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, excluding annexes
Submission deadline 30 April 2009

Darwin Project Information

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)
Host country 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)
Other 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 Mar 200y) and annual report number (1,2,3..)	01 April 2008 – 31 March 2009 Annual Report 3
Project Leader Name	Dr. Carlos Garcia de Leaniz
Project website	www.biodiversity.cl
Author(s) and main contributors, date	Carlos Garcia de Leaniz, Gonzalo Gajardo, Kyle Young; 30 April 2009

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 the second world's largest 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 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, 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 work well during the past year (April 2008-March 2009). As in previous occasions, it benefited from fluent and frequent email and telephone contacts, in addition to visits by Chilean staff (Dr. Gajardo, Dr. Young) to the UK, six Departmental Seminars in UK, US, Canada and Spain and presentation of results at the international conference *Managing Alien Species for Sustainable Development of Aquaculture & Fisheries* (MALIAF, Florence, Italy 04-08 Nov. 2008) http://www.dbag.unifi.it/maliaf/MALIAF_abstracts.pdf during this period (full details in **Annex 4**).

Contacts between partners were also fruitful in several other ways. For example, several partners commented and gave valuable feedback on four manuscripts, two already published (Young et al 2008. Biological Invasions DOI 10.1007/s10530-008-9372-5; Buschmann et al 2009. Ocean & Coastal Management DOI 10.1016/j.ocecoaman.2009.03.002), one recently submitted (Stephenson et al. 2009) and one soon to be submitted for publication (Young et al 2009; **Annex 3**). They also gave advice on GIS, development of the database, and experimental design. Partnership and collaborations made during the past year of the Darwin Initiative were also instrumental in helping to develop a joint MSc project at the University of Valparaiso (Prof K. Whitlock) and Falkland Islands Development Corporation. A MSc thesis (Paul Howes) on recognition of salmonid predators by native galaxiid fishes was jointly supervised by Darwin partners and staff from U. Valparaiso and FIDC and this resulted in top qualifications (Distinction) at Swansea University. Likewise, an undergraduate dissertation by Vivien Schröder on the use of stable isotopes for discriminating farmed from free-living salmonids in Chile was also awarded top marks (90%, first class) at SU. As in previous occasions, the summaries of these have been uploaded into the project webpage, and are presented in **Annex 3.**

During the past year we continued advising national and international NGO's involved in protecting freshwater biodiversity in Chile. Thus, Mrs. Paula Moreno, the aquaculture coordinator for the World Wildlife Fund Chile, commissioned a report on the impacts of aquaculture escapees in Chile based on an outline proposed by Dr. Young (see previous annual report) We also provided WWF with a qualitative summary of the Darwin Project results to date and identified outstanding questions for future research. Dr. Young advised Aaron Sanger, an attorney leading the International Rivers campaign against hydroelectric development in southern Patagonia, and prepared a technical assessment of the 'Aquatic Ecosystem' section of the Environmental Impact Statement. He also met Mr. Sanger at Bellingham, WA on 1 December, 2008 while Dr. Young was travelling to present project results at two departmental seminars in Canada.

We continued interacting with Cristián Correa, a Chilean PhD student at McGill University (Canada) and Dr. Brian Dyer (Universidad de Viña del Mar, Chile), and agreed to combine our different data sets into a common Chilean atlas of native and exotic fish species, that would be made available through the Darwin project website. To achieve this aim, we also contacted partners in a large EU-funded project http://www.nasco.int/sas/salseamerge.htm (SALSEA-MERGE), who provided suitable software code and are helping us to produce an interactive version of the fish atlas, currently under development. We also helped a Chilean PhD student from PUCV at Valparaiso (Daniela Atria Gomez), to apply for funding to come to Dr. Consuegra's laboratory at University of Wales to work on MHC genetic screening of chilean farmed salmonids. Likewise, the Darwin Initiative supported a grant application by Dr. Guido Plaza (also from PUCV) to the Chilean funding agency FONDECYT entitled Daily growth ptterns, sawning oigin and degree of anadromy in free-living Salmonids in Southern Chile. Finally, our partnership was successful in obtaining a 3 year PhD studentship (£45,000) for Delphine Vanhaeke (University of Wales Aberystwyth) entitled 'Landscape genetics of salmonid invasions in the southern hemisphere' beginning in October 2008. On February 2009 Delphine went to sample in Chile for two months under the umbrella of the Darwin Initiative.

Other collaborations developed through the project partners included contacts with GESA in Argentina (Dr. Javier Ciancio) and U. La Laguna in Spain (K. Toledo, Prof. A. Brito), and these

proved instrumental in developing a toolkit for distinguishing wild from farmed fish based on isotopic signatures (see results below) and for applying for additional sources of funding. We also continued liaising with Mr. Don Staniford, from 'Pure Salmon Campaign' in the USA (http://www.puresalmon.org/) and this resulted in a fruitful exchange of information on the extent of salmon escapees, and their potential impacts upon native biodiversity in Chile and elsewhere. Mr Staniford has agreed to advise us on developing a Code of Best Practices to reduce the escape and impact of farmed salmonids in Chile, and will participate in our last workshop and final project meeting, scheduled for 2-5 September 2009 .

3. Project progress

The key activity milestone for the past year 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, distribution , and impact of exotic and naturalized salmonids). We continued monitoring the distribution and abundance of native fish in relation to exotic and naturalized salmonids, and prepared a manuscript on the impact of exotic salmonids on native fish biodiversity. We also experimentally investigated prey-predator interactions between exotic salmonids and native galaxiids, initiated a study on the trophic ecology of salmonids, and began applying the molecular toolkit to the identification of salmonids escaping from fish farms.

(1). Monitoring the impact of exotic salmonids on native fish biodiversity. We have continued our field monitoring program on Chiloe Island, as this has the highest concentrations of net pen aquaculture in Chile, and also around the Osorno area. Amongst native galaxiids, *Aplochiton zebra* and *Galaxias maculatus* dominate our sampling sites, whereas rainbow trout (*Oncorhyunchus mykiss*) and brown trout (*Salmo trutta*) are the most locally abundant exotic species in our sampling sites. There is large variation among sites in the relative abundance of wild (naturalized) and escaped salmonids. It is apparent that some streams support self-sustained salmonid populations, whereas others are probably in the initial stages of a salmonid invasion (**Figure 1**).

