Darwin Initiative – Final Report

*(To be completed with reference to the Reporting Guidance Notes for Project Leaders (http://darwin.defra.gov.uk/resources/reporting/) - it is expected that this report will be a **maximum** of 20 pages in length, excluding annexes)*

Darwin project information

Project Reference	EIDPO041
Project Title	Protecting native galaxiids from salmonid invasions in Chile and the Falklands.
Host country(ies)	Chile, Falkland Islands
UK Contract Holder Institution	Swansea University Department of BioSciences
UK Partner Institution(s)	Aberystwyth University IBERS
Host Country Partner Institution(s)	Universidad de los Lagos Laboratorio de Genética, Acuicultura y Biodiversidad (Chile) Falkland Islands Government, Environmental Planning Department (Falkland Is.) Oregon State University Department of Fisheries & Wildlife, Corvallis (USA) US Geological Survey (USGS) FRESC , Corvallis Research Group , Corvallis (USA)
Darwin Grant Value	£XXX
Start/End dates of Project	01-04-2010 to 31-08-2012
Project Leader Name	Carlos Garcia de Leaniz
Project Website	www.biodiversity.cl
Report Author(s) and date	Dr. Carlos Garcia de Leaniz (SU) Dr Sofia Consuegra (AU) Dr Gonzalo Gajardo (ULA) Mr Daniel Fowler (FIDC) Mr Nick Rendell (EPD) Mr Jose Sanzana (ULA) Dr. Guillermo Giannico (OSU) Dr. Jason B Dunham (USGS) 23 March 2013

1 Project Background

Salmonids are not naturally present in the Southern Hemisphere and constitute one of the most important threat to native freshwater fishes in Chile and the Falkland Islands, countries which are committed to the implementation of the CBD through the development of Species and Habitat Action Plans for endangered species. The main aim of this post-project award was to inform such Action Plans for endangered native galaxiid fishes through (1) the training of Chilean and Falkland officers on the development of both *in-situ* and *ex-situ* conservation measures, and (2) the building of capacity to implement such conservation measures.

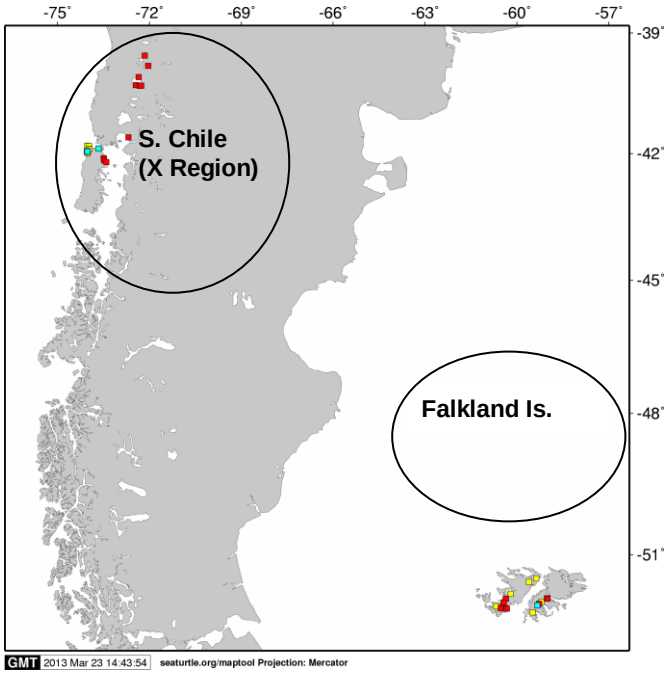


Figure 1. Project Site Map.

The Post-project Award took place in two of the places most affected by salmonid invasions, Southern Chile (X Region – Los Lagos) and the Falklands Islands.

Shown are the main sampling sites; full list available in the online Darwin Fish Atlas http://www.biodiversity.cl/salmon/

2 Project support to the Convention on Biological Diversity (CBD)

Exotic salmonids are at the heart of modern aquaculture and are also widely translocated to support and create sport fisheries. Exotic fish represent an enormous source of wealth for Chile and to a lesser extent for the Falklands, but when they become invasive they can also cause significant losses of biodiversity and impair ecosystem functioning. Both host partners are signatories of the Convention on Biological Diversity (CBD), and Chile is also a signatory of several trade agreements with the US and Asian-Pacific (APEC) countries that will likely promote the further expansion of alien species.

The initial Darwin Initiative and the Post-project award have provided support for the implementation of articles 7-10, 12-14 and 16-17 of CBD, with special emphasis on Introduced Species (30%), Biosafety (10%), Inland Waters Biodiversity (20%), Marine and Coastal Biodiversity (20%), and Sustainable Use (20%) themes. They have also helped to deliver the aspirations of the CBD's five Strategic Goals of the Aichi Biodiversity Targets, in particular Aichi Target No. 9 on alien species, which specifies that by 2020 'alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment.'

An urgent compromise for CBD subscribers is to halt the loss of biodiversity by 2020 (Strategic Plan for Biodiversity 2011–2020, the so-called Aichi Targets for 'Living in Harmony with Nature'). Islands are especially vulnerable to fish introductions because (a) fish invaders can spread rapidly when they are unconstrained by landscape features, and (b) invaded fish communities are at a particularly high risk of extinction in isolated waterways as evolutionary rescue is usually impossible. Chile can be considered as an island from an ecological and biogeography perspective, isolated as it is by the Pacific and Atlantic Oceans in the west and the south, by the Andes in the East, and by the driest desert in the world (the Atacama desert) in the North.

In this sense, this project has contributed to a better understanding of the impact of exotic salmonids on native fish biodiversity, a topic that would have been extremely difficult or impossible to fund solely with national resources. Exotic salmonids have made Chile the second largest salmon world producer, and make a major and decisive contribution to Chile's economy through fish farming and sport fishing, whereas in the Falklands Islands they provide opportunities for attracting anglers and generate wealth in rural communities. Darwin funding has facilitated the advancement of knowledge on exotic impacts, in line with Aichi target 20 that invites governments to mobilize resources to implement the 2011-2020 strategic plan agreed in 2010 at Nagoya.

On the other hand, the international network established by the Darwin Initiative and the post-project award in Chile and the Falklands Islands demonstrates the value of transnational cooperation and the merits of an integrative approach, where the viewpoints of different stakeholders are considered. Building of capacity and infrastructures, as well as knowledge transfer to partners in Chile and the Falklands, were also an important output of this post-project award.

3 Project Partnerships

The partnership continued to be a strong one and built on the links established during the original Darwin Initiative grant with ULA in Chile and the Scoping awards with EPD in the Falklands. It benefitted from fluent and frequent contacts via email, skype and telephone, as well as from face to face contacts during visits. The two UK partners made an extended visit to both Chile and the Falkland Islands during the post-project award, and there were also shorter visits by the Chile partner to the Falkland Islands, by the Falklands partner to Chile, and from the two US partners to Chile. We also met at two international conferences (Luarca Spain, and Seattle USA) where we presented results of the project. A memorandum of understanding was established between the UK lead Institution and the two host partners.

The partnership resulted in a number of joint activities and proposals. For example ULA supported the application of Jose Sanzana (the Chilean project officer) to enrol for an MSc degree at Aberystwyth University (the UK partner institution) while EPD supported a similar application by Daniel Fowler (the project officer in the Falklands) to study for an MRes at the UK lead institution. The Chilean application was ranked highly but was ultimately unsuccessful in securing funding from the Chilean government. The application from the Falklands, on the other hand, was successful and after spending a term at Swansea University undertaking the course component, Dan Fowler returned to the Falklands and is currently writing his dissertation for the MRes in Sustainable Aquaculture & Fisheries degree based on the work carried out during the post-project award (expected submission date June 2013).
The partnership was also successful in securing funding for a PhD at Aberystwyth University, and this resulted in a successful viva by Delphine Vanhaecke during 2012 (Conservation Genetics of Native Galaxiid Fish Threatened by Invasive Salmonids in the Southern Hemisphere) based on work supported by the initial Darwin Initiative and the Post-project Award. Other examples of strong collaboration during the partnership include joint presentations at several international conferences (International Symposium on Ecology of Stream Salmonids Luarca, Spain – May 2010; Annual Meeting of The Fisheries Society of the British Isles, Bournemouth, UK – July 2011; Annual Conference of the American Fisheries Society Seattle USA - September 2011; European Aquaculture Society Annual Meeting, Rhodes, Greece – October 2011; World Aquaculture Society Conference, Natal, Brazil - June 2011; Annual Conference of The American Fisheries Society, Minneapolis, USA - August 2012), as well as joint grant applications. Thus, ULA submitted a research grant to the Chilean Government with support from the two UK partners, while the two UK partners teamed up with EDP Falklands Islands and other institutions and the resulting consortium submitted a grant application entitled 'Managing Alien Fish in the Outermost Regions and Territories' to the EU BEST scheme.

The partnership was reciprocal in many respects. For example, active exchange of ideas, working hypotheses and experimental protocols allowed to produce a working protocol for assessing the impact of invasive salmonids with active involvement of relevant stakeholders.

Following active discussion between partners a sampling protocol was agreed and implemented in both Chile and the Falklands. Work during this period also resulted in a number of joint peer-reviewed publications (**Annex 5**) which benefitted greatly from the input received from local collaborators and advisors, as well as from the local land owners in Chiloe and the Falklands who provided valuable logistic support.

