Cyfoeth Naturiol Cymru
Natural Resources Wales

Monitoring Allis and Twaite Shad: quality assurance and species identification using molecular techniques

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NRW Evidence Report No 53

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We work to support Wales' economy by enabling the sustainable use of natural resources to support jobs and enterprise. We help businesses and developers to understand and consider environmental limits when they make important decisions.

We work to maintain and improve the quality of the environment for everyone and we work towards making the environment and our natural resources more resilient to climate change and other pressures.

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- Having a well resourced proactive programme of evidence work;
- Continuing to review and add to our evidence to ensure it is fit for the challenges facing us; and
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Mae gwangod (*Alosa* spp.) yn bysgod sy'n ymdebygu i benwaig ac yn cael eu diogelu gan y Gyfarwyddeb Gynefinoedd a'u monitro drwy ddefnyddio cic-samplu ar gyfer eu hwyau yn ystod y tymor silio. Fodd bynnag, mae wyau rhywogaethau pysgod eraill sydd heb fod yn darged yn cael eu dal yn y ffordd hon ambell waith yn ogystal. Prif nod y prosiect hwn oedd sicrhau ansawdd y rhaglen samplu wyau gwangod ym masnau afonydd Cymru (Gwy, Wysg a Thywi) drwy ddefnyddio technegau genetig er mwyn penderfynu ai gwangod oedd sampl cynrychioladol o wyau a gasglwyd.

Casglwyd 162 o wyau gwangod tybiedig o Afon Tywi, Sir Gaerfyrddin gan dîm maes a benodwyd gan Cyfoeth Naturiol Cymru (CNC). Roedd y samplau wyau wedi cael eu sefydlogi a'u storio mewn ethanol 95% a'u cludo i Labordy Cefas yn Weymouth. Oherwydd tywydd gwael, ni chasglwyd unrhyw wyau o Afonydd Gwy ac Wysg.

Tynnwyd DNA o wyau unigol a'u sgrinio drwy adwaith cadwynol polymeras (ACP) drwy ddefnyddio set ysgogi benodol ar gyfer *Alosa* spp. sy'n targedu'r genyn mitocondriaidd, Sytocrom b, er mwyn penderfynu a oedden nhw'n rhywogaethau gwangod. Dau sampl yn unig (1.2%) a oedd yn negyddol ar gyfer dilyniant targed Sytb sy'n benodol i Alosa drwy ddefnyddio ACP. Roedd yr un samplau yn negyddol wrth ddefnyddio prawf ACP generig ar gyfer y genyn Sytocrom ocsidas I (SOI), ac yn dangos bod methiant y prawf penodol yn fwyaf tebygol yn ganlyniad i brinder o'r DNA targed yn y sampl. Cynhyrchodd y 160 o wyau a oedd yn weddill signal penodol ar gyfer *Alosa* spp.

Gwnaed dadansoddiad dilyniant ar gynhyrchion mwyhau a gynhyrchwyd yn gyfochrog drwy ddefnyddio coctel ysgogi generig SOI er mwyn cadarnhau tarddiad y wyau a oedd yn cynhyrchu arwyddion ACP gwannach sy'n benodol i *Alosa*. O'r 15 o wyau a brofwyd, cafodd dau ddilyniant gwahanol eu hadnabod ac roedden nhw'n rhannu 99% o unfathiant y niwcleotid gyda'i gilydd. Roedd y ddau ddilyniant yn tarddu o wangod, yn seiliedig ar unfathiant y niwcleotid. Roedd y dilyniannau genyn mitocondriaidd (SOI) ar gyfer y tri wy o gynefin 4 ym Mhenddaulwyn, Cynefin 8 yn White Mill a Phont Nantgaredig yn tarddu o *Alosa fallax*, ac roedd y dilyniannau a gafwyd o'r 12 wy a oedd yn weddill o Gynefin 4 ym Mhenddaulwyn, Glantowylan, Cynefin 8 yn White Mill, Pont Llandeilo ac ar y terfyn Llanwol yn tarddu o *Alosa alosa*.