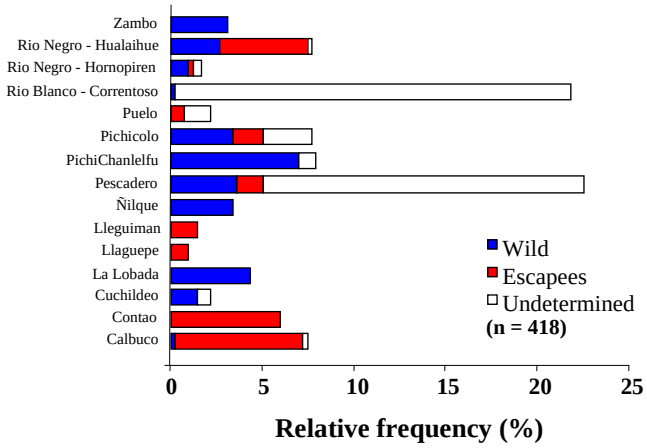


Figure 1. Likely origin of 418 free-living salmonids sampled at 15 locations during February 2009 based on external appearance and internal dissection. Overall, 30% of salmonids were classified as wild (naturalized) fish, 24% were classified as recent escapees, and 46% could not be classified.

We are in the final stages of submitting a manuscript entitled "A trial of two trouts: comparing the impacts of rainbow and brown trout on a native galaxiid." This paper was presented at the recent MALIAF conference in Italy (http://www.dbag.unifi.it/maliaf/MALIAF_abstracts.pdf) and provides to our knowledge the first quantitative comparison of the impacts of rainbow and brown trout on a native fish. The abstract (given in section 10 below) summarizes our key findings.

(2). Prey-predator interactions between invasive salmonids and native galaxiids. Working with Dr. Kathleen Whitlock at University of Valparaiso, a MSc student from Swansea University (Paul Howes) and a Darwin volunteer (Jessica Stephenson) investigated experimentally the olfactory ecology of prey-predator interactions between an invasive salmonid (brown trout) and two native galaxiids, *Galaxias maculatus*, and *Aplochiton zebra*. We found that following the addition of alarm cues, *G. maculatus* tended to school more but showed no tendency to move less or hold lower in the water column (**Figure 2**). These results suggest that *G. maculatus* does not produce skin alarm cues, or that it responds to alarm cues in ways not detected in our experiment. This has been submitted as a short communication to the *Journal of Fish Biology*.

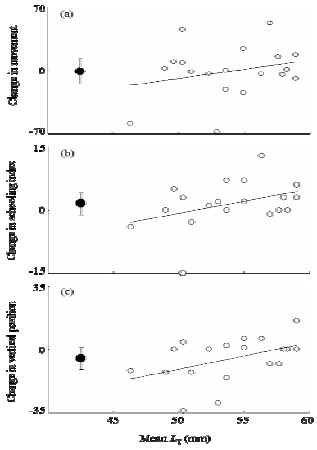


Figure 2. Relationships between mean total length (L_T) and change in (a) movement, (b) schooling and (c) vertical position, calculated as the score after the addition of skin extract minus the score before the addition of skin extract. A score of 0 = no change in recorded behaviour. Open circles represent the mean L_T of the three fish used in each trial. Filled circles (±95% CI) are the means of the 19 trials (Stephenson, J.F. et al. Testing for alarm cue response in a circumpolar galaxiid *Galaxias maculatus.* submitted to *J. Fish Biol.*)

We next used a 'y-maze' to test the hypothesis that juvenile *G. maculatus* would avoid the odour of a sympatric predator but would ignore the odour of a novel predator. Our hypothesis was supported by a highly significant population by odour interaction (**Figure 3**).

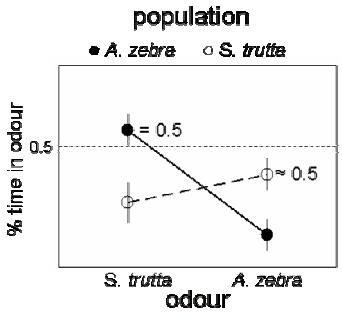


Figure 3. The mean (SE) percent of time spent by the galaxiid *G. maculatus* in the arm of a y-maze scented with the odour of a native predator (*Aplochiton zebra*) or the odour of an exotic predator (*Salmo trutta*). Fish from the stream with *A. zebra* avoided the scent of *A. zebra* but not that of *S. trutta*. Fish from the stream with *S. trutta* avoided the scent of *S. trutta* but not that of *A. zebra*, though the pattern was weaker (Howes, P.N. 2008. MSc thesis. Swansea University, 100 p.)

In a second experiment we found that predator diet strongly affected the anti-predatory response of *G. maculatus. G. maculatus* from a stream with *A. zebra* avoided the predator odour regardless of diet, but those from a stream with *S. trutta* avoided the predator odour only when it was fed conspecifics (**Figure 4**).

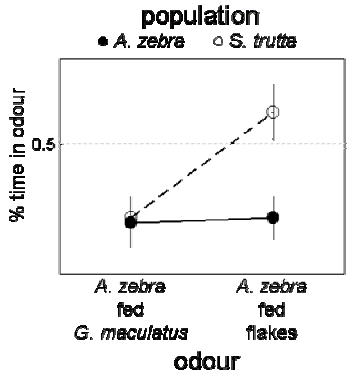


Figure 4. The mean (SE) percent of time spent by *G. maculatus* in the arm of the y-maze treated with the scent of *A. zebra* fed either conspecifics or fish flakes. Fish from the stream with the native predator *A. zebra* avoided the scent of *A. zebra* regardless of diet. Fish from the stream with the exotic predator *S. trutta* (without the native predator *A. zebra*) avoided the scent of *A. zebra* only when it had eaten other *G. maculatus*. (Howes, 2008.)