As in previous occasions, the work of the host project coordinators, Gonzalo Gajardo in Chile and Nick Rendell in the Falklands, were key in securing the necessary logistic support. The two US partners secured additional funding to carry out the field work in Chiloe, and also to develop the analysis of the Darwin online Fish Atlas and Database generated during the project.

4 Project Achievements

4.1 Impact: achievement of positive impact on biodiversity, sustainable use or equitable sharing of biodiversity benefits

Our project has had a major and positive impact on conservation of endemic freshwater fish biodiversity in Chile and the Falkland Islands in several different ways:

1. It developed novel molecular markers for identification of the endangered *Aplochiton* sp. and their hybrids

2. It identified a new galaxiid species in the Falkland Islands (*Aplochiton taeniatus*) and helped to delineate it from the morphologically similar *Aplochiton zebra*, present in both Chile and the Falklands

3. It developed morphological criteria for the visual identification of the two *Aplochiton* species in the wild to aid in captive breeding

4. It provided estimates of effective population size genetic diversity, and gene flow among invaded galaxiid populations (*Galaxias maculatus*) as well as among invading fish (Rainbow trout)

5. It demonstrated a positive relationship between the number and proximity to salmon farms, the incidence of salmon escapees, and the probability of salmonid invasions

6. It showed that salmonids escaping from fish farms survive and outcross with naturalized trout, thereby increasing genetic diversity and facilitating the invasion process

7. It also demonstrated the existence of metapopulation structure among the native fish *Galaxias maculatus*, documented the existence of sources and sinks differentially affected by salmon farming and illustrated the passive dispersal of the species

8. It pilot trialled the *ex*-situ conservation of *Aplochiton* sp. and was successful in rearing wild-caught fish to maturity in captivity, to secure the spawning of broodstock, and to achieve the hatching of eggs and the development of embryos until first feeding.

9. It produced the Darwin Fish Atlas, a large online database holding over 19,000 records of fish present in Chile and the Falklands, and freely available at the project website

10. It provided training and education to local students, volunteers, and research officers and helped to disseminate the CBD targets and the plea of native fishes threatened by biological invasions

The project benefitted from an extensive network of collaborators and its impact can also be demonstrated by the attention given to our joint publications, as shown in **Table 1** below.

Table 1. Impact metrics of peer-reviewed publications generated during the Darwin Initiative (2006-2009) and the Post-project Award (2010-2012) measured as number of citations in Google Scholar (http://scholar.google.com/citations) and number of page views in Academic.edu (http://www.academia.edu/).

Publication	Google Scholar citations	Academia.edu page views
Vanhaecke et al (2012a). *Freshwater Biology* **57**: 1241-1252.	2	17
Vanhaecke et al. (2012b). *PLoS ONE* **7** (3): e32939.	5	56
Consuegra et al (2011). *Evolutionary Applications* **4**: 660-671.	8	159
Schröder & Garcia de Leaniz (2011). *Biological Invasions* **13**: 203-213.	11	222
Vanhaecke et al (2011). *Molecular Ecology Resources* **11**: 219-222.	7	139
Garcia de Leaniz et al (2010). *Systematics and Biodiversity* **8**: 447-459.	10	244
Young et al (2010) *Animal Conservation* **13**: 399-410.	16	237
Young et al (2009). *Biological Invasions* **11**: 1955-1961.	16	46
Buschmann et al (2009). *Ocean & Coastal Management* **52** :243-249.	33	-

4.2 Outcomes: achievement of the project purpose and outcomes

The results obtained demonstrate that the project has achieved most of its purposes and delivered the majority of the agreed outputs and outcomes. The only exception is the re-introduction in the wild of *Aplochiton* sp reared in captivity. Although wild-caught parents were successfully reared in captivity and made to spawn, the resulting alevins could only be maintained until the first feeding stages. First feeding proved very challenging and although different diets were tried, none was entirely successful for the rearing of *Aplochiton* larvae. Part of the problem rests on the lack of information on this species, as practically nothing is known about its feeding habits and early ecology. An additional problem, revealed by this project , is that the two *Aplochiton* species frequently occur in sympatry and are easily misidentified by visual criteria. It is hence possible that the lack of success of the captive breeding programme may have been in part due to poor hybrid performance due to the accidental crossing of the two parental species in captivity. In this sense, the molecular tools (Vanhaecke et al. 2012b. PLoS ONE 7 (3): e32939) and improved morphological criteria (Woodhall 2012 MSc thesis) developed during this project will help in the identification of the two species, the selection of broodstock and the breeding in captivity.

The project has certainly raised awareness of the issues surrounding introduced salmonids and native galaxiids and there is now momentum that should carry forward and help to protect native freshwater fishes. Knowledge generated through the project in the form of publications is freely available at our project website (and other sources) and has already informed (and helped to shape) environmental policy, both in the Falklands (Action plan for *Aplochiton* and policy on the sale of exotic brown trout) and in Chile (tools for the assessment of the impact of salmon farms and for identification of salmonid escapees). For example, the Project Officer in the Falklands is currently drafting the Species Action Plan (SAP) for *Aplochiton* species in the Falkland Islands. Once ratified by the Falkland Islands Government this document will be the defining action plan for management and protection of galaxiids in the Falklands, and will increase the pressure on the Government to take appropriate action to safeguard the future of these fish. This SAP could not have been completed without the research conducted by the project.

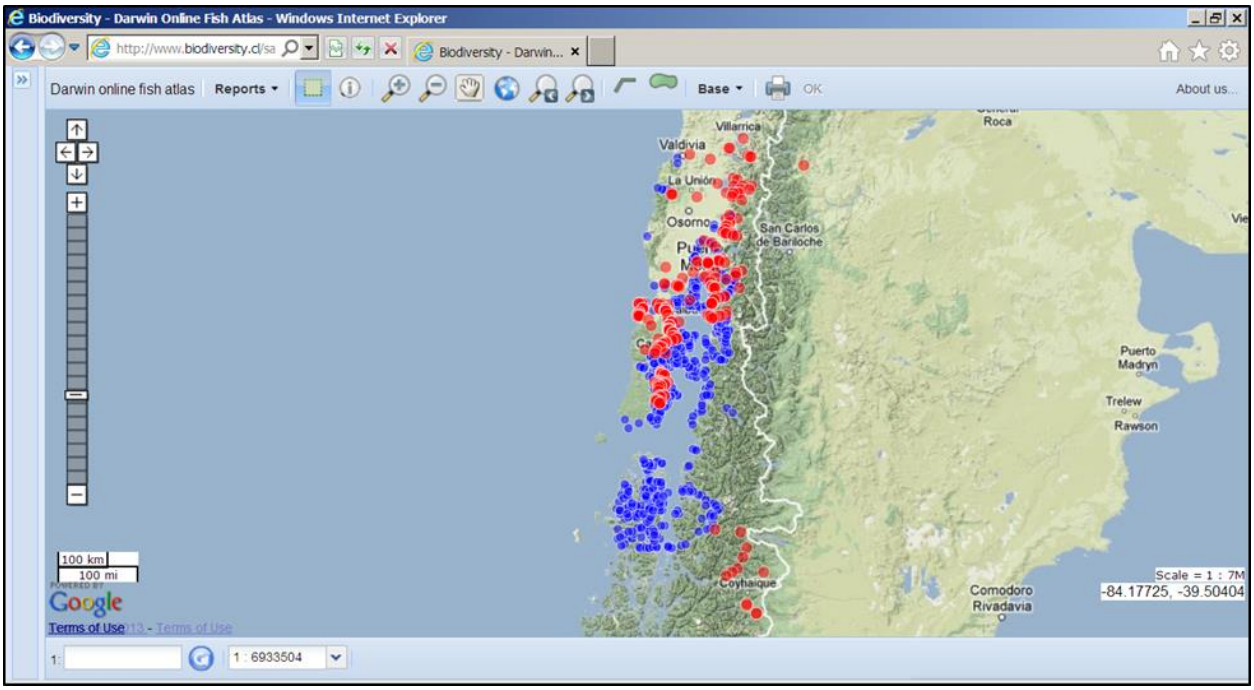
4.3. Outputs (and activities)

Progress towards meeting the project outputs via the activities set out in the logical framework was as follows:

Output 1. Estimates of effective population size, genetic variation, degree of isolation, and risk of salmonid encroachment for target galaxiid populations in Chile and the Falklands Islands

Activity 1.1. Sampling of endangered galaxiids, habitat data, and collection of broodstock

During the two years of the post-project award we continued sampling in Chile and the Falklands, collecting data on habitat distribution, as well as samples for analysis and for the captive breeding programme. In total 4,268 fish were sampled in Chile and the Falklands (of which 630 corresponded to *Aplochiton* sp. the main target species for this project) and entered these on the **Darwin Fish Atlas** (available online at our project website). These numbers add to the database of the previous project which now holds 19,068 fish records from 466 sampling stations corresponding to 143 watersheds. To our knowledge, this is the most comprehensive database of both invading salmonids and invaded native galaxiids. The database also includes habitat description (**Figure 2**). Results show that roughly 52% of the all the fish sampled were exotic salmonids (7,684) and 48% were native fishes (7,116). This information has been the source of several primary publications (**Annex 3**) , as well as of one review, recently submitted (Arismendi et al. Differential invasion success of exotic salmonids in Southern Chile: patterns and hypotheses. *Reviews in Fish Biology and Fisheries* -under review).