Yn gyffredinol, mae data'r dilyniant yn dangos y gallai 100% o'r wyau y gellid eu hadnabod wedi cael eu hadnabod yn gywir fel eu bod yn tarddu o wangod, gyda dau o wyau heb eu hadnabod yn bendant oherwydd ansawdd gwael y DNA. Ni fyddai unrhyw un o'r 9 safle a gafodd eu harchwilio ar Afon Tywi yn 2014 wedi cael eu cofnodi'n anghywir fel eu bod yn cefnogi silio gwangod drwy gael eu hadnabod yn y maes yn unig. Cadarnhawyd silio gwangod yn Nantgaredig, lle profodd samplau a gasglwyd yn 2013 i fod yn rhai pilcod. Mae'r canlyniadau hyn yn cymharu'n ffafriol gyda'r gwaith blaenorol ac maen nhw'n cynrychioli cyfradd uchel o lwyddiant.

2. Executive Summary

Shads (*Alosa* spp.) are herring-like fish that are protected by the Habitats Directive and monitored using kick sampling for their eggs during the spawning season. However, eggs of other non-target fish species are also sometimes caught in this way. The primary aim of this project was to quality assure the shad egg sampling programme on Welsh river basins (Wye, Usk and Tywi) by using genetic techniques to determine whether a representative sample of eggs collected were shad.

162 putative shad eggs were collected from the River Tywi, Carmarthenshire by the Natural Resources Wales (NRW) appointed field team. Egg samples were fixed and stored in 95% ethanol and forwarded to the Cefas Weymouth Laboratory. Due to bad weather no eggs were collected from the River Wye and Usk.

DNA was extracted from individual eggs and screened by polymerase chain reaction (PCR) using an *Alosa* spp.-specific primer set targeting the mitochrondrial gene, Cytochrome b, to determine if they were shad species. Only two samples (1.2%) were negative for the *Alosa*-specific Cytb target sequence by PCR. The same samples were also negative when using a generic PCR assay for the Cytochrome oxidase I (COI) gene indicating that the failure of the specific assay was most likely the result of insufficient target DNA in the sample. All the remaining 160 eggs produced an *Alosa* spp. specific signal.

Sequence analysis was undertaken on amplification products generated in parallel using the COI generic primer cocktail to confirm the origin of eggs producing the weaker *Alosa*-specific PCR signals. Of the 15 eggs tested, two distinct sequences were identified sharing 99% nucleotide identity with each other. Both sequences were shad in origin based on the nucleotide identities. The mitochrondrial (COI) gene sequences for three eggs from Habitat 4 at Penddaulwyn, Habitat 8 at White Mill, and Nantgaredig Bridge were *Alosa fallax* in origin, and the sequences obtained for the remaining 12 eggs from Habitat 4 at Penddaulwyn, Glantowylan, Habitat 8 at White Mill, Llandeilo Bridge and at the Tidal limit were *Alosa alosa* in origin.

Overall, the sequence data indicates that 100% of the eggs that could be identified were correctly identified as shad in origin, with the identity of two eggs being uncertain due to poor DNA quality. None of the 9 sites surveyed on the R.Tywi in 2014 would have been erroneously recorded as supporting shad spawning using field identification alone. Shad spawning was confirmed at Nantgaredig, where samples collected in 2013 had proved to be minnow. These results compare favourably with previous work and represent a very high success rate.

3. Introduction

3.1. Background

The twaite shad *Alosa fallax*, and the allis shad *Alosa alosa*, are clupeid fish once found in a large number of rivers in the south of England and Wales (Aprahamian & Aprahamian 1990). Although they were once common in the rivers such as the Thames, the populations have been reduced primarily through pollution and barriers to migration such as dams and weirs (Aprahamian *et al.* 2003, Maitland & Hatton-Ellis 2003). As a result, shad populations have declined to such an extent that they are protected under Annexe II of the Habitats Directive. Today, the principal strongholds are limited to the rivers of south-west Britain, including the Rivers Wye, Usk and Tywi in south Wales. All three rivers are designated as Special Areas of Conservation (SAC) for both species.

