Report on project to undertake genetic analysis of Allis shad (*Alosa alosa*)

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Project Specification (as supplied):
Research and set up genetic analysis markers for Allis shad (*Alosa alosa*); use the markers developed to analyse 100 samples of Allis shad from Plymouth Sound and Estuaries Special Area of Conservation. Produce a research report on the findings of the analysis.

Overview

Allis shad, *Alosa alosa*, is a member of the herring family. It is now rare in the UK and although previously known to spawn in several British rivers, the only recently confirmed spawning site is in the Tamar Estuary (Plymouth Sound and Estuaries SAC). The status of previously recorded spawning populations in the Solway Firth area (Maitland & Lyle 2001) and the Severn (Carstairs 2000) are currently unknown. The species is also now rare throughout much of its former range across western Europe and the Mediterranean, and major breeding populations are now confined predominantly to rivers in western France and Portugal. From a legislative perspective (EU Habitats Directive) allis shad are an Annex II species and, while their presence in the Tamar is a qualifying feature, it is not a primary reason for designation of Plymouth Sound and the Tamar estuary as a Special Area of Conservation (Plymouth Sound and Estuaries SAC).

Previous genetic work on a number of anadromous fish species has highlighted how (in contrast to catadromous species and many ocean spawning fish) the tendency to return to their natal river to breed and spawn can increase in-river population relatedness and drive intra-specific structuring, often within relatively small catchments, e.g. in shad (Alexandrino et al 2006), Atlantic salmon (Garant et al 2000; Griffiths et al 2010), brown trout (Griffiths et al 2009) and Pacific salmonids (Beacham et al 1985; Shaklee et al 1991). The aim of the study was to determine whether the sample of putatively allis shad scales analysed were representative of a single inter-breeding population or whether we could detect evidence of population structure within the sample.

This report outlines the genetic characterisation of DNA from 110 putatively Allis shad samples collected in the Tamar estuary Devon/Cornwall, southwest England. DNA was extracted from shad scales and analysed with 16 published microsatellite loci. Of the 110 samples available, DNA from 98 fish amplified successfully. A few scales appeared markedly different from the majority and may not have been shad; this may account for some samples not amplifying with these shad-specific primers. Detailed analysis was conducted at eight microsatellite loci on 98 fish.

Results of genetic characterisation suggested no significant differences between fish related to year class, and no grouping of fish into distinct genetic groups (as might be expected if intra-specific in-river population structure was present and/or Twaite shad, *Alosa fallax*, had been sampled). As far as we are able to discern, no *A. fallax* were sampled.

Materials and Methods

Shad sampling

Allis shad were sampled from the Tamar estuary over a 10-year period. Scale samples were taken from fish caught in the Environment Agency run fish-trap at Gunnislake Weir (NGR SX437711).

Year	No.
2004	5
2005	17
2006	26
2007	11
2008	4
2009	5
2010	12
2011	15
2012	1
2013	2

Table 1. Number of shad samples taken each year from the Tamar estuary for the 10-year period between 2004–2013.

DNA extraction and PCR Amplification

DNA was extracted from shad scales using the HotSHOT method (Truett et al. 2000). Extracted DNA was tested and found to amplify with 16 published microsatellite loci: Alo1, Alo6, Alo7, Alo9, Alo15, Alo26, Alo29, Alo32, Alo33, Alo43, Alo45 (Rougement et al. 2015); Aa20, Af20 (Faria et al. 2004); AsaC051, AsaD021, AsaD055 (Julian & Barton 2007). A few scales looked somewhat different from the majority and, although they yielded DNA, they did not amplifying with these shad-specific microsatellite primers; it appears probable that these scales were not from shad, as Twaite shad or Twaite x Allis hybrids would be expected to amplify with all of these loci (see Rougement et al. 2015).