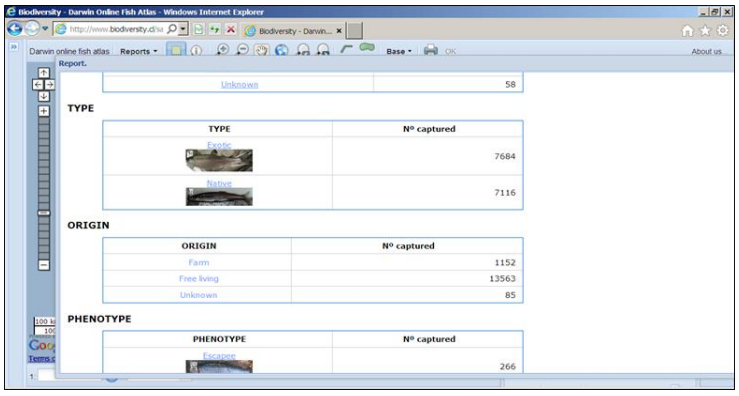


Figure 2. Screen-shots of the Online **Darwin Fish Atlas** developed during the project and available at the project website (www.biodiversity.cl). Shown are the location of salmonid farms (blue points) and sampling sites (red points). Each sampling site has an associated database with information on the identity of fish species present as well as their main characteristics. The Atlas currently holds 19,068 fish records collected at 466 sampling sites

Activity 1.2. Genetic analysis and estimates of effective population size and gene flow

We developed novel molecular markers for species identification of the endangered *Aplochiton* sp. (Vanhaecke et al. 2011. *Molecular Ecology Resources* 11: 219-222; Vanhaecke et al 2012a. *PLoS ONE* 7 (3): e32939) and estimated the effective population sizes and extent of gene flow for three native galaxiid fishes: *Galaxias maculatus* (Vanhaecke et al. 2012b. *Freshwater Biology* 57: 1241-1252); *Aplochiton zebra* and *Aplochiton taeniatus* (Vanhaecke et al., in preparation), and one exotic salmonid, rainbow trout (Consuegra et al. 2011. *Evolutionary Applications*. 4: 660-671). Connectivity estimates for galaxiids indicated an asymmetric pattern of gene flow and allowed us to identify population sources (net donors of fish) and population sinks (net recipients of fish – **Figure 3, Table 2**)

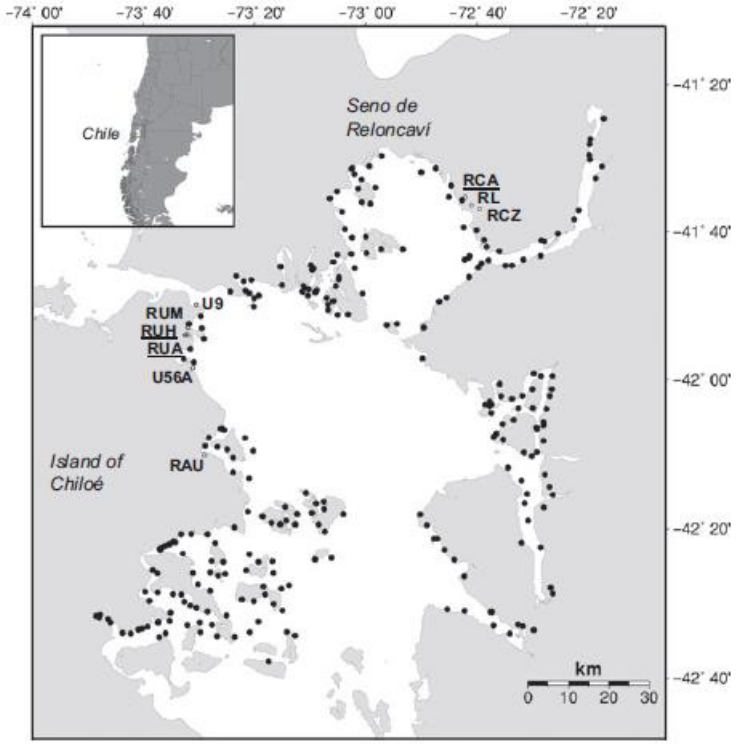


Figure 3. Map of the Gulf of Ancud (Chiloe Interior Sea) showing the location of salmonid farms (dots) and *Galaxias maculatus* populations acting as genetic sources (river codes underlined, n = 3) and genetic sinks (river codes in normal typeface, n = 6) according to the direction and magnitude of gene flow. From: Vanhaecke et al. 2012b. *Freshwater Biology* **57**: 1241-1252.

Table 2. Number of migrants per generation among the nine study populations of *Galaxias maculatus* estimated using MIGRATE. Sources and sinks were estimated according to the net exchange of migrants for each population. Taken together, Chiloe populations acted as a net source (average emigrants–immigrants = 2.35) and populations in mainland Chile (Seno Reloncavı) acted as a net sink (average emigrants–immigrants = - 4.7). From: Vanhaecke et al. 2012b. *Freshwater Biology* 57: 1241-1252.

	Seno Reloncaví			Chiloé						
Donor	RCA	RL	RCZ	RAU	U9	RUM	RUA	RUH	U56A	Total
Recipient										
RCA	*	1.3	1.6	3.3	3.5	5.8	19.7	25.2	12.1	72.50
RL	0.74	*	0.78	4.72	4.32	19.35	17.41	13.57	25.90	86.79
RCZ	0.92	1.08	*	8.91	13.31	5.42	24.32	41.33	5.31	100.60
RAU	13.43	8.10	1.07	*	3.74	1.60	13.13	7.42	2.26	50.75
U9	11.99	15.13	12.03	3.09	*	1.10	4.31	2.19	5.91	55.75
RUM	15.63	5.00	18.95	0.89	2.56	*	1.22	1.03	1.86	47.14
RUA	9.41	11.89	11.09	8.34	1.14	0.94	*	0.52	2.57	45.90
RUH	15.69	16.75	23.35	8.26	3.01	0.60	0.45	*	2.60	70.71
U56A	12.22	16.68	20.97	4.71	3.31	1.98	2.59	3.99	*	66.45
Total	80.03	75.93	89.84	42.22	34.89	36.79	83.13	95.25	58.51	
Type	Source	Sink	Sink	Sink	Sink	Sink	Source	Source	Sink	

Activity 1.3. Isotopic & elemental analysis for estimates of connectivity

We field-tested methods for the identification of invasive salmonids escaping from fish farms based on their isotopic signatures (Schröder,V. & Garcia de Leaniz, C. 2011. *Biological Invasions* **13,** 203-213) and begun to estimate the extent of resource overlap between native galaxiids and exotic salmonids by comparing the $\delta^{13}C$ and $\delta^{15}N$ SIS under allopatric and sympatric conditions (**Figure 4**).

We also collected otoliths of galaxiids for potential analysis of Sr/Ca ratios by Laser Ablation ICP-MS which might reveal marine signatures, and thus discriminate between diadromous and resident populations.

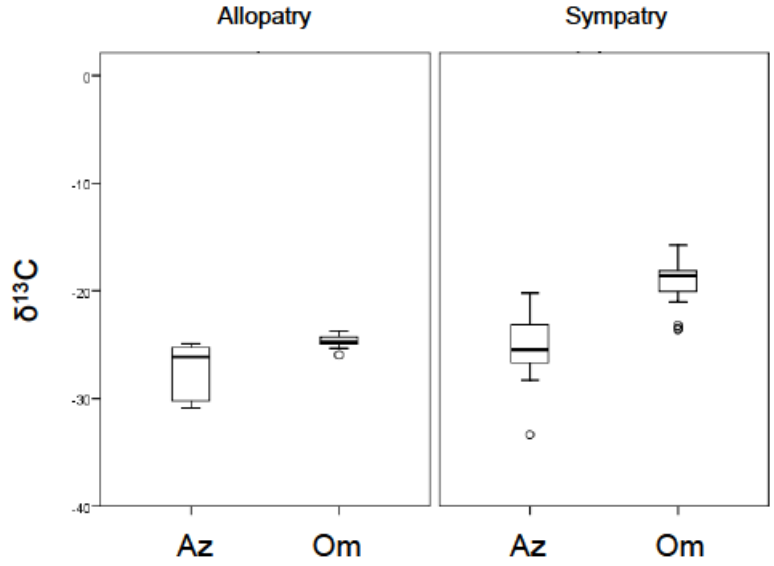


Figure 4. $\delta^{13}C$ SIS of native *Aplochiton zebra* (Az) and the invasive rainbow trout (Om) living in allopatry or in sympatry in Chile.

Activity 2.1. Modelling and integration of ecological/genetic data for landscape approach with help from US partners

With help from the US partners at OSU and USSG the Darwin database was combined with other fish records and used to generate a global model to explain the differential invasion success of salmonids in Chile. This resulted in a review paper led by Dr Ivan Arismendi (a Chilean colleague associated to the Darwin Project and now at Oregon State University), which emphasized the interaction between invader, the invaded ecosystems and human dimension , as shown in **Figure 5** below.

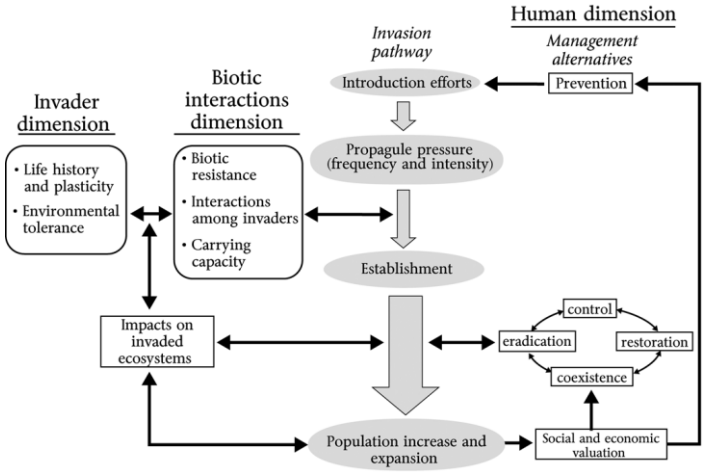


Figure 5. Conceptual framework of biological invasion processes including the three core dimensions (invader, biotic interactions, and human influences). Arrows represent links between main drivers. Source: Arismendi et al. Differential invasion success of exotic salmonids in Southern Chile: patterns and hypotheses. *Reviews in Fish Biology and Fisheries* – (under review).