In a third series of experiments, carried out in the Falklands, we examined the effects of olfactory and visual cues on anti-predatory behaviour of *G. maculatus.* As in Chile, we compared the responses of *G. maculatus* originating from two populations, one cohabiting with the exotic predator, *S. trutta,* and one cohabiting with the native predator, *A.zebra.* The results suggest that, as in Chile, each population of *G. maculatus* responds most strongly to the scent of the predator it lives with (**Figure 5**).

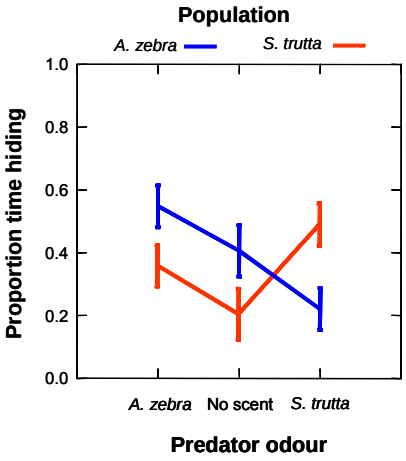


Figure 5. The mean (SE) percent of time spent by *G. maculatus* hiding in a circular test arena scented with blank water (no scent), the odour of a native predator (*Aplochiton zebra*), or the odour of an exotic salmonid predator (*Salmo trutta*). *G. maculatus* in the Falklands respond most strongly to the scent of the sympatric predator. (Howes, 2008)

The results also indicate that the combination of visual and chemical cues always elicits a stronger hiding response in *G. maculatus* than the smell of a predator alone, regardless of population of origin, or predator type (**Figure 6**).

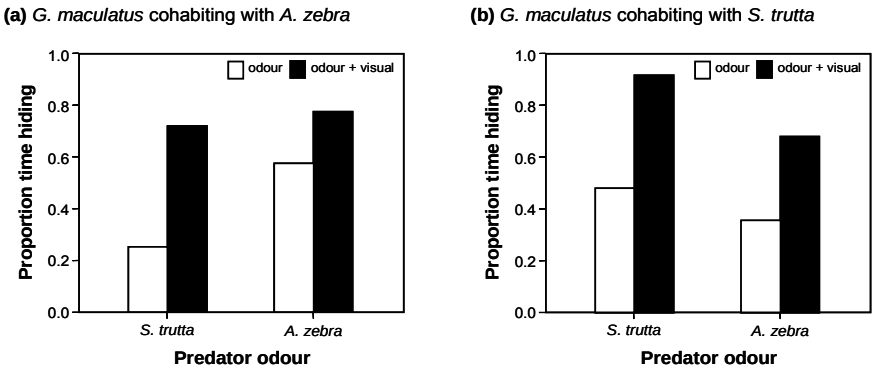


Figure 6. The proportion of time spent hiding by *G. maculatus* in a circular test arena when exposed to the odour and sight of a predator. The sight and smell of a predator elicit a stronger hiding response than the smell of a predator alone, regardless of population of origin, or type of predator. (Howes. 2008)

Together these experiments suggest that while *G. maculatus* may not display a strong innate alarm cue response, it can quickly learn to identify and avoid the odour of sympatric predators, perhaps through association with the odours of preyed conspecifics. Given that exotic salmonids dominate many fish communities in cool temperate waters of the Southern Hemisphere and that predation is the mechanism by which exotic species most often drive natives to local extirpation, understanding the olfactory ecology of galaxiid-salmonid interactions is a global conservation priority. Our project, hence, is contributing to an increasingly important field and laying the basis for future collaborations in Chile and elsewhere.

(3). Trophic ecology of exotic salmonids escaping from fish farms. Ecologically similar species may compete through interference competition for habitat and/or exploitation competition for consumable resources. We are examining the scope for trophic competition between exotic salmonids and native galaxiids in two ways, indirectly through the analysis of stable isotopes in muscle tissue, and directly through stomach analysis.

Analysis of stable isotopes. First we compared isotopic signatures of farmed and wild-caught salmonids and found significant differences between them, which may make it possible to identify farm escapes from wild (naturalized) fish (**Figure 7**).

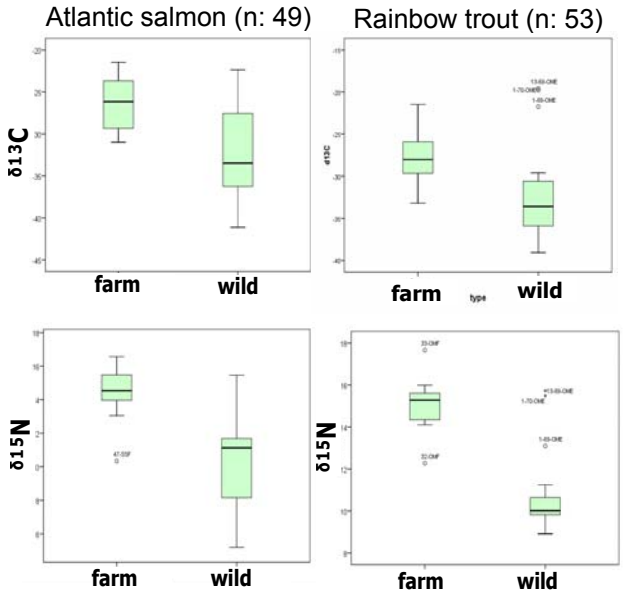


Figure 7. ^{13}C and ^{15}N isotopic signatures for Atlantic salmon and rainbow trout collected in Chiloe. Farmed salmonids show significant carbon and nitrogen enrichment compared to free-living fish (V. Schröder. 2009, Exotic salmonids in Chile – A stable isotope approach. Marine Biology dissertation, Swansea University).

Our pilot data also indicates that there is some overlap in isotopic signatures between naturalized salmonids and native galaxiids, particularly between rainbow trout (*Oncorhynchus mykiss*) and *Aplochiton zebra* at $\delta^{15}N$ (**Figure 8**). This may be indicative of trophic overlap, and perhaps of competition for resources.

