and interest and foster capacity for biodiversity. As the salmong industry is facing serious problems due to the ISA virus, the project will capitalize on the need to produce systematic and reliable science - not currently available to attack this problem.

#### 8. Dissemination

Dissemination activities during the last six months of the project were substantial and included the organization of the second training workshop, as well as 12 oral presentations and other activities by partners (**Annex 4**). A paint contest is being prepared in collaboration with the School of Design at ULA to raise awareness among school children on the impact of exotic species and their effects on native biodiversity. It is anticipated that the best twelve paintings will illustrate the 2009/10 calendar. We are also preparing our participation at the forthcoming Conference on 'Managing Alien Species

for Sustainable Development of Aquaculture and Fisheries' and beginning work on the organization of the Third Darwin Workshop.

#### 9. Project Expenditure

# Project expenditure <u>during the reporting period</u> (Defra Financial Year 01 April to 31 March) – 01 April 2007 – 31 March 2008

Item	Budget (please indicate which document you refer to if other than your project schedule)	Expenditure	Balance
Rent, rates, heating,			
overheads etc			
Office costs (eg postage,			
telephone, stationery)			
Travel and subsistence			
Printing			
Conferences, seminars, etc			
Capital items/equipment			
Others			
Salaries (specify)			
TOTAL			

The main differences in the balance lie in the expenditure for capital equipment, which was less than anticipated, and the costs of transport and consumables for field work which was more expensive. These are preliminary figures, subject to final auditing.

# 10. OPTIONAL: Outstanding achievements of your project during the reporting period (300-400 words maximum). This section may be used for publicity purposes

I agree for ECTF and the Darwin Secretariat to publish the content of this section

Abstract of MS submitted to Biological Invasions

# **Exotic salmonid diversity does not affect their competitive impact on a native galaxiid** Kyle A. Young et al

**Abstract** We used an invaded stream fish community in southern Chile to experimentally test the hypothesis that exotic species diversity affects their competitive impact on a native species. In artificial enclosures an established invasive, rainbow trout, *Oncorhynchus mykiss*, and a potential invader, Atlantic salmon, *Salmo salar*, reduced the growth rate of native zebra trout, *Aplochiton zebra*, by the same amount. In enclosures with both exotic salmonids, the growth rates of all three species were the same as in single exotic treatments. While neither species identity or diversity appeared to affect competitive interactions in this experiment, the impact of exotic salmonid diversity may vary with the type of interspecific interaction and/or the co-evolutionary histories of the exotics. Our study links two prominent concepts in invasion biology by testing whether the result of invasional meltdown, an increase in exotic species diversity, affects their impact through interspecific competition, the mechanism invoked by the biotic resistance hypothesis.

**Keywords** Exotic species diversity  $\cdot$  Interspecific competition  $\cdot$  Salmonids  $\cdot$  Galaxiids  $\cdot$  Stream enclosure experiment  $\cdot$  Aquaculture