Output 2. Deployment of 2 Recirculation Aquaculture Systems (RAS) for captive breeding of endangered galaxiids in the host countries (one in Chile, one in Falklands).

Activity 2.2. Training on RAS technology, molecular and isotopic techniques and enrolment in MRes in Aquatic Ecology and Conservation in UK

During the visit of the Chilean RA (Mr. Jose Sanzana) to Swansea University at the end of the previous Darwin Project we provided training on Recirculation Aquaculture Systems (RAS) at our Freshwater Research Unit (**Figure 6**). The two RAS systems purchased for the project and deployed in Chile and the Falklands are identical to the ones used at Swansea, which has considerably facilitated capacity building and the transfer of expertise on these systems.
The US and UK partners came down to the Falklands and/or Chile during January 2011 and we provided training to the RA's on sampling techniques and survey methods. The Falkland project officer also travelled to Chile to help set-up the RAS there and to train the Chilean project officer on the running and maintenance of RAS systems.

Figure 6. Jose Sanzana (left), the Chilean RA from U. de los Lagos, at Swansea University where he received training in laboratory techniques and in recirculation technology at Swansea's Freshwater Research Unit. Dan Fowler (right) , the Falkland RA, enrolled in the MRes at Swansea and then went back to the Falklands to carry the field work for his thesis.

Both RA's applied for enrolment in the MRes programme in Sustainable Aquaculture and Fisheries at Swansea University and were given conditional offers for the 2011/12 entry. One condition for entry for the Chilean RA was achieving a score of 6.5 in the IELTS and he started taking English classes in earnest, though he was ultimately unsuccessful in securing a scholarship. The Falklands RA (Dan Fowler) was successful in securing the necessary funding in the Falklands and is now writing up his MRes thesis, having passed the taught component in January 2013. At the end of the project, the Falklands RAS was rehoused at the newly formed South Atlantic Environmental Research Institute, where is it available for further work on galaxiid conservation as well as for ecological research on aquatic species in general. The RAS in Chile is housed at the Universidad de los Lagos (Osorno), where it is also used for research and teaching (**Figure 7**).

Figure 7. Facilities used for rearing the first *Aplochiton sp.* at the Falklands FIDC station (left) and at the University of Los Lagos (Chile) (right).

Output 3. Development and implementation of captive breeding and reintroduction programmes for endangered galaxiids in host countries

Activity 2.3. Captive breeding and rearing of juveniles in host countries

Aplochiton sp. broodstock were collected and successfully made to spawn in captivity in both Chile and the Falklands. In Chile, 18 *Aplochiton* juveniles (average length 24 cm) were initially caught with nets with the help of local fishermen in Lake Llanquihue during 2010 and these were brought to the Osorno aquaculture facilities. We also brought 49 juveniles from Rivers Futangue and R. Quiman. These were fed on dried artemia and although they appeared to be feeding properly, all died after 18 days for unknown reasons. More success was achieved on January 2011, when 146 juveniles were transported from R. Blanco to the University Pescado hatchery. There they were fed twice a day on salmon pellets many survived for over a year. Later on, more fish (up to a total of 200) *A. zebra* and *A. taeniatus* were collected from five different watersheds and reared at the University hatchery at Rio Pescado. A group of 50 individuals were reared for 22 months in captivity and a second group of 150 individuals were reared for 10 months and were maintained on artificial trout diet, using small-sized pellets suitable to the small mouths of these individuals. Offspring was produced in the winter of 2011 (May-June) and in May 2012, but few of the latter embryos survived. Hatched alevins averaged 4mm total length at the moment of yolk sack reabsorption (**Figure 8**). Similar problems with first feeding were experienced in the Falklands.

Rearing of *Aplochiton* in the Faklands has been going on for several years at the FIDC facilities with very good results, and 30 broodstock spawned an estimated 50,000 eggs during 2010. However the problem is, like in Chile, the feeding and survival of juveniles past the initial stages, and the difficulty of identifying the two *Aplochiton* species based on phenotype traits alone (see below). Galaxiids in the Falklands are annual spawners, so in a two year project only two attempts at captive breeding were possible. Both attempts produced large numbers of fertilised eggs, and in turn, large numbers of fry. First feeding of fry was difficult, and few fish survived for long once the yolk sacs were absorbed, and none survived past 21 days. This was another instance of knowledge gaps holding back project achievements, as it is not known if fry feed in fresh or salt water. In any event, our results demonstrate that the rearing of wild-caught *Aplochiton* in captivity is feasible and that this could be used as a live gene bank, using readily available trout pellets as food. However, although feeding of *Aplochiton* in captivity is not problem once they have reached a certain size, it is still a formidable challenge to feed recently-hatched alevins and to rear the species from egg to sexual maturation entirely in captivity.

Figure 8. Spawning of *Aplochiton* in captivity at the Falkland Islands hatchery facility (top). Arrow points to a large mass of sticky eggs laid down in a tank fitted with an artificial substrate. Development of *Aplochiton* eggs and embryos hatched in Chile (below, middle photo) and the Falkland Islands (below, right photo)

Activity 3.1. First reintroductions of captive bred galaxiids in host countries

In an effort to circumvent the difficulties experienced during first feeding, newly hatched fry and late-term eggs were distributed into salmonid-free areas in the hope of establishing *Aplochiton* refugia in the Falklands.

The timescale of the project did not allow us to follow up sampling to ascertain whether stocked alevins had survived or not (finding small fish is very difficult, finding larger fish is easier), but the Falklands Project Officer will sample in the refugia as soon it is practicable.

Broodstock from the project (**Figure 9**) were also translocated to a large landlocked pond to assess whether refugia could be established in this manner. Again, follow-up surveys looking for evidence of survival, spawning and reproductive success will be conducted at an appropriate time.

Figure 9. *Aplochiton* broodstock reared in the Falklands (left and centre), and in Chile (right) used for pilot translocation trials.

Output 4. Field-testing of in-situ salmonid control measures designed to prevent salmonid colonization and encroachment of galaxiid refuge areas

Activity 3.2. Assessment of in-situ conservation measures to prevent salmonid dispersal and encroachment

Pilot research was conducted in both Falklands and in Chile on the effects of culverts and other obstacles on habitat and fish passage from both the invader (salmonid) and invaded (galaxiids) perspectives, looking specifically at how these structures can affect population connectivity and potentially slow down salmonid spread, but perhaps also negatively affect galaxiid dispersal. This work resulted in the identification of several sites for landlocked galaxiids in the Falklands (that could be used as refugia to prevent salmonid encroachment), and two contributions (as two case studies) to a comprehensive book on fish passage and migrations (summarised below):

Sanzana, J. Gajardo, G. & Garcia de Leaniz, C. (2012). Assessing the impact of barriers on connectivity of endangered native fishes in the face of salmonid invasions in Southern Chile. In Gough, P., Philipsen, P., Schollema, P.P. & Wanningen, H. (Eds.). From Sea to Source; International Guidance for the Restoration of Fish Migration Highways. Regional Water Authority Hunze en Aa's. Postbus 195, 9640 AD Veendam, The Netherlands. pp 48-49.

Fowler, D. & Garcia de Leaniz, C. (2012). Assessing the impact of culverts on population connectivity of endangered galaxiid fishes in the Falkland Islands. In Gough, P., Philipsen, P., Schollema, P.P. & Wanningen, H. (Eds.). From Sea to Source; International Guidance for the Restoration of Fish Migration Highways. Regional Water Authority Hunze en Aa's. Postbus 195, 9640 AD Veendam, The Netherlands. pp 58-59.

There are an estimated 786 km of road tracks in the Falkland Islands crisscrossing a dense network of streams, lakes and ponds. Most of these tracks have been built over the last two decades and use culverts, rather than bridges, to negotiate stream crossing. The actual number of culverts is not known, but a conservative estimate would be in the hundreds. In a

pilot survey, Ross reported finding culverts in 22 of 38 sampling locations (58%) in both West and East Falkland, but this is almost certainly an underestimate (Ross, 2009). Concerns have been raised about the potential impacts of culverts on the connectivity of the three native galaxiid fishes, *Galaxias maculatus* (Falklands minnow) and the endangered *Aplochiton zebra* and *Aplochiton taeniatus*., which rely on a marine larval phase for completing their life cycle, as well as on the introduced sea trout (*Salmo trutta*) which forms the basis of a valuable sport fishery. Costs, and not fish passage, has been the overriding criterion for designing such culverts, and assessing their impact has been flagged as a high conservation priority, particularly for the endangered *Aplochiton*.

Our surveys indicate that culverts in the Falklands are widespread and vary widely in size, from 30 to 200 m in diameter, and can be over 15 m long. They are now being favoured over bridges to create causeways over all types of waterways, from head streams as small as 1.5 m wide, to stream mouths under tidal influence 155 m wide. Small culverts are made of PVC, but large ones tend to be made of corrugated iron. Average bottom water velocities (at 5 cm from the bottom) were 60 cm/s (range 0-125 cm/s) and 55 cm/s (range 0-113 cm/s) at the upstream and downstream ends of nine culverts, respectively. Similar values for water depth were 34.6 cm at the upstream end and 35.2 cm at the downstream end. We found instances where culverts were dry because water was infiltrating under rock gabions, as well as perched culverts that made fish passage impossible.