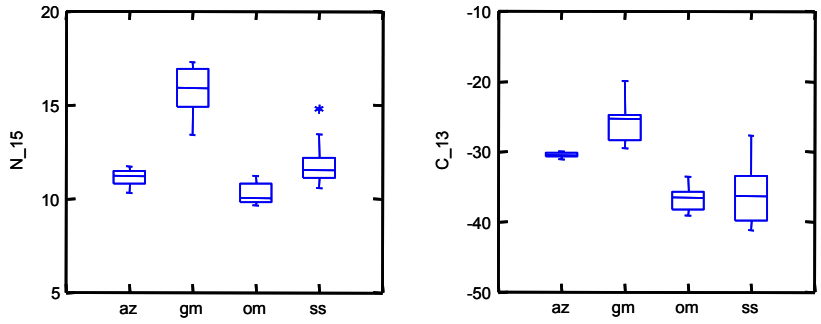


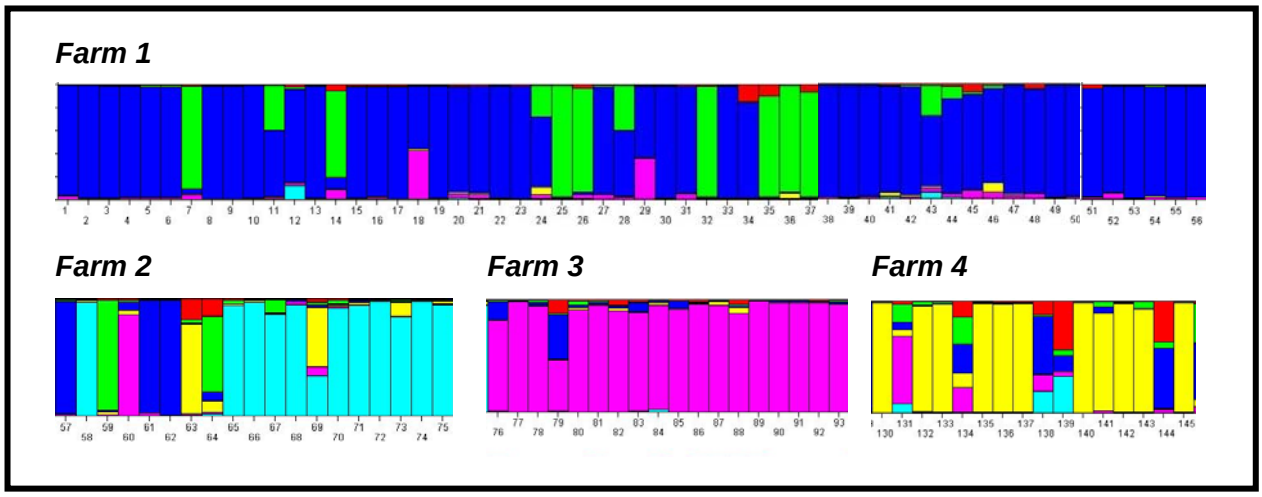
Figure 8. Muscle isotopic signatures ($\delta^{15}N$ and $\delta^{13}C$) of two native galaxiids (az: *Aplochiton zebra*; gm: *Galaxias maculatus*) and two exotic salmonids (om: *Oncorhynchus mykiss*; ss: *Salmo salar*) collected in streams in Chiloe.

Stomach analysis. While the marine ecology of exotic salmonid escapes has been studied in British Columbia and Chile, to our knowledge there are no published reports on the impacts of escapees in freshwater. To address this question Gabriel Orellana, a 3rd year aquaculture student at Universidad Austral (Puerto Montt) , is doing his thesis on the freshwater ecology of salmonid escapees within the framework of the Darwin project. Gabriel's project is comparing the stomach contents of wild (naturalized) and escaped salmonids, to gain an indication of likely trophic impacts. During the summer and fall of 2008 he collected and analyzed 260 stomachs, finding 12 taxa of insects, molluscs and fish. The most relevant items were Ephemeroptera larvae, freshwater snails (Chilina) and Plecoptera larvae. This data, in addition to that obtained previously by two MSc students (Ben Perry, Paul Howes), is being analyzed and will be compared with indirect evidence obtained from isotopic analysis **Figure 8** above). It is expected that this will form the basis of a publication on the trophic ecology of salmonid escapes in freshwater.

(4). Genetic assignment of salmonid escapees.Over the last year we developed and field-tested a molecular toolkit for discriminating farmed fish in Chile, both for Atlantic salmon and rainbow trout. This was greatly aided by collaborations between Chilean and UK partners, and also by input and contributions from an EU-funded project. Preliminary results for A. salmon are encouraging and indicate that there may be enough genetic structuring amongst salmon farms

to be able to identify escapees, and perhaps also to assign them to the farm of origin (**Figure 9**).