· ·		Progress and Achievements April 2007 - March 2008	Actions required/planned for next period		
work with local partners in countries rich achieve The conservation of biological di	odiversity from within the United Kingdom to a in biodiversity but constrained in resources to versity,The sustainable use of its components, benefits arising out of the utilisation of genetic	See also <u>www.biodiversity.cl</u>	(do not fill not applicable)		
aquatic biodiversity	to exotic species, introductions and protection of native aquatic biodiversity 3. Increased understanding and public awareness of threats to native bio-diversity resulting from foreign fish introductions		<ol> <li>Organization of third training workshop, dissemination</li> <li>Continuation of sampling program</li> <li>Development of databases</li> <li>Testing of molecular methods for GSI</li> <li>Submission of 2 MS: 1 popular science, 1 scientific paper</li> </ol>		
<b>Output 1.</b> Abundance, distribution, and impact of exotic and naturalized salmonids assessed	1. Findings endorsed by the scientific community and stakeholders	distribution of exotic and naturalized sa development of (and reference to) datal community and stakeholders	base, in addition to endorsement by scientific		
Activity 1. Research & monitoring of exotic a	Activity 1. Research & monitoring of exotic and naturalized fish species		A sampling field protocol involving a combination of snorkeling and electro-fishing was developed, as well as protocols for collecting samples and carrying out genetic analysis. Two salmon farms, and 216 sites in 114 watersheds were sampled. Work in the next period will involve expanding the coverage of fish farms and sampling sites, as well as the beginning of isotopic analysis		
<b>Output 2.</b> A monitoring and impact assessment programme with trained personnel established	2. GIS database & molecular and isotopic toolkits for identification of exotic and farmed fish species developed, tested, and at least 2 staff trained	s Development of GIS database in progress. Development of molecular protocols a			
Activity 2. Capacity building and training in a	assessing impact of exotic invasions	As per activity 1, involving 2 hired staff (RA's), 2 technicians and three volunteers. Work in the next period will involve further training and recruitment of additional volunteers/students.			
Output 3. MAP, CBP, and possible exclusion zones for protecting aquatic biodiversity from exotic invasions3. MAP and CBP peer reviewed and presented at international conference		Discussed with stakeholders at first workshop, though this output is expected to be completed by end of project. Continuing dialogue with stakeholders will help to develop (and ensure necessary endorsement of) MAP and CBP			
Activity 3. Research & monitoring of exotic	and naturalized fish species	As per activity 1.			
	4. Participation of fish farmers and rest of stakeholders in educational events, Darwin project featured in media	participated actively in in second works conferences and seminars and featured	tput. As in previous occasions, stakeholders shop. Darwin project was also publicized at in the media.		
Activity 4. Education programme and dissem	ination of results	developed. Two manuscripts so far have others are in preparation. Plans are bein	e during the next period, as per logical able Aquaculture to launched at ULA is being been submitted for publication, and two ang made to attend a conference on 'Managing nent of Aquaculture and Fisheries' next		

## Annex 1. Report of progress and achievements against Logical Framework for Financial Year: 2007/08

## Annex 2. Project's full current logframe

Project summary	Measurable Indicators	Means of verification	Important Assumptions
Purpose To build, in collaboration with government, industry and other stakeholders, the capacity for assessing, monitoring, and reducing the impact of the accidental or deliberate introduction of exotic fish species on Chilean aquatic biodiversity	<ol> <li>New knowledge on the distribution and abundance of exotic fish species and their impact upon native aquatic biodiversity</li> <li>Endorsement of a Management Action Plan (MAP) and Code of Best Practice (CBP) in relation to exotic species, introductions and protection of native aquatic biodiversity</li> <li>Increased understanding and public awareness of threats to native bio-diversity resulting from foreign fish introductions</li> </ol>	<ol> <li>Project reports, workshop proceedings and publications in peer-reviewed journals</li> <li>Documentation and correspondence for MAP and CBP</li> <li>Records of educational programme and training workshops. Development of professional curricula on sustainable aquaculture. Students trained under programme pass their courses</li> </ol>	<ol> <li>Project findings are understood and accepted by the salmon industry, regulatory agencies and all stakeholders.</li> <li>Policy makers use findings to help reduce the impact of exotic species on local biodiversity</li> <li>Market forces and increased recognition on the impact of exotics facilitate the shift towards more sustainable fish farming</li> <li>Curriculum development is implemented by ULL with the launch of a MSc in Sustainable Aquaculture</li> </ol>
Outputs 1. Abundance, distribution, and impact of exotic and naturalized salmonids assessed 2. A monitoring and impact assessment programme with trained personnel established 3. MAP, CBP, and possible exclusion zones for protecting aquatic biodiversity from exotic invasions 4. Educational events and media coverage for local people, fish farmers, and rest of stakeholders	<ol> <li>Findings endorsed by the scientific community and stakeholders</li> <li>GIS database &amp; molecular and isotopic toolkits for identification of exotic and farmed fish species developed, tested, and at least 2 staff trained</li> <li>MAP and CBP peer reviewed and presented at international conference</li> <li>Participation of fish farmers and rest of stakeholders in educational events, Darwin project featured in media</li> </ol>	<ol> <li>Publication of results in peer reviewed, national and international scientific journals</li> <li>Project reports, scientific papers, abundance and distribution maps, species database, fish escape assessment protocol, project website</li> <li>Workshop proceedings, MAP and CBP published and distributed, copies sent to Darwin Initiative</li> <li>Educational leaflets, press releases, media coverage, reports</li> </ol>	<ol> <li>Sampling strategy and logistic support are appropriate for project objectives and facilitate the collection of data</li> <li>Adequate performance of molecular and isotopic diagnostic toolkits to produce desired results</li> <li>Successful liaison with industry, government agencies and rest of stakeholders for project support</li> <li>Links to educational media and NGO's are established (already in place via ULL)</li> </ol>
Activities 1. Capacity building and training in assessing impact of exotic invasions 2. Research & monitoring of exotic and naturalized fish species 3. Education programme and dissemination of results	<ul> <li>r1. Initial meetings with stakeholders. Develop GIS database, molecular and isotopic iagnostic toolkits and run training workshops, establish sampling strategy and field rotocols. Follow-up training workshops in Yr2 &amp; Yr3.</li> <li>r1.Field test diagnostic methods for species identification, stock assignment and trophic iche overlap, begin screening. Yr2 &amp; Yr 3. Continue screening, workshops to discuss esults. Yr 3. Writing of scientific publications, MAP and CBP.</li> <li>r1. Develop education programme, establish website, and attract media interest. Yr2-Yr3. evelop MSc curriculum, presentation of results at international conferences. All years:</li> </ul>		<ul> <li>Assumptions</li> <li>Program receives required support from University (in place).</li> <li>Research methods and tools are adequate. Required baseline information is provided by industry and regulatory bodies</li> <li>Successful liaison with stakeholders and media interest. Support from University</li> </ul>