Culverts are widespread in the Falklands, and yet their impacts on native fauna have not been addressed. As old culverts need replacement and new tracks are being planned, it is essential to develop a sound system for assessing their impacts, and for suggesting mitigation or alternative measures. We used a simple field inventory to identify potential barriers for fish migration, and will make use of genetic data to estimate levels of gene flow to infer connectivity between populations. No information is available on the swimming stamina of native galaxiids (and was not, therefore, a factor that could have been taken into account in their design), but studies on 7 fish species indicate that water velocities in culverts should not exceed 92 cm/s for any species, and should not exceed 45 cm/s for brown trout (Tudorache et al., 2008), the species that most closely resembles the native *Aplochiton* sp. On this basis, it would thus appear that many of the culverts in the Falklands constitute a significant barrier for fish migration. We also encountered, just like Ross, dry and perched culverts that were impossible for fish to ascend under all conditions (Ross, 2009). Data are still very limited, but we suspect that culverts may pose one of the biggest threats to the conservation of native galaxiid fishes in the Falkland Islands.

We also conducted a review of the main threats faced by galaxiids confronted with salmonid invasions, as well as on the most suitable methods to prevent salmonid encroachment (Garcia de Leaniz et al. 2010. *Systematics & Biodiversity* **8**: 447-459). From our analysis, it is clear that exotic salmonids tend to occupy the same trophic level as many native galaxiids, and that the large size of salmonids is probably the biggest threat to the much smaller galaxiids (**Figure 10**). As salmonid eradication campaigns are unlikely to be viable (or successful), we concluded that reducing the escape of salmonids from fish farms, stopping the deliberate stocking of salmonids for sport fishing , and preventing salmonids from colonizing galaxiid streams by deploying suitable physical barriers, appear to be the best options available to managers to protect galaxiids from salmonid invasions.

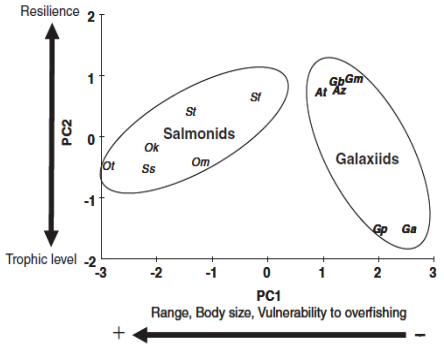


Figure 10. PCA of the first two principal components accounting for 86% of variation in life history traits of invasive salmonids and native galaxiid fishes. Body size, range, and vulnerability to overfishing are the traits that separate the two groups the most. Source: Garcia de Leaniz et al. 2010. *Systematics & Biodiversity* **8**: 447-459

Output 5. Capacity building, training and education to draw attention to the conservation needs of galaxiids and the threats posed by salmonid invasions

Activity 3.3. Education and dissemination programmes, presentation of results and media coverage

During the first year of the post-project award all the partners engaged in a number of dissemination activities, including the publication and presentation of results, and contacts with the media. The latter included articles in The Wool Press, The Penguin News, and The Falklands Conservation Newsletter. These activities are summarised in **Table 3.** .Due to delays in the start of the project, the decision was taken to enrol the research assistants in the MRes course in the second year, not the first. The Falklands research assistant enrolled at Swansea University in September 2011, and is due to submit his thesis in the summer of 2013.

Education was carried out in the Falklands largely through two public presentations on the project's work (**Figure 11**), and a series of articles in the local press highlighting galaxiid issues and reporting on project progress.

Figure 11. Dr. Sofia Consuegra (Aberystwyth University) giving a talk at the Falkland Islands Fisheries Department on the identification of *Aplochiton* species in the project through the application of DNA barcoding, January 2011.

The Wool Press is written for the rural and agricultural community, and is therefore a direct link to the landowners around the Falklands. The Penguin News is the national weekly newspaper of the Falklands, and the submitted articles were an introduction to the project, including an advert to recruit volunteers for the project locally, as well as coverage of the visit to the Falkland Islands by the UK and Chilean project partners. The Falklands Conservation Newsletter is the newsletter of the principal environmental NGO in the region (Falklands Conservation), and the submitted article discussed recent project work.

A project steering group was formed in the Falklands for the purpose of advising the project and help in the dissemination of results to the widest possible number of stakeholders, The group consists of two government fisheries scientists, the CEO of the main conservation NGO in the Islands (Falklands Conservation), the government environmental officer (and project partner), a representative form the aquaculture industry and a representative from the angling community.

As in our previous project, Swansea MSc and MRes 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 of Aquatic Resources. In total, 42 Darwin grant applications were submitted to a University panel and talks disseminating the various student Darwin Initiatives were given. This has served to disseminate the Darwin Initiative in general, and our project in particular. In January 2011 reciprocal staff exchange was produced between the Falklands and Chile to share experiences and advances. Two Chilean volunteers students did their professional practice in the framework of the project: Daniel Velasquez, a Marine Biology student and Jorge Becker, a student of Aquaculture Engineering. Also, four foreign volunteers were trained in field and lab activities: Adrian Padilla and Kate Miner from Oregon State University, USA, and Camille Crespe, Maud Baudequin (2012) from VetAgroSup University. Clermont-Ferrand. France.

Table 3. Summary of main disseminating activities during the post-project award (excl. peer- reviewed publications, tabulated separately).

Date	Type	Presenter/Author	Location/Publisher	Topic
May 2010	Conference	S. Consuegra	Luarca, Spain	Salmonid Invasions
May 2010	Conference	C.Garcia de Leaniz	Luarca, Spain	Galaxiid response to salmonids
Sep 2010	Media	D. Fowler	The Wool Press	Introduction to Project
Oct 2010	Conference	G. Gajardo	Nagoya, Japan	Native gene pools in S. America
Oct 2010	Conference	G. Gajardo	V Mar, Chile	Salmonid Invasions
Oct 2010	Seminar	S. Consuegra	Cardiff, UK	Project and Salmonid Invasions
Oct 2010	Media	D. Fowler	Penguin News	Project Introduction, recruitment
Nov 2010	Seminar	J. Sanzana	U Lagos, Chile	Salmonid impacts on galaxiids
Nov 2010	Seminar	C.Garcia de Leaniz	Swansea, UK	Biological Invasions
Jan 2011	Seminar	S. Consuegra	Falklands Is.	Molecular identification *Aplochiton*
Jan 2011	Media	D. Fowler	Penguin News	Visit by partners
Feb 2011	Media	D. Fowler	FC Newsletter	Recent project work
Mar 2011	Seminar	C.Garcia de Leaniz	Bangor U, UK	Salmonids as Invasives
Jun 2011	Conference	G. Gajardo	Natal, Brazil	Alien salmonids in Chile
Jul 2011	Conference	C.Garcia de Leaniz	Bournemouth, UK	Salmonids agents of selection
Sep 2011	Conference	C.Garcia de Leaniz	Seattle, USA	SIA signatures invasives
Sep 2011	Conference	S. Consuegra	Seattle, USA	Winning the invasion roulette
Oct 2011	Conference	C.Garcia de Leaniz	Rhodes, Greece	SIA signatures invasives
Oct 2011	Media	C.Garcia de Leaniz	Darwin News	Tracing invasive salmonids
Nov 2011	Seminar	C.Garcia de Leaniz	Swansea, UK	Biological Invasions
Feb 2012	Media	C.Garcia de Leaniz	Darwin News	Salmon farming and CBD 2020
Jun 2012	Survey	J. Sanzana	website	Native fish public awareness
Jul 2012	Media	C.Garcia de Leaniz	Darwin News	Salmonid impacts
Jul 2012	Media	C.Garcia de Leaniz	Version Diferente	Salmon farming and CBD 2020
Aug 2012	Conference	I.Arismendi	Minneapolis, USA	Salmonid invasion success
Sep 2012	Video	J. Sanzana	U. Lagos, Chile	Darwin Project in Chile
Nov 2012	Seminar	G. Gajardo	Rupanco, Chile	Wings for Science
Apr 2013	Media	C.Garcia de Leaniz	Darwin News	Protecting native fish

4.4 Project standard measures and publications

Project measures are quantified in **Annex 4** and full details of publications are given in **Annex 5**. The post-project award directly generated 2 full-time jobs and the data generated supported the research of two post-doctoral visitors to the UK (Caty Monzon–Arguello, Daniela Gomez). It also resulted in the training of over 30 undergraduate students and volunteers, 4 students at post-graduate level (including one PhD), and produced 6 peer-reviewed scientific publications (with 3 more currently under review), 2 book chapters, and 5 dissemination articles.

4.5 Technical and Scientific achievements and co-operation

Technical and scientific achievements have already been described and are summarised in **Annexes 4 and 5.** In addition to these, the network established during the post–project award resulted in an application entitled ‘Fish Aliens on Islands (FINS) - Managing Alien Fish in the Outermost Regions and Territories’ submitted in June 2012 to the EU ‘Preparatory Action "Best" – Voluntary Scheme for Biodiversity and Ecosystem Services in Territories of the EU Outermost Regions and Oversees Countries and Territories’. The application was submitted by a consortium led by Swansea University and that includes two of the Darwin partners (Aberystwyth University UK; Falklands Environmental Planning Department). Although it was not selected for funding , feedback received was very useful and will form the basis of a resubmission.