Each DNA sample was then screened in detail for variation at eight microsatellite loci, using the primers of Rougemont et al (2015). PCR amplification was carried out using a BIO-RAD MyCycler Thermal Cycler in 10µl reaction volumes containing 1µl of extracted DNA (c. 30 ng DNA), 3µL RNase-Free Water, 5µL of QIAGEN HotStarTaq Plus Master Mix, and 1µL of primer mixture, in two microsatellite multiplexes of five loci (Alo1, Alo6, Alo26, Alo29 and Alo43) and three loci (Alo7, Alo32 and Alo45), respectively. PCR conditions were as follows: an initial denaturing step at 95°C for 5 mins was followed by 20 cycles of touchdown PCR consisting of 30 secs at 94°C, a 30 sec annealing step starting at 55°C and decreasing by 0.5°C each cycle until a touchdown temperature of 45°C was achieved, and an elongation step of 72°C for 30 secs, followed by 15 cycles comprising 94°C for 30 secs, 45°C for 30 secs and 72°C for 30 secs. This was followed by a final 10 min extension step at 72°C. Genotyping was performed on a Beckman Coulter CEQ™ 8000 Genetic Analysis System.

Microsatellite Analysis (post-amplification screening)

Basic measures of genetic diversity (number of alleles per locus and per population, observed heterozygosity and expected heterozygosity (H_O and H_E, respectively) were calculated. To test for population structure within the data set a Principal Coordinate Analysis (PCA), based on a matrix of pairwise genetic distances (Peakall et al. 1995) between samples. All analyses were performed using GenAlEx v6.501 (Peakall & Smouse 2012).

Results

A total of 62 alleles were found at the eight loci (Table 2). Number of alleles per locus ranged from six (Alo6, Alo26, Alo29, Alo32 and Alo45) to 13 (Alo1). Expected heterozygosity was lowest for Alo29 (0.351) and highest for Alo43 (0.707). Data for the French population of Allis Shad, taken from Rougemont et al (2015), are also presented in Table 2.

Table 2. Basic measure of genetic diversity for Tamar and French *Alosa alosa.*

Locus	Na Tamar	Na France	Ho Tamar	Ho France	He Tamar	He France
Alo1	13	11	0.633	0.767	0.688	0.751
Alo6	6	5	0.622	0.767	0.615	0.650
Alo7	10	5	0.378	0.333	0.366	0.425
Alo26	6	6	0.602	0.621	0.563	0.646
Alo29	6	5	0.378	0.517	0.351	0.523
Alo32	6	4	0.694	0.633	0.694	0.607
Alo43	9	7	0.713	0.724	0.707	0.709
Alo45	6	7	0.594	0.724	0.549	0.725

Na – number of alleles
Ho – observed heterozygosity
He – expected heterozygosity

Principal Coordinates Analysis

PCA analysis of the microsatellite data showed no obvious structuration within the data (Fig. 1). No sub-groupings related to, for example, year of collection or possibly to the presence of cryptic other species, e.g. Twaite shad, were noted. We conclude that the fish sampled represent a single, freely inter-breeding (panmictic) population of Allis shad with genetic profiles stable across the period of time covered by these samples, i.e. 10 years.