REFERENCE SAMPLES



TEST SAMPLES

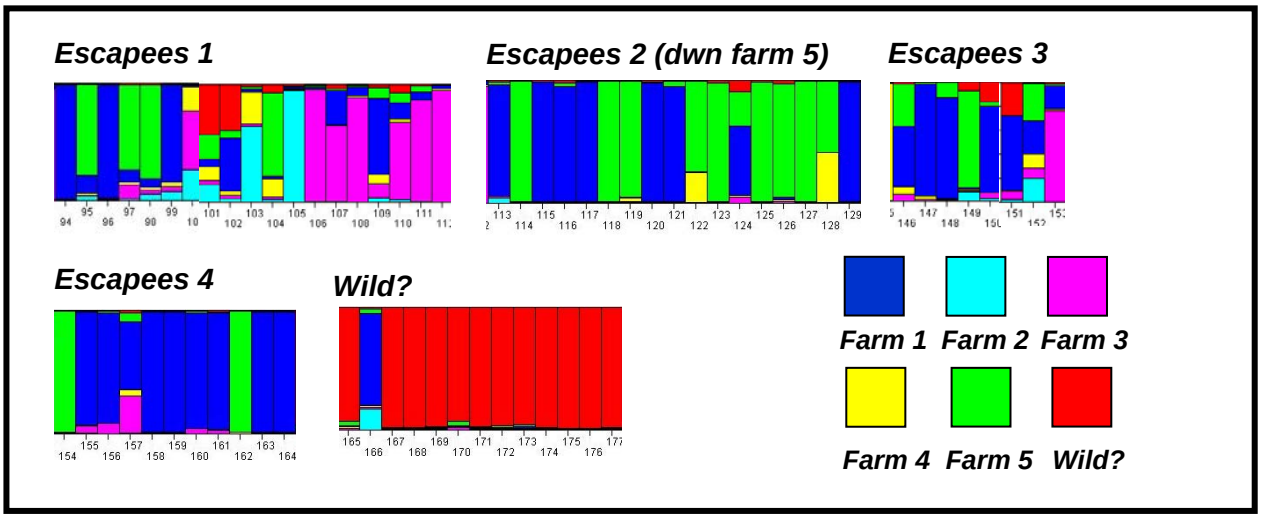


Figure 9. Genetic structuring of 177 Chilean Atlantic salmon screened with 16 microsatellite DNA loci. Genetic variation was screened for samples from 4 farms (plus one downstream of a hatchery) and 5 batches of free-living fish (presumed escapees or wild) captured in the study rivers by electro-fishing. Maximum likelihood suggests that the most likely number of distinct groups in the sample is 6, represented by six different colours. Each bar represents one fish, the height of each colour being proportional to the probability of belonging to one of the six putative origins. Preliminary assignments of 55 salmon escapees indicate that 49% of escapees probably originated from Farm No. 1, 29% originated from farm No. 5, 14% from farm No. 3, and 4% from farm No. 2. No escapees were assigned to farm No. 4, and 4% of fish could not be reliably assigned.

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). During the last year we continued training and supervising personnel, students and volunteers in the field. This included one international volunteer (Jessica Stephenson), one MSc student from Swansea University (Paul Howes), and a 3rd year Chilean Biology student at University Austral (Gabriel Orellana), At the Osorno laboratory, the local host coordinator Dr. Gajardo, continued supervising an undergraduate thesis by Hector Venegas entitled "Genetic structure of Rainbow and Brown trout in four localities of southern Chile (Region X and XI)". With input from partners in UK, Dr. Gajardo has also continued supervising the work of a PhD student, Soledad Cortez, at University Austral Valvidia entitled "Effect of geographic isolation and the migratory strategy on the genetic structure of resident and diadromous *Galaxias maculatus* in the Rio Valdivia basin".

After Dr. Kyle Young left the Project on December 2008, a new field team was quickly assembled, which now has recorded valuable information in a number of new sites, while at the same time re-sampled some previous locations for comparison. During this period, a massive salmonid escape occurred in Calbuco, near Puerto Montt (region X, the salmon epicenter), event extensively covered by the media as the area has a high concentration of salmon cages. We took advantage of this escape and collected escapees from a "pool" of hatcheries. Sampling was done in the period January-February (summer time) and March-April (Fall). The new team is led by Josè Sanzana, an experienced Fisheries Engineer, who had previously conducted a thorough survey of salmonids in the wild. Mr. Sanzana represents a timely addition to the project, and has the local expertise needed to ensure the legacy of Darwin Initiative after our project finishes.

We were also able to use resources from an ongoing EU project (SALSEA-MERGE) for the genetic analysis of salmonid escapes in Chile. A panel of 17 microsatellites was transferred from UK partners to ULA and 6 man-months (covered by the UK leader) were spent in capacity building and in developing and field-testing the molecular toolkit for genetic stock identification. Dr. Consuegra continued providing training to Chilean staff (via telephone, emails, and a visit to Wales) on Genetic stock identification of escaped salmonids, as well as parentage analysis. In collaboration with staff at the Institute of Environment Sustainability at Swansea University, we trained an Erasmus student (Viviene Schröder) on preparation and analysis of salmonid and galaxiid samples for stable isotopes, as well as on development of individual growth profiles using scale image analysis. In Canada, Dr. John Volpe provided training on the use of GIS for risk assessment and mitigation of salmon escapes (Kris Kloehn), as well as on differential salmonid impacts on native Chilean fauna (Valerie Mucciarelli). Details of these and other training activities provided by each partner are given in **Annex 4**.

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 the last year 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. Thus, the lead partner and the Chilean coordinator met with the Academic Registrar (Mr. Huw Morris) and the Head of the School of Environment & Society at Swansea University on October 2008, signed a declaration of intent (**Annex 3**), and discussed the development of the joint MSc curriculum, in particular with respect to the recently announced Erasmus Mundus II scheme (see http://eacea.ec.europa.eu/)

One of the US partners, Dr Guillermo Giannico, is assisting in the development of the joint MSc programme capitalizing on his outreach and third mission experience at OSU.
During the reporting period, the local host coordinator Dr. Gajardo hosted an Open Day at the Osorno Genetics & Aquaculture Laboratory, organised by the National Commission for Scientific and Technological Research (CONICYT). During the open day, the goals of the Darwin Project were explained, and students extracted salmon DNA at the laboratory furbished by the Darwin project. The Darwin project continued to be publicized at conferences and seminars and featured in several media events. One article featuring our Darwin Project will

appear next May in the National Chilean newspaper La Nacion, on a special edition marking the 70th anniversary of CORFO (The Chilean Economic Development Agency, **Annex 3).**

Our project website 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 seminars and conferences in UK, Italy, Spain, USA and Canada. A powerpoint slide show outlining the background, aims and methodology of project was developed and presented at several seminars. During the reporting period we gave several national and international presentations in relation to the project (full details are given in **Annex 4**), presented two conference abstracts and one oral talk at the International Conference *Managing Alien Species for Sustainable Development of Aquaculture & Fisheries* (MALIAF, Florence, Italy 04-08 Nov. 2008).