Monitoring shad in a cost-effective manner is challenging, and various approaches have been tried including catch records from anglers and netsmen, hydroacoustic fish counters and seine netting of juveniles (Aprahamian *et al*. 2003; Hillman 2003; Hillman *et al*. 2003; Noble *et al*. 2007). The Countryside Council for Wales (CCW) and the Environment Agency in Wales have carried out kick sampling for shad eggs during the spawning season, which is a simple and cost-effective technique that has provided good semi-quantitative information on shad spawning activity and distribution (Thomas & Dyson 2012a,b, Garrett *et al*. 2013). However, a weakness of this method is that fish eggs are not easily identified, and some eggs being sampled by the may be from non-target species. Variability in egg size has been reported, suggesting either that two shad species are involved, or that eggs of non-target taxa are being sampled.

In 2013, eggs from 12 sampling sites from the Wye, Usk and Tywi were analysed using molecular techniques. A total of 226 eggs were successfully genotyped. 85% of eggs sampled were shad in origin and the remaining non-*Alosa* eggs were identified as belonging to minnow (*Phoxinus phoxinus*) and chub (*Squalius cephalus*) (Hardouin *et al.* 2013). This suggests that although a high proportion of eggs are correctly identified, there is nevertheless the risk that some material is misidentified. In some cases, there were potential implications for the findings of the survey (Hardouin *et al*. 2013). Consequently, regular quality assurance of the monitoring using genetic screening is beneficial, especially where the results may be used to influence significant decisions.

3.2 Project objectives

The key objectives of the current study were:

- To quality assure the taxonomic identification of eggs collected by the kick sampling procedure; confirming that a subsample were re either twaite shad *Alosa fallax*, or allis shad *Alosa alosa* in origin by analysis of the Cyt b polymerase chain reaction (PCR) assay (Alexandrino *et al*. 2006)

- To confirm the origin of any non-shad eggs by analysis of the cytochrome oxidase subunit 1 (COI) mitochrondrial gene using methods described by Ivanova *et al.* (2007)

4. Materials and Methods

4.1. Sample collection and sample processing

During the spawning season (late May and early June 2014), NRW staff collected individual eggs by kick sampling from 15 sampling sites in the Tywi (Table 1) using a standard protocol (Appendix A). Due to bad weather and consequent high flows, no egg samples were obtained from any of the sampling sites on the Wye or the Usk.

Sampling staff were issued with clear instructions (Appendix A) and standard field equipment including pre-labelled 1.5ml Eppendorf tubes containing 95% ethanol. Briefly, all suspected shad eggs collected from each sampling site were placed carefully in the appropriate pots using a clean pair of forceps, taking care not to burst the egg. Eggs that appeared to be close to hatching were also not collected as these were likely to hatch in the alcohol and thereby posed an increased risk of cross contamination. New pots were used for each sampling site, and no than 30 eggs were placed in a single pot. Multiple pots for the same sample site were used if required. When sampling was completed, the lids of the individual sample tubes were sealed using the parafilm to reduce the risk of ethanol leakage and/or evaporation. Samples were maintained at 4°C and sent by overnight courier to Cefas in a cool box.

4.2. Primer design

The primers used in analysis are those already published (Alexandrino et al. 2006, Ivanova et al. 2007).
The *Alosa* genus-specific primers,
alocytbf1 (CCTTCTAACATTTCAGTCTGATG) and
alocytbr1 (AGGATTGTGGCCCCTGCAATTAC) were used to amplify a partial fragment of the mtDNA cytochrome b gene (Alexandrino et al. 2006).