4.6 Capacity building

The capacity of the host partners to further protect native galaxiid fishes has been increased in many ways, as indicated in the standard measures. Through strong relations with project partners, both the Chilean and Falklands partners have now a wealth of experience to call upon for guidance and advice with regards to salmonid impacts and galaxiid conservation, and freshwater ecology in general.

Two local project officers have been trained in freshwater ecology methods and have a greater understanding of the issues surrounding conservation of native fish species. The Falklands RA is also due to complete his MRes shortly and is currently seeking funding opportunities for further work that would build upon the project's foundations.

The resources provided by the Darwin funding (particularly the Online Fish Atlas, the molecular toolkit and the two RAS facilities) constitute important research tools for the conservation of native galaxiids in Chile and the Falklands. Lastly, a Species Action Plan for Falklands native Galaxiids is being drafted, and once completed and ratified, it will be an essential tool for the management of invasive salmonids and the conservation of native fishes , and should also facilitate the provisioning of additional funding and resources to meet their conservation needs.

4.7 Sustainability and Legacy

The sustainability and legacy of the project has been achieved in different ways:

1. The **Online Darwin Fish Atlas** (first conceived and developed during the Darwin Initiative, but significantly expanded and made available online during the post-project award) represents a powerful management tool, as well as an enduring example of the contribution of our project to native fish conservation. This will continue to be used in the future by managers and researchers alike and testify to the value and lasting legacy of the Darwin Initiative.

2. Two dedicated **Recirculation Aquaculture Systems** for the rearing of endangered galaxiids were built and these will continue to be used in conservation and research of native fish biodiversity in Chile and the Falklands. The Chilean host-partner secured additional funds for a wet laboratory at the University of Los Lagos, Osorno campus (**Figure 7**) which will be used for research on the food of galaxiid larvae, targeting one of the specific problems encountered during the captive-breeding of this species. Such infrastructures, along with valuable lessons learned on the reproduction and rearing of the native *Aplochiton* sp., will facilitate *ex*-situ conservation of endangered galaxiids in the host countries.

3. **Knowledge exchange** achieved through the training of two Project Officers, students and volunteers, as well as through multiple dissemination activities, will persist beyond the duration of the project and will continue to raise public awareness and change attitudes regarding the threats posed by invasive species and the conservation needs of native fish

4. **Scientific and Technical solutions** developed during this project included a molecular toolkit for the identification of the endangered galaxiid fish *Aplochiton* sp. as well as an isotopic toolkit for the identification of salmonid escapees. These will outlast the project and will enable managers and researchers in the host countries to refine their conservation plans and to react to the threats posed by invasive salmonids

5. Using powerful DNA barcoding and molecular markers specifically developed during this project, a **new species of galaxiid**, *Aplochiton taeniatus* was **detected in the Falkland Islands**, along with the existence of *A. zebra* x *A. taeniatus* hybrids. These represent important **additions to the biodiversity** of the host countries and will have a direct effect on the development of Species Action Plans.

Key staff at the host countries have permanent jobs and will continue the work started during the Darwin Initiative and the post-project award at the host institutions. We keep (along with other project partners) in regular contact and will use the DI and post-project award as a spring board for future grants . For example we applied for a EU BEST project and are now developing further applications.

5 Lessons learned, dissemination and communication

Scarcity of suitable baseline data was highlighted by all stakeholders during our meetings as one of the most important threats to native fish biodiversity and for achieving sustainability in the salmon industry. Thus, we feel that to a large extent the long-lasting success of our project will be measured by the quality and timeliness of our data on the origin and impacts of exotic salmonids upon native 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 and a blind faith in the continuous growth paradigm, our project has capitalized on the need to produce systematic and reliable science - not currently available to attack this problem. As in our previous project, we saw the main challenges and difficulties of the post-project in the logistics, which in Chile are particularly complicated (and even more so in the Falklands), and the extreme difficulty of sampling remote aquatic ecosystems, particularly in autumn and winter. We continued throughout the post-project to attract highly motivated international volunteers, and after initial delays, we also succeeded in recruiting local students and volunteers to work in the field. There were also a number of students helping at the Osorno Laboratory in Chile.

Dissemination activities during the duration of the project were numerous and substantial, and included the organization of several seminars, as well as newspaper articles, a video, talks, posters and presentations by partners. Progress in disseminating our project to the scientific community have also been successful: six peer-reviewed papers and two book chapters were published, three are currently under review, and two more manuscripts are in the final stages of preparation. We participated in four major international Conferences in Spain, USA, Greece, and Brazil, including the European Aquaculture Conference 2011, and the World Aquaculture Conference 2012, thus ensuring good dissemination of project outputs.

5.1 Darwin identity

As we did during the original project, we have strived during the post-project award to advertise the Darwin Initiative by stressing three key issues: (1) how the project fulfilled the aims of the CBD, as well as the National Biodiversity Strategies in the host countries, (2) the uniqueness and fragility of freshwater ecosystems in Chile and the Falklands, and (3) the socio-economic importance of exotic salmonids and how the project would reduce their impacts on native galaxiid fishes. Because Chile is a world leader in salmon farming (an activity increasingly under attack), our project attracted considerable public attention (as evidenced by the number of visits to our website). In addition, an increasing number of tourists and anglers are being attracted to the rivers and lakes of southern Chile and to a lesser extent also to the Falklands and this, along with the work of the University of Los Lagos in Chile and the Environmental Planning Department in the Falklands, provided numerous opportunities for promoting the Darwin Initiative and the dissemination of results. The Darwin name and logo have appeared in the ULL and the Falklands RAS and wetlabs, in the 4WD vehicle, in all the dissemination outputs and contacts with the media, in presentations, publications, as well as in the project website. In addition, MSc students at Swansea University are asked every year to write Darwin Grant proposals as part of their course work for a module in Conservation of Aquatic Resources, an activity which has proved very popular and which serves to expose them to the Darwin Identity.

A questionnaire carried out during the post-project award indicates that 26% of the 112 interviewees (most of whom were artisanal fishermen, 45%) were aware of the Darwin Project in Chile (see http://www.e-encuesta.com/answer.do?testid=mUjDijy1M9g=&chk=1). Although we have no baseline data carried out at the time the project started, we consider this to be a very positive indicator of the impact achieved by the project, as well as of its legacy.

6 Monitoring and evaluation

Other than the difficulties outlined above in relation to the rearing of the endangered *Aplochiton* in captivity and the difficulties in securing a scholarship from the Chilean Government to enable the Chilean Project Officer to enrol for an MRes at the UK lead institution, there were no major changes to the logframe. In general, logframe indicators have proved useful and effective, forcing us to remain focused to achieve the project objectives, as per annual reports. Much of our work has been published or is being submitted for publication in its various forms (theses, papers) and has therefore gone through extensive, independent evaluation.

6.1 Actions taken in response to annual report reviews

NA (No reviews were received from annual report)

7 Finance and administration

7.1 Project expenditure

Concept	Expenditure	Budget	Balance	% Difference
Salaries	XXX	XXX	XXX	-1.1%
Travel	XXX	XXX	XXX	+2.6%
Operating Costs	XXX	XXX	XXX	-11.2%
Capital Equipment	XXX	XXX	XXX	-14.0%
Overheads	XXX	XXX	XXX	-10.8%
Total	**XXX**	**XXX**	**XXX**	-5.0%

Overall the project underspent by 5%. The main difference in the balance lies in the under-expenditure for Capital equipment due to the high cost of purchasing and transporting the chiller from the UK (a heavy piece of equipment) which motivated the Chilean partner to source it locally, and the under-expenditure of operating cost which were in due to the in-kind contribution of the host partners. These figures are undergoing auditing.

7.2 Additional funds or in-kind contributions secured

Below is a breakdown of the in-kind contributions secured during the duration of the project (28 months):

Time spent in project (all partners) – 1,732 hrs	£XXX
Use of vehicle by host-partner during field sampling in Chile (2 yrs)	£XXX
Use of vehicle by host-partner during field sampling in the Falklands (2 yrs)	£XXX
Scholarship and additional in-kind contribution for RA in the Falklands (1 yr)	£XXX
Use of private accommodation during field work in the Falklands (1 week, 2 people)	£XXX
Travel costs contributed by US partners for field work in Chile (2 people)	£XXX
Use of fish laboratory facilities and equipment contributed by UK lead partner	£XXX
Use of molecular laboratory and equipment contributed by UK partner	£XXX
Use of aquaculture facilities and equipment contributed by host partner in Chile	£XXX
Use of aquaculture facilities contributed by host partner in the Falklands (2 yrs)	£XXX
Total	**£XXX**

7.3 Value of DI funding

DI funding was essential for this project; its main value was fourfold:

1. It provided the resources necessary for the development of a diagnostic molecular toolkit for the identification of the endangered galaxiid *Aplochiton* as well as for estimating the genetic diversity and connectivity of native galaxiid populations

2. It enabled us to equip a modern aquaculture facility for ex-situ conservation of endangered galaxiids in the two host countries and to hire and train staff who can now continue to carry the monitoring and diagnostic work

3. It facilitated meetings with stakeholders, as well as the development of the training and education components of the project, including an online Darwin Fish Atlas to aid in the conservation of native fishes in the host countries

4. It was instrumental in forging a network of national and international researchers working on the conservation of fish biodiversity in the two host countries, and to enable us to apply for further funding

Annex 1 Report of progress and achievements against final project logframe for the life of the project