As per last year, an MSc thesis co-supervised by the UK-leader and Chilean staff was produced in relation to the project (Paul Howes. *Predator recognition of invasive salmonids by native galaxiids*"), with funding from Swansea University, Falklands Islands Development Corporation, and University of Valparaiso (through Dr. Kathlee Whilock). The study was presented at a one-day Symposium at Swansea on October 2008, was awarded a *Distinction* mark and its summary and conclusions posted in the project website. It will also be presented as a poster entitled *Invasive salmonids as agents of selection* at the forthcoming 2009 Conference of the European Society for Evolutionary Biology (ESEB, Torino, Italy, 24-29 August 2009). Likewise, the 3rd year Marine Biology dissertation entitled *Invasive salmonids in Chile - A stable isotope approach* by Vivien Schröder was awarded first class in April 2009, is now posted in the project website, and it will be presented as an oral talk at the Conference *The Impact of Invasive Species on the Aquatic Environment*, hosted by Institute of Fisheries Management during the Wales Biodiversity Week (6-14 June 2009).

As in the previous 2 years, Swansea MSc Students were asked to write Darwin Initiative grant applications modelled on our current project, as part of their course work for a module in Conservation Biology. In total, 16 Darwin grant applications were submitted to a University panel and posters summarising the various student Darwin Initiatives were posted on campus. This has served to disseminate the Darwin Initiative in general, and our project in particular.

We have started to organize and advertise the third training workshop which will be held in Puerto Varas from 2 to 3 of September 2009, followed by the final project meeting, where we intend to present a draft of a *Code of Best Practices*, as well as a Management Action Plan to try to reduce the impacts of exotic aquaculture in Chile (flyer in **Annex 3**)

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) the MAP and CBP towards the end of the project. A lot of useful and timely data is being collected, and we are in this way filling important gaps in knowledge. By publishing and presenting our results we are ensuring that the project is properly disseminated and that the information is freely available to the public. Considerable effort is being spent in the training of students, personnel and volunteers. We are satisfied that the education programme is meeting our targets, and that our student are engaging in quality projects, as their marks and achievements testify.

3.3 Standard Measures

Table 1. Project Standard Output Measures (same codes as per last report)

Code No.	Description	Year 3 (Apr-Mar
Training outputs		
2A	UG Chilean students undertaking training	2
4B,D	Training	24 weeks
4C	PG Chilean undertaking training PG Non-Chilean students receiving training	2 2
5	RA's training	2
6A,B	Attendance at seminars & presentations	150
7	Education/training material produced: information leaflet, PowerPoint slideshow, protocols, website, flyers on exotic salmonids	6
Research outputs		
8	Time spent in Chile by UK partner students (days) Time spent in UK by Chilean partner & staff (days)	90 16
11A,B	Peer-reviewed MS published Peer-reviewed MS submitted Peer-reviewed MS in preparation	2 1 2
Dissemination outputs		
14A	Project progress meeting	1
14B	Presentation conferences/seminars	15
15A,B	Press coverage in Chile	3
16A	Newsletter	2
17B	Project website, regularly updated and linked to all partners	1
23	ULA cash contribution field work Courier Service & contributions from other projects (e.g. FONDEF) UWS contribution to stable isotope analysis (consumables) UWS contribution to molecular toolkit (3 man-months, consumables) Air fare and housing paid by FIDC and U. Valparaiso 3-year PhD studentship secured at U. Wales Aberystwyth Other contributions made by partners & stakeholders, including in kind Total	£1,000 £500 £500 £7,750 £1,000 £45,000 £36,918 £92,668

Table 2. Publications

Type * (eg journals, manual, CDs)	Detail (title, author, year)	Publishers (name, city)	Available from (eg contact address, website)	Cost £
Theses				
UG Thesis	V. Schröder. 2009. Invasive salmonids in Chile - A stable isotope approach	Swansea University	Summary and conclusions in project webpage www.biodiversity.cl	
MSc thesis	P.Howes. 2008. Predator recognition of invasive salmonids by native galaxiids	Swansea University	Summary and conclusions in project webpage www.biodiversity.cl	
Peer-reviewed Papers published				
Journal	Buschmann AF, Cabello F, Young KA, Carvajal, J, Varela, D & L. Henriquez. 2009. Salmon aquaculture and coastal ecosystem health in Chile: Analysis of regulations, environmental impacts and bioremediation systems	*Ocean & Coastal Mgmt*	DOI 10.1016/j.ocecoaman. 2009.03.002 Paper posted in project webpage www.biodiversity.cl	
Journal	K.A. Young, J. Stephenson, A. Terreau, A.-F. Thailly, G. Gajardo & C. Garcia de Leaniz. 2008. The diversity of juvenile	*Biological Invasions*	DOI 10.1007/s10530-008-9372-5 Paper posted in	

	salmonids does not affect their competitive impact on a native galaxiid		project webpage www.biodiversity.cl	
Papers submitted				
Journal	Stephenson, JF, Young, KA, Whitlock, KE. Testing for alarm cue response in a circumpolar galaxiid *Galaxias maculatus*.	*Journal of Fish Biology*	*Submitted October 2008*	
Journal	K. A. Young, J.B. Dunham, J. F. Stephenson, A. Terreau, A. F. Thailly, G. Gajardo & C. Garcia de Leaniz. 2009. A trial of two trouts: comparing the impacts of rainbow and brown trout on a native galaxiid	*Animal Conservation*	*Submitted April 2009*	
Conference Abstracts				
Conference Abstract	C Garcia de Leaniz, P. N . Howes, & K.E. Whitlock. 2009. Invasive salmonids as agents of selection	*ESEB 2009 website*	http://www.eseb2009.it /uk/	
Conference Abstract	K.A. Young, J. Stephenson, A. Terreau, A.-F. Thailly, G. Gajardo & C. Garcia de Leaniz. 2008. One trout, two trout, new trout: the ecology of historic and aquaculture driven salmonid invasions in southern Chile	*MALIAF website*	http://www.dbag.unifi.it /maliaf/.	
Conference Abstract	B J. Perry, KA Young, B.Gara, G. Gajardo & C. Garcia de Leaniz. 2008. Effects of non-native salmonids on native fish fauna in Chile and the Falkland Islands	*MALIAF website*	http://www.dbag.unifi.it /maliaf/.	