A cocktail of 4 primers (C_FishF1t1/C_FishR1t1),
VF2_t1 (TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC),
FishF2_t1 (TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC),
FishR2_t1 (CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA)
and FR1d_t1 (CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA)
was used to amplify a partial COI sequence for confirmation of the species by sequence analysis (Ivanova et al. 2007).

4.3. DNA extraction and amplification

Individual eggs were examined using a Leica M125 dissecting microscope for the presence of an embryo. Individual eggs containing an embryo were then digested overnight in 500 µl of ATL buffer (Qiagen) containing proteinase K and the DNA extracted from 200 µl of the digest using the DNA Investigator Kit and the Universal BioRobot (Qiagen) following the QIAamp DNA tissue UNIV rcv31 extraction protocol. DNA from individual eggs was eluted in a 50 µl volume in a 96 well format.

Amplifications were performed in a 96 well format using the Cyt b (alocytbf1 / alocytbr1) and CoxI primer cocktail (C_FishF1t1 / C_FishR1t1). PCR reactions for the Cyt b assay were performed in a 50 µl reaction volume consisting of 1x GoTaq flexi

buffer (Promega, UK), 2.5 mM $MgCl_2$, 1 mM dNTP mix, 50 pmol of the forward and reverse primers, 1.25 units of GoTaq DNA Polymerase (Promega, UK) and 2.5 µl of the purified DNA template. The reaction mix was overlaid with mineral oil and after an initial denaturing step (5 min at 95°C), was subjected to 40 temperature cycles (1 min at 95°C, 1 min at 60°C and 1 min at 72°C) in a Peltier PTC-225 thermal cycler followed by a final extension step of 10 min at 72°C. A negative control extraction and amplification was included for every 10 eggs processed. Conditions used for the COI gene assay were the same as above, with the exception that the annealing temperature was reduced from 60°C to 48°C according to the published protocol.

To determine which of the eggs were from shad, 15 µl of the reaction products generated using the genus-specific and universal COI primer sets were visualised on 1.5% agarose gels stained with ethidium bromide. By resolving the reaction product from both the Cyt b and CoxI assays it was possible to identify samples that failed to generate products from both assays due to problems with the integrity of the DNA sample.

4.4. Sequencing

COI gene sequence analysis was applied to those samples that produced a product with COI assay only, together with samples that produced only weak products when using the *Alosa* spp.-specific Cyt b assay. The latter were selected to rule out the possibility of cross reactivity between the primers used in the *Alosa* spp.-specific Cyt b assay and non-target fish species.

PCR products generated using the COI primers cocktail were extracted and purified by ethanol precipitation. Both DNA strands of the amplicon were sequenced using the ABI PRISM BigDye terminator cycle sequencing system (Life Technologies) and the M13 primers corresponding to the tag sequences on the COI primers used in the initial amplification. Sequencing reactions were analysed on an ABI 3130 genetic analyser. A consensus sequence (with primer derived sequences removed) was determined using Sequencer software (Gene Codes Corporation, Ann Arbor, MI) and the origin of the amplicon sequence identified using the Basic Local Alignment Search Tool (BLAST) facility available at the National Centre for Biotechnology Information (NCBI).

Multiple sequence alignments and phylogenetic analysis were performed using a 242 nucleotide partial COI gene sequence obtained for the 15 of the eggs that produced weak amplification products when using the *Alosa* spp.-specific primer set. A partial COI gene sequence from the American shad, *Alosa sapidissima* (KC015147) was used as an outgroup. Multiple alignments were performed using Clustal W (Thompson *et al*. 1997) with the following Clustal parameters: a gap opening penalty of 15 and gap extension penalty of 6.66. Phylogenetic analyses were conducted using MEGA version 4 (Tamura *et al*. 2007).The neighbour-joining tree was constructed using a maximum composite likelihood model, and the robustness of the tree was tested using 1000 bootstrap replicates.