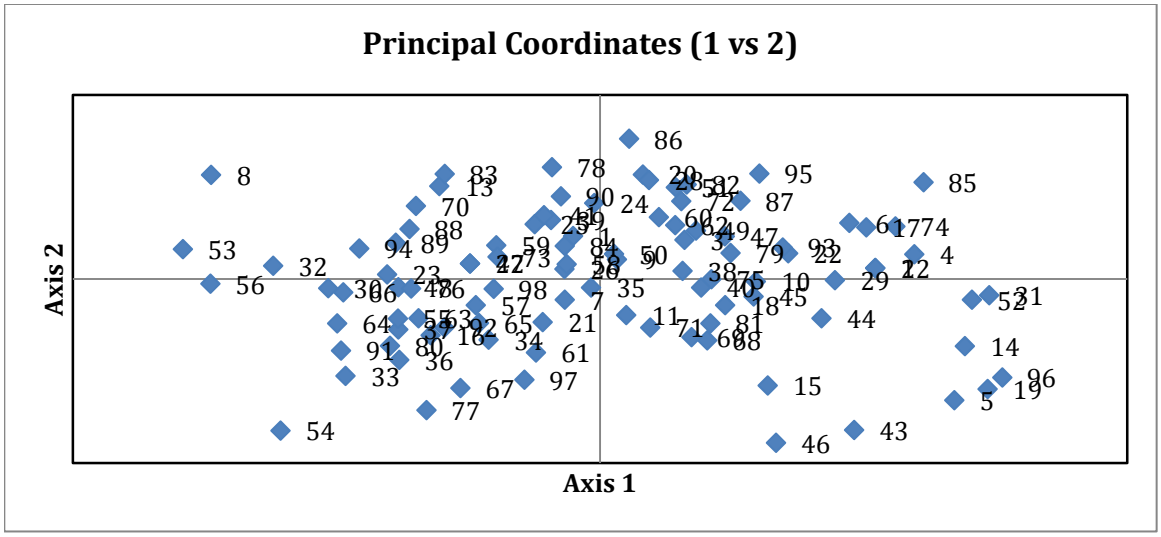

Figure 1. PCA analysis of eight shad microsatellite loci

Discussion

DNA from 110 putatively Allis shad (*Alosa alosa*) samples was analysed with 16 published microsatellite loci. Of the 110 samples available, DNA from 98 fish amplified successfully. Detailed characterisation analysis was conducted at eight microsatellite loci on these 98 fish. Results suggested no differences between fish related to year class, and no grouping of fish into distinct genetic groups (as might be expected if intra-specific in-river population structure was present within Allis shad and/or if Twaite shad had been sampled). Without the inclusion of known Twaite shad samples in the study, the data are not able to inform on the presence of possible Allis x Twaite hybrids, though previous analysis of Tamar shad by Jolly et al. (2012) reported approximately 13% of Allis shad analysed from the Tamar as having apparently hybrid ancestry (with no non-hybrid Twaite shad present).

Genetic analysis of fish analysed in this study showed comparable allele size ranges to those detected in French shad (Rougemont et al. 2015), though sizes are not directly comparable as (for study-specific reasons) Rougemont et al. attached a 19bp M13 fluorescently-labelled primer tail to their primers to allow for subsequent size fractionation on an ABI Prism 3130xl Genetic Analyser. A higher number of alleles were found in the Tamar sample (Table 2), but this likely reflects the higher number of individuals screened from the Tamar (Tamar 98 fish, France 30 fish).

The findings of this work suggest that Allis shad sampled from the river in any given year constitute a single panmictic (i.e. freely interbreeding) population. More precisely, the fish sampled are the offspring of a previous freely interbreeding parental group and (assuming they then mate at random with other Allis shad in the river at the time of sampling) they represent the continuation of such a freely interbreeding population. Similarly, a lack of apparent genetic differentiation between year groups suggests that fish from different year groups are not sufficiently reproductively isolated from each other to allow genetic drift to act to drive genetic differentiation between year classes. Or, to put it another way, genetic exchange between fish from different year classes remains sufficiently frequent to prevent genetic drift between year groups. These findings accord with the results of a recent study of Allis shad in French rivers (Martin et al 2015) which found weak genetic structure in this species and evidence of a substantial flow of fish straying between river basins both in the immediate vicinity or at longer distances. Statistics relating to genetic diversity (heterozygosity; Table 2) suggest that diversity within the Tamar population is not markedly different (either higher or lower) than the genetic diversity of a healthy population of Allis shad in the Vilaine river, Brittany, northwest France (Rougemont et al 2015).

Important caveats to these findings: we assume that our genetic markers (microsatellite loci) are of sufficient resolution to be able to detect relevant patterns of genetic variance. Previous research with these loci suggests this to be the case; see Rougemont et al (2015) and Martin et al (2015). Sample sizes per year class are frequently small (ranging between 1–26) and the extent to which the accuracy of resulting statistics may have been compromised is unknown.

Overall, these findings suggest that Allis shad sampled from the Tamar over the 10-year period 2004–2013 are effectively one population and can be managed as such. Statistics relating to genetic diversity (heterozygosity) suggest that diversity in the Tamar population is on a par with that observed in a population of Allis shad in the Vilaine river in northwest France; as such, the Tamar population does not appear to be at any greater immediate risk

of inbreeding than a comparable French population, though the isolation of the Tamar Allis shad population and the much greater geographical separation from the nearest healthy spawning Allis shad population necessitates that the genetic heath of the Tamar population be regularly monitored. Monitoring should also attempt to increase the sample size of yearly samples to improve statistical accuracy. Given the relative genetic health of the current population, the onus is on good river management to maintain spawning habitat, and river navigability and access for these migratory fish. In particular, the relatively lesser ability of these fish –compared to Atlantic salmon and sea trout– to traverse even relatively minor in-river barriers during up-stream migration should be borne in mind.

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