3.4 Progress towards the project purpose and outcomes

Overall progress towards achieving the project's ultimate goal can be considered satisfactory. The recent downturn in the Chilean salmon industry has served to highlight the inherent risks of farming exotic species in open net cages, and the inability by industry to self-regulate its own growth. Thus, recent problems with the Chilean salmon industry have only served to highlight the lack of sustainability of some farming practices. The basic assumptions of the project 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 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 and students, who see the project filling an empty niche in Chile (see statement after first workshop: www.biodiversity.cl/workshop, and our recent newspaper article in La Nacion - **Annex 3**).

It is worth pointing out that important officers of governmental bodies, such as CONAMA (Natl. Commission for the Environment), and SUBPESCA (Undersecretariat for Fisheries), have continued to express to the UK and Chilean partners their full support and endorsement of the project. Likewise, support and collaboration from the Chilean salmon industry, most notably from Marine Harvest and Salmones MultiExport, have been instrumental in our sampling surveys. By liaising with other groups working on reducing the impact of exotic species in

Aquaculture (e.g. WWF, EU-Impasse project, NASCO, Pure Salmon Campaign) we are also ensuring that the results of our project are properly disseminated and transcend the Chilean scenario.

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. More recently, problems caused by massive escapes (Aysen, Calbuco), the ISA virus, and difficulties with the export market have made the Chilean salmon industry more cautious. 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 two years, and the help of additional volunteers and personnel have contributed to make our work more efficient.

However, there have also been some drawbacks, most notably with the application of the molecular toolkit, which forced us to terminate the contract of one of the paid RA's in Chile, and with the departure of Dr Young on January 2009. The work of the RA was taken up by staff at the Osorno Laboratory with additional help and input from UK partners. Arrangements were also made during the visit of the host coordinator to the UK to ensure that the analyses of molecular data are carried out in accordance with the project objectives. To this end, additional human resources were committed to the project in the form of time spent by a Postdoc and a new PhD student, funded from other sources . On the other hand, the departure of Dr Young in January 2009 was compensated by the recruitment of an eager and experienced Chilean fish biologist (Mr Jose Sanzana) who quickly assembled an efficient field team, who have continued to carry out and expand the monitoring programme. An agreement with the Natural Isotopes Laboratory at the Millenium Centre (Swansea University) has proved very successful for the analysis of our samples, a student was fully trained and this formed the basis of a research dissertation . Similarly, the help of highly motivated and skilled volunteers has continued to be particularly fruitful, and valuable results were obtained in a MSc thesis, which wad awarded top marks.

We have continued to attract highly motivated international volunteers, and after initial delays, we have finally also succeeded in recruiting Chilean volunteers to work in the field . There are also a number of Chilean students helping at the Osorno Laboratory. 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 took the necessary actions in response to previous comments made by the reviewer. There were no new comments to be addressed.

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 salmonid 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

Popular dissemination activities during the past year of the project were substantial and included the organization of two Seminars, as well as a newspaper article, talks, and presentations by partners (**Annex 4**). Progress in disseminating our project to the scientific community also continued to be successful: two peer-reviewed papers were published, two are under review, and two more are in the final stages of preparation. We participated in a major Conference on **'**Managing Alien Species for Sustainable Development of Aquaculture and Fisheries' on November 2008, are now preparing our participation in the 2009 Conference of Society for Evolutionary Biology, and begun work on the organization of the Third Darwin Training Workshop and the Final Project meeting with all stakeholders.

9. Project Expenditure

Table 1 Project expenditure <u>during the reporting period</u> (Defra Financial Year 1 April 2008 to 31 March 2009)

Item	***Budget*** *(please indicate which document you refer to if other than your project application or annual grant offer letter)*	*Expenditure*	*Variance*
Rent, rates, heating, overheads etc			
Office costs (eg postage, telephone, stationery)			
Travel and subsistence			
Printing			
Conferences, seminars, etc			
Capital items/equipment (specify)			
Others (specify)			
Salaries (specify by individual)			
TOTAL			

(* partially funded from other sources)

The main difference in the balance lies in the under-expenditure for 'Others' which include the external laboratory costs for molecular and isotopic analysis (which have not yet been charged in full to the project) and the over-expenditure in the salary of the Molecular RA to carry out the molecular analysis in Wales, which has been partially funded from other sources. 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 LTS and the Darwin Secretariat to publish the content of this section

We reproduce below the abstract of the MS submitted for publication to *Animal Conservation* (K. A. Young, J.B. Dunham, J. F. Stephenson, A. Terreau, A. F. Thailly, G. Gajardo & C. Garcia de Leaniz. 2009. A trial of two trouts: comparing the impacts of rainbow and brown trout on a native galaxiid)

Abstract. Exotic fishes are one of the principal threats to global freshwater biodiversity. Rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* are the world's two most widespread exotic fishes and now dominate most fish communities in the cold-temperate waters of the southern hemisphere, where they are implicated in population declines and local extirpations of native fish species. Here we provide the first direct comparison of the impacts of rainbow and brown trout on populations of a native fish by quantifying the three components of exotic species impact-range, abundance, and per-capita effect. We surveyed 54 small streams on the island of Chiloé in Chilean Patagonia and found rainbow trout have colonized significantly more streams and have a wider geographic range and than brown trout. The two species have similar post-yearling abundances in allopatry and sympatry, and their abundances depend similarly on reach-level variation in physical habitat. The species have dramatically different per-capita effects on the native drift-feeding peladilla *Aplochiton zebra*, which is virtually absent from streams invaded by brown trout but shares a broad sympatric range with rainbow trout. Within this range the species' post-yearling abundances vary independently before and after controlling for variation in physical habitat. In the north of the island peladilla inhabit streams that remain uninvaded over a century after the introduction of exotic trouts. With pristine native fish communities and a zone of peladilla-rainbow trout sympatry, the streams of Chiloé represent a regional conservation priority and an unprecedented opportunity to study the invasion biology of one of the world's most important exotic species. **Keywords** : non-native species; invasion; fishes; freshwater; Chile; *Oncorhynchus mykiss*; *Salmo trutta*; *Aplochiton zebra*.