Project summary	Measurable Indicators	Progress and Achievements April 2010 – August 2012	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 develop practical, proactive measures to help reverse the widespread decline of native galaxiids in Chile and the Falkland Islands caused by salmonid invasions	1. Data on salmonid pressure, habitat connectivity, and galaxiid population structuring and gene flow is generated to prioritize galaxiid populations for conservation 2. Ex-situ captive breeding programme for endangered galaxiid is tested 3. Field-testing of in-situ measures to prevent salmonid colonization and encroachment of galaxiid refuge areas and (subject to stakeholder consent) eventual removal of invasive salmonids at selected sites. 4. Capacity building and training to on conservation needs of endangered galaxiids	Continued the development of the Online Darwin Fish Atlas and obtained additional data on salmonid pressure, and population connectivity. Six peer-reviewed papers and two book chapters (case studies) were published, three MS are under review and two more MS are under the last stages of preparation. Ex-situ conservation programme was developed and *Aplochiton* broodstock were maintained for up to 20 months, making now use of molecular species diagnostic kit. Culvert survey started to provide assessment of barriers as potential salmonid exclusion devices (in –situ conservation). Capacity building and training of RA's, students and volunteers	NA
Output 1. Estimates of effective population size, genetic variation, degree of isolation, and risk of salmonid encroachment for target galaxiid populations in Chile and the Falklands Islands	Results published in six peer-reviewed papers (details in Annex 5)	Estimates obtained of effective population size, genetic variation, and degree of isolation in *Galaxias maculatus*, Rainbow trout, and *Aplochiton.* Estimates also obtained of risk of salmonid encroachment (based on salmonid propagule pressure and degree of isolation) for target galaxiid populations in Chile and the Falklands.	
Activity 1.1. Sampling of endangered galaxiids, habitat data, and collection of broodstock.		Data on abundance and habitat distribution of exotic and naturalized salmonids incorporated into Darwin Fish Atlas. Over 300 Aplochiton broodstock collected for ex-situ conservation	
Activities 1.2 – 1.3. Genetic and isotopic analysis and estimates of effective population size and gene flow		Estimates of effective population size and connectivity obtained for two native galaxiid species and one invasive salmonid.	
Activity 2.1. Modelling and integration of ecological/genetic data for landscape approach with help from US partners		Darwin Fish database used to generate a global model to explain differential invasion success of invasive salmonids emphasizing interactions between invader, invaded ecosystems and humans (paper led by I. Arismendi under review)	

Output 2. Deployment of 2 Recirculation Aquaculture Systems (RAS) for ex-situ conservation of endangered galaxiids in host countries (one in Chile, one in Falklands).	RAS deployed in both Chile and the Falklands	Ex-situ captive breeding programme expanded during year 2. Testing of new diets and treatments carried out to improve post-hatching alevin survival. Successful reproduction and survival of alevins for up to 21 days post-hatching
Output 3. Development and implementation of captive breeding and reintroduction programmes for endangered galaxiids in host countries	*Aplochiton* Captive breeding programme developed in both Chile and the Falklands	Success in artificial reproduction. First *Aplochiton* eggs hatched in captivity in both Chile and the Falklands. First introduction of captive-bred eggs of *Aplochiton* in salmonid-free refugia in the Falklands. First translocations of *Aplochiton* broodstock in salmon-free areas in the Falklands. Progress made on keeping broodstock in captivity (over 2 years) and on extending alevin survival.
Activity 2.3. Captive breeding and rearing of juvenile galaxiids Activity 3.1. First reintroductions of captive bred galaxiids in host countries		
Output 4. Field-testing of in-situ salmonid control measures designed to prevent salmonid colonization and encroachment of galaxiid refuge areas	Pilot data on potential use of in-situ control measures published (Sanzana et al 2012; Fowler & Garcia de Leaniz 2012). Modelling of impacts of salmonid encroachment published (Garcia de Leaniz et al 2010) or under review (Arismendi et al.). Details in Annex 5.	Surveys carried out during both year 1 and year 2. Potential galaxiid refugia identified and extent of salmonid propagule pressure and encroachment quantified in selected rivers in both Chile and the Falklands.
Activity 3.2. Assessment of *in-situ* conservation measures		
Output 5. Capacity building, training and education to draw attention to the conservation needs of galaxiids and the threats posed by salmonid invasions	Dissemination activities, publications	Dissemination activities during the project included presentations at seminars, conferences, and articles in magazines and newspapers, as well as the production of a video and an online questionnaire. Progress in disseminating results to the scientific community were also successful with six peer-reviewed papers published, two book sections, two under review, and three more are in the final stages of preparation. UG and PG students and volunteers were trained.
Activity 2.2. Training of Research Assistants and enrolment in MRes in UK.		Training provided to two RA's. who were both accepted at MRes programme in Sustainable Aquaculture & Fisheries at Swansea University. Falklander RA secured PG scholarship and was able to enrol in MRes course (expected submission summer 2013)
Activity 3.3. Education and dissemination programmes, presentation of results and media coverage		We will continue with our successful dissemination campaign, prepare additional scientific papers and speak at three major international conferences after project is finished

Annex 2 Project's final logframe, including criteria and indicators

Project summary	Measurable Indicators	Means of verification	Important Assumptions
Goal: Effective contribution in support of the implementation of the objectives of the Convention on Biological Diversity (CBD), the Convention on Trade in Endangered Species (CITES), and the Convention on the Conservation of Migratory Species (CMS), as well as related targets set by countries rich in biodiversity but constrained in resources.			
Sub-Goal: Endangered galaxiid populations in urgent need of protection from salmonid invasions are identified, screened, and targeted for conservation efforts in both host countries	Four project components are completed successfully, as per outputs below	Project reports, publications in peer-reviewed journals. independent DI review and checking against outputs and deliverables	[redacted]
Purpose The purpose of the project is to develop practical, proactive measures to help reverse the widespread decline of native galaxiids in Chile and the Falkland Islands caused by salmonid invasions	1. Data on salmonid pressure, habitat connectivity, and galaxiid population structuring and gene flow is generated to prioritize galaxiid populations for conservation 2. Ex-situ captive breeding programme for endangered galaxiid is tested 3. Field-testing of in-situ measures to prevent salmonid colonization and encroachment of galaxiid refuge areas and (subject to stakeholder consent) eventual removal of invasive salmonids at selected sites. 4. Capacity building and training to on conservation needs of endangered galaxiids	1. Project reports, presentations, and publications in peer-reviewed journals 2. Records of captive breeding programme 3. Documentation and correspondence, field results 4 Records of educational programme and training. Staff trained under programme meet agreed standards and achieve qualifications	Increasing public awareness of the impact caused by invasive salmonids on endangered native galaxiids will lead to more support for the conservation of native freshwater fishes and the development of more proactive measures
Outputs (add or delete rows as necessary) 1. Estimates of effective population size, genetic variation, degree of isolation, and risk of salmonid encroachment for target galaxiid populations in Chile and the Falklands Islands	Field sampling and genetic/isotopic analyses completed in year 1. Conservation status assessed by year 2	Project reports, presentations, and publications in peer-reviewed journals	Molecular and isotopic markers prove informative, resolve population structuring and uncover degree of connectivity

2. Deployment of 2 Recirculation Aquaculture Systems (RAS) for captive breeding of endangered galaxiids in the host countries (one in Chile, one in Falklands)	Acquisition and installation of RAS by mid year 1	Project reports, correspondence, auditing	Availability of suitable sites and required infrastructure is in place
3. Development and implementation of captive breeding and reintroduction programmes for endangered galaxiids in host countries	Reproduction of endangered Aplochiton in captivity, survival and development of larvae, release of first juveniles by year 2	Project reports, correspondence, media coverage, presentations	Collection of galaxiid broodstock , reproduction, and rearing in captivity are successful
4. Field-testing of in-situ salmonid control measures designed to prevent salmonid colonization and encroachment of galaxiid refuge areas	Assessment of effects of barriers to salmonid migration and removal measures during year 1 and 2	Project reports, media coverage, presentations, and publication in popular and peer reviewed journals	Stakeholder consent to salmonid control measures at selected pilot sites
5. Capacity building, training and education to draw attention to the conservation needs of galaxiids and the threats posed by salmonid invasions	Presentations to schools, two appointed RA enrolled in MRes/MSc programmes by year 1. Theses submitted by year 2. Results of projected presented at conference by end of year 2	Project reports, correspondence, achievement of training benchmarks, and academic qualifications (MRes/MSc)	Public awareness is maintained and stakeholders remain engaged over the course of the project
Activities (details in workplan) 1.1 Sampling of endangered galaxiids, habitat data, and collection of broodstock 1.2 Genetic analysis and estimates of effective pop size and gene flow 1.3 Isotopic & elemental analysis for estimates of connectivity 1.4 Modelling and integration of ecological/genetic data for landscape approach with help from US partners 1.5 Training on RAS technology, molecular and isotopic techniques and enrolment in MRes in Aquatic Ecology and Conservation in UK 1.6 Captive breeding and rearing of juveniles in host countries 1.7 First reintroductions of captive bred galaxiids in host countries 1.8 Assessment of in-situ conservation measures to prevent salmoinid dispersal and encroachment 1.9 Education and dissemination programmes, presentation of results and media coverage			
Monitoring activities: Indicator 1 Number of samples collected, collection and analysis of data Indicator 2 Genetic estimates Indicator 3 Isotopic & elemental analysis estimates Indicator 4 Connectivity estimates and quantification of salmonid impact risk Indicator 5 PG Enrolment and completion of training during term 1 Indicator 6. Production of galaxiids Indicator 7. Reintroductions figures for galaxiids Indicator 8 Fiel sampling and analysis of results Indicator 9. Number of presentations, articles and dissemination outputs			