Annex 3. Photographs

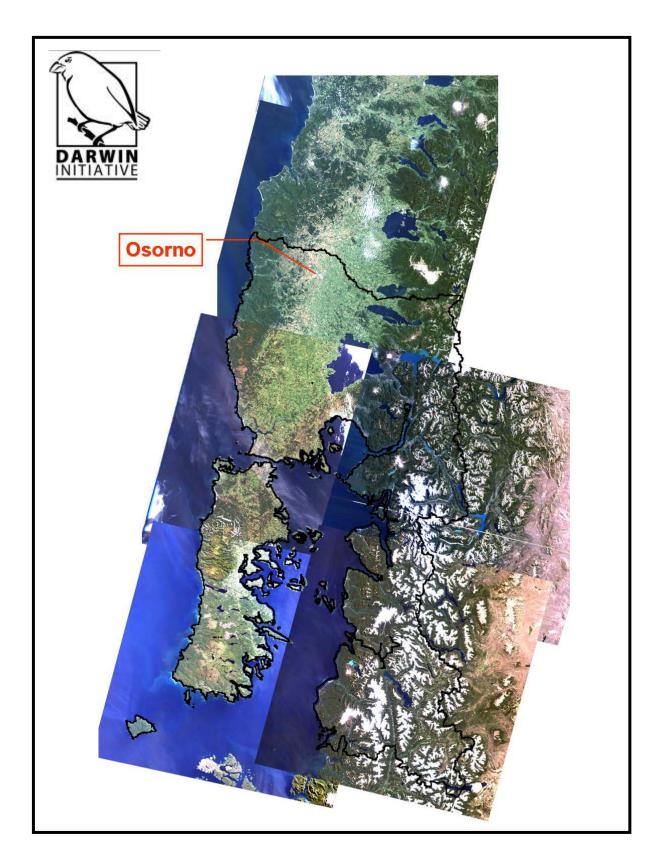


Photo 1. Satellite map of Region X (Los Lagos) where most of the Chilean salmon farming is concentrated

Photo 2. Some of the participants and public who ttended the Second Darwin Workshop on Molecular Ecology of Aquatic Invasions, held at Vina del Mar (17-19 December 2007)





Photo 3. Juveniles of brown trout (top) and Atlantic salmon (bottom) in the River Chaica, south of Puerto Montt. If confirmed, the presence of young A. salmon in this river (where there is no hatchery and no record of recent deliberate introductions) would represent the first evidence of natural reproduction of Atlantic salmon in Chile.



#### Photo 4. Flyer for collecting data from the public on the distribution of exotic salmonids in Chile



Annex 4. Details of individual contributions