Annex 1 Report of progress and achievements against Logical Framework for Financial Year: 2008/09

Project summary	Measurable Indicators	Progress and Achievements April 2007 - March 2008	Actions required/planned for next period
Goal: *To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but constrained in resources to achieve The conservation of biological diversity,The sustainable use of its components, and The fair and equitable sharing of the benefits arising out of the utilisation of genetic resources*		*See also www.biodiversity.cl*	*(do not fill not applicable)*
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	1. New knowledge on the distribution and abundance of exotic fish species and their impact upon native aquatic biodiversity 2. 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 3. Increased understanding and public awareness of threats to native bio-diversity resulting from foreign fish introductions	Training of volunteers, students an personnel and continuing interest in our project. Two peer-review papers published and two other submitted for publication. Presentation of results at international conferences and seminars	1. Organization of third training workshop, dissemination 2. Continuation of sampling program 3. Development of Atlas and databases 4. Application of molecular and isotopic toolkits for GSI 5. Submission of further MS: 1 popular science, 2 scientific papers
Output 1. Abundance, distribution, and impact of exotic and naturalized salmonids assessed	1. Findings endorsed by the scientific community and stakeholders	Good progress continues to be made. Assessment of abundance and distribution of exotic and naturalized salmonids will he helped by development of online Atlas and database, in addition to endorsement by scientific community and stakeholders	
Activity 1. Research & monitoring of exotic and naturalized fish species		Sampling field protocols by combination of angling and electro-fishing were developed, as well as protocols for carrying out genetic and isotopic analysis. Over 8,000 fish have now been sampled from 300+ sites, and the toolkits were full tested and validated in the field. Work in the next period will involve continuing with the assignment of exotic salmonids escaping from fish farms and expanding the coverage of fish farms for the baseline database	
Output 2. 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	Network of sampling stations and development of molecular and isotopic protocols completed. Trained staff in place. Testing of toolkit for molecular and isotopic stock identification completed. Development of online GIS database in progress.	
Activity 2. Capacity building and training in 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 invasions	3. MAP and CBP peer reviewed and presented at international conference	Discussed with stakeholders at first two workshops, though this output is expected to be completed by end of project. Continuing dialogue with stakeholders is helping to develop (and ensure necessary endorsement of) MAP and CBP. Contacts were also made with international players with experience in developing CBP in other regions & scenarios.	
Activity 3. Research & monitoring of exotic and naturalized fish species		As per activity 1.	
Output 4. Educational events and media coverage for local people, fish farmers, and rest of stakeholders	4. Participation of fish farmers and rest of stakeholders in educational events, Darwin project featured in media	Progress continues in relation to this output. Stakeholders are being invited to participate in third training and final project workshop. Darwin project was also publicized at conferences and seminars and featured in the media.	
Activity 4. Education programme and dissemination of results		MSc curriculum on Sustainable Aquaculture is being developed. Two manuscripts were published, and two others are being submitted. Results will be presented at two international conferences, on June and August 2009	

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	1. New knowledge on the distribution and abundance of exotic fish species and their impact upon native aquatic biodiversity 2. 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 3. Increased understanding and public awareness of threats to native bio-diversity resulting from foreign fish introductions	1. Project reports, workshop proceedings and publications in peer-reviewed journals 2. Documentation and correspondence for MAP and CBP 3. Records of educational programme and training workshops. Development of professional curricula on sustainable aquaculture. Students trained under programme pass their courses	1. Project findings are understood and accepted by the salmon industry, regulatory agencies and all stakeholders. Policy makers use findings to help reduce the impact of exotic species on local biodiversity 2. Market forces and increased recognition on the impact of exotics facilitate the shift towards more sustainable fish farming 3. Curriculum development is implemented by ULL with the launch of a MSc in Sustainable Aquaculture
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	1. Findings endorsed by the scientific community and stakeholders 2. GIS database & molecular and isotopic toolkits for identification of exotic and farmed fish species developed, tested, and at least 2 staff trained 3. MAP and CBP peer reviewed and presented at international conference 4. Participation of fish farmers and rest of stakeholders in educational events, Darwin project featured in media	1. Publication of results in peer reviewed, national and international scientific journals 2. Project reports, scientific papers, abundance and distribution maps, species database, fish escape assessment protocol, project website 3. Workshop proceedings, MAP and CBP published and distributed, copies sent to Darwin Initiative 4. Educational leaflets, press releases, media coverage, reports	1. Sampling strategy and logistic support are appropriate for project objectives and facilitate the collection of data 2. Adequate performance of molecular and isotopic diagnostic toolkits to produce desired results 3. Successful liaison with industry, government agencies and rest of stakeholders for project support 4. Links to educational media and NGO's are established (already in place via ULL)
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	Activity Milestones **Yr1.** Initial meetings with stakeholders. Develop GIS database, molecular and isotopic diagnostic toolkits and run training workshops, establish sampling strategy and field protocols. Follow-up training workshops in **Yr2 & Yr3.** **Yr1**.Field test diagnostic methods for species identification, stock assignment and trophic niche overlap, begin screening. **Yr2 & Yr 3**. Continue screening, workshops to discuss results. **Yr 3**. Writing of scientific publications, MAP and CBP. **Yr1.** Develop education programme, establish website, and attract media interest. **Yr2-Yr3**. Develop MSc curriculum, presentation of results at international conferences. All years: annual reports, workshops proceedings, update webpage. At least 2 scientific peer-reviewed papers submitted by end of Yr 3		Assumptions 1. Program receives required support from University (in place). 2. Research methods and tools are adequate. Required baseline information is provided by industry and regulatory bodies 3. Successful liaison with stakeholders and media interest. Support from University