Annex 3 Project contribution to Articles under the CBD

Project Contribution to Articles under the Convention on Biological Diversity

Article No./Title	Project %	Article Description
7. Identification and Monitoring	15	Identify and monitor components of biological diversity, particularly those requiring urgent conservation; identify processes and activities that have adverse effects; maintain and organise relevant data.
8. In-situ Conservation	10	Establish systems of protected areas with guidelines for selection and management; regulate biological resources, promote protection of habitats; manage areas adjacent to protected areas; restore degraded ecosystems and recovery of threatened species; control risks associated with organisms modified by biotechnology; control spread of alien species; ensure compatibility between sustainable use of resources and their conservation; protect traditional lifestyles and knowledge on biological resources.
9. Ex-situ Conservation	15	Adopt ex-situ measures to conserve and research components of biological diversity, preferably in country of origin; facilitate recovery of threatened species; regulate and manage collection of biological resources.
10. Sustainable Use of Components of Biological Diversity	5	Integrate conservation and sustainable use in national decisions; protect sustainable customary uses; support local populations to implement remedial actions; encourage co-operation between governments and the private sector.
12. Research and Training	15	Establish programmes for scientific and technical education in identification, conservation and sustainable use of biodiversity components; promote research contributing to the conservation and sustainable use of biological diversity, particularly in developing countries (in accordance with SBSTTA recommendations).
13. Public Education and Awareness	15	Promote understanding of the importance of measures to conserve biological diversity and propagate these measures through the media; cooperate with other states and organisations in developing awareness programmes.
14. Impact Assessment and Minimizing Adverse Impacts	10	Introduce EIAs of appropriate projects and allow public participation; take into account environmental consequences of policies; exchange information on impacts beyond State boundaries and work to reduce hazards; promote emergency responses to hazards; examine mechanisms for re-dress of international damage.
15. Access to Genetic Resources	5	Whilst governments control access to their genetic resources they should also facilitate access of environmentally sound uses on mutually agreed terms; scientific research based on a country's genetic resources should ensure sharing in a fair and equitable way of results and benefits.
16. Access to and Transfer of Technology	5	Countries shall ensure access to technologies relevant to conservation and sustainable use of biodiversity under fair and most favourable terms to the source countries (subject to patents and intellectual property rights) and ensure the private sector facilitates such assess and joint development of technologies.
17. Exchange of Information	5	Countries shall facilitate information exchange and repatriation including technical scientific and socio-economic research, information on training and surveying programmes and local knowledge
Total %	100%	Check % = total 100

Annex 4 Standard Measures

Code	Description	Totals
Training Measures		
1b	Number of PhD qualifications obtained	1
2	Number of Masters qualifications obtained	3 (one in progress)
3	Number of other qualifications obtained	2
4a	Number of undergraduate students receiving training	+30 (incl. Chile, Falklands and UK)
4b	Number of training weeks provided to undergraduate students	10
4c	Number of postgraduate students receiving training (not 1-3 above)	4
4d	Number of training weeks for postgraduate students	16
5	Number of people receiving other forms of long-term (>1yr) training not leading to formal qualification(ie not categories 1-4 above)	2
6a	Number of people receiving other forms of short-term education/training (ie not categories 1-5 above)	3
6b	Number of training weeks not leading to formal qualification	4
7	Number of types of training materials produced for use by host country(s)	3
Research Measures		
8	Number of weeks spent by UK project staff on project work in host country(s)	4
9	Number of species/habitat management plans (or action plans) produced for Governments, public authorities or other implementing agencies in the host country (s)	1
10	Number of formal documents produced to assist work related to species identification, classification and recording.	1
11a	Number of papers published or accepted for publication in peer reviewed journals	5
11b	Number of papers published or accepted for publication elsewhere	3
12a	Number of computer-based databases established (containing species/generic information) and handed over to host country	1
12b	Number of computer-based databases enhanced (containing species/genetic information) and handed over to host country	1
13b	Number of species reference collections enhanced and handed over to host country(s)	1
Dissemination Measures		
14a	Number of conferences/seminars/workshops organised to present/disseminate findings from Darwin project work	3
14b	Number of conferences/seminars/ workshops attended at which findings from Darwin project work will be presented/ disseminated.	10
15a	Number of national press releases or publicity articles in host country(s)	2
15b	Number of local press releases or publicity articles in host country(s)	4
15c	Number of national press releases or publicity articles in UK	4
17a	Number of dissemination networks established	2
Physical Measures		
20	Estimated value (£s) of physical assets handed over to host country(s)	£XXX
21	Number of permanent educational/training/research facilities or organisation established	2
23	Value of additional resources raised for project	£XXX
Other Measures used by the project and not currently including in DI standard measures		
NA	15 min HD Dissemination Video	1

Annex 5 Publications

Type	Details (citation)	Available from	£cost
Peer reviewed paper	Vanhaecke,D.; Garcia de Leaniz,C.; Gajardo,G.; Thomas,C.J.; Consuegra,S. (2012). Metapopulation dynamics of a diadromous galaxiid fish and potential effects of salmonid aquaculture. *Freshwater Biology* 57: 1241-1252.	www.biodiversity.cl	-
Peer reviewed paper	Vanhaecke,D.; Garcia de Leaniz,C.; Gajardo,G.; Young,K.; Sanzana,J.; Orellana,G.; Fowler,D.; Howes,P.; Monzon-Arguello,C.; Consuegra,S. (2012). DNA barcoding and microsatellites help species delimitation and hybrid identification in endangered galaxiid fishes. *PLoS ONE* 7 (3): e32939.	www.biodiversity.cl	886
Peer reviewed paper	Vanhaecke, D., Allainguillaume, J., Croxford, A., Garcia de Leaniz, C., & Consuegra, S. (2011). Development of 13 microsatellite markers for the threatened galaxiid fish *Aplochiton zebra* (Jenyns, 1842). *Molecular Ecology Resources* 11: 219-222.	www.biodiversity.cl	-
Peer reviewed paper	Consuegra, S., Phillips, N., Gajardo, G., and Garcia de Leaniz, C. (2011). Winning the invasion roulette: escapes from fish farms increase admixture and facilitate establishment of non-native rainbow trout. *Evolutionary Applications* **4**: 660-671	www.biodiversity.cl	
Peer reviewed paper	Schröder, V. & Garcia de Leaniz, C. (2011). Discrimination between farmed and free-living invasive salmonids in Chilean Patagonia using stable isotope analysis. *Biological Invasions* 13: 203-213.	www.biodiversity.cl	
Peer reviewed paper	Garcia de Leaniz,C., Gajardo, G., & Consuegra, S. (2010) From Best to Pest: changing perspectives on the impact of exotic salmonids in the Southern Hemisphere. *Systematics and Biodiversity* **8**, 447-459.	www.biodiversity.cl	
Peer reviewed book chapter (case study)	Sanzana, J. Gajardo, G. & Garcia de Leaniz, C. (2012). Assessing the impact of barriers on connectivity of endangered native fishes in the face of salmonid invasions in Southern Chile. In Gough, P., Philipsen, P., Schollema, P.P. & Wanningen, H. (Eds.). From Sea to Source; International Guidance for the Restoration of Fish Migration Highways. Regional Water Authority Hunze en Aa's. Postbus 195, 9640 AD Veendam, The Netherlands. pp 48-49.	www.biodiversity.cl http://www.fromseatosource.com/?page=TEAM	-
Peer reviewed book chapter (case study)	Fowler, D. & Garcia de Leaniz, C. (2012). Assessing the impact of culverts on population connectivity of endangered galaxiid fishes in the Falkland Islands. In Gough, P., Philipsen, P., Schollema, P.P. & Wanningen, H. (Eds.). From Sea to Source; International Guidance for the Restoration of Fish Migration Highways. Regional Water Authority Hunze en Aa's. Postbus 195, 9640 AD Veendam, The Netherlands. pp 58-59.	www.biodiversity.cl http://www.fromseatosource.com/?page=TEAM	-
Popular science article	Gajardo, G & C. Garcia de Leaniz (2013). Protecting native fish in the face of salmonid invasions: What have we achieved? Darwin News April 2013.		-
Popular science article	Gajardo, G & C. Garcia de Leaniz (2012). Breaking myths on salmon impact on Chilean Biodiversity (19-029). Darwin News July 2012. p. 22	http://darwin.defra.gov.uk/newsletter/Darwin%20News%202012-07.pdf	-
Popular science article	Garcia de Leaniz, C. & G. Gajardo (2012). Between a rock and a hard place: doubling salmon farming in Chile while complying with CBD 2020. (EIDP041) Darwin News February 2012.	http://darwin.defra.gov.uk/newsletter/	-
Popular science article	García de Leániz, C., Consuegra, S., & Gajardo, G. (2012). El desafío de doblar la producción de salmones y cumplir los acuerdos de biodiversidad requiere una versión diferente basada en el valor de los ecosistemas. Version Diferente (Chile) [in Spanish] July 2012.		1,000
Popular science article	Garcia de Leaniz, C., Gajardo, G., Consuegra, S., Sanzana, J., Fowler, D., Rendell, N. Giannico, G. & Dunham, J. (2011). Tracing the origin of invasive salmonids. DEFRA Darwin Initiative website	http://darwin.defra.gov.uk/featured-project/2011-10-EIDPO041/	-

Annex 6 Darwin Contacts

Ref No	EIDPO041
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