5. Results

5.1. Egg Collection Locations

A total of 162 putative *Alosa* spp. eggs were collect from the River Tywi for genetic analysis (Table 1). Eggs were obtained from 9 sites, Penddaulwyn, Penddaulwyn Habitat 4 and Habitat 5, Glantowylan, White Mill Habitat 8, Nantgaredig Bridge, Cothi confluence, Llandeilo Bridge and the Tidal limit). No eggs were obtained from Cothi Bridge, Dryslwyn, Cilsan Bridge, Manordeilo, Llanwrda and Llwynjack , Llanegwad, Llandovery and Dolauhirion were not sampled. Only three sites (Nantgaredig Bridge, White Mill and Cothi Confluence) were sampled in 2013 (Hardouin *et al* 2013); all of these were resampled in 2014.

5.2. *Alosa*-specific Cyt b gene amplification

DNA was extracted from individual eggs and screened using the *Alosa* spp.- specific primers as described above, and the PCR products were resolved by agarose gel electrophoresis (Figure 1). In the majority of cases a strong PCR product was obtained indicating that the eggs were *Alosa* spp. in origin. However, two eggs (0.61%) did not produce an *Alosa*.-specific product; sample 2A/15 from Habitat 4 at Penddaulwyn and sample 10/3 from Llandeilo Bridge suggesting these egg samples represent a different fish species. A further 19 eggs produced weak products suggesting that while they were *Alosa* spp. in origin, the DNA yields and/or the quality of the DNA was poor.

5.3. COI gene amplification

Duplicate DNA samples for all 162 eggs were screened using the COI primer cocktail (C_FishF1t1 / C_FishR1t1). The quality of the amplification was poor relative to the *Alosa* spp.-specific primer set, possibly due to the length of the primers and PCR conditions used but nonetheless, in most cases samples generated a product (Figure 2). Importantly, the samples from Habitat 4 at Penddaulwyn (2A/15) and from Llandeilo Bridge (10/3), that failed to yield an amplification product when using the *Alosa* spp.-specific primer set also failed to produce a product when using the a product COI primer cocktail Samples 4a/12 to 4a/18 also produced a weak product with the COI primers (Figure 2) but since a strong product was obtained when using the *Alosa* spp.-specific primer set it can be concluded that they are *Alosa* spp. in origin.

Table 1: Summary of the results of DNA analysis of eggs sampled from different locations in the River Tywi. Details of the number of eggs collected at each of the sampling points and the results PCR tests undertaken using the *Alosa*-specific primers set taken from Alexandrino *et al* (2006). The results of COI sequencing are also shown. NS= not sampled

Site No.	**NGR**	**Site Name**	**Total No.Eggs**	***Alosa* specific PCR**			**COI sequence data**	**Allis shad *A. alosa***	**Twaite shad *A. fallax***
				strong positives	negative	weak positives			
16	SN 44780 20495	Tidal limit	22	16	0	6	2	2	
2	SN 46231 20402	Penddaulwyn	4	4	0	0			
2a	SN 46363 20537	Habitat 4	25	19	1	5	4	3	1
2b	SN 46812 20700	Habitat 5	25	25	0	0			
3	SN 469 210	Glantowylan	32	31	0	1	1	1	
4	SN 46740 21494	White Mill	NS						
4a	SN 47146 21161	Habitat 8	24	20	0	4	4	3	1
5	SN 493 203	Nantgaredig Bridge	7	7	0	0	1		1
6	SN 49902 20108	Cothi confluence	6	6	0	0			
6a	SN 50504 20250	Cothi Bridge	0						
8	SN 55031 20345	Dryslwyn	0						
9	SN 59168 21463	Cilsan Bridge	0						
10	SN 62653 21991	Llandeilo Bridge	17	12	1	4	3	3	
11	SN 68759 26802	Manordeilo	0						
12	SN 71809 31006	Llanwrda	0						
13	SN75488 33138	Llwynjack	0						
Total			162	140	2	20	15	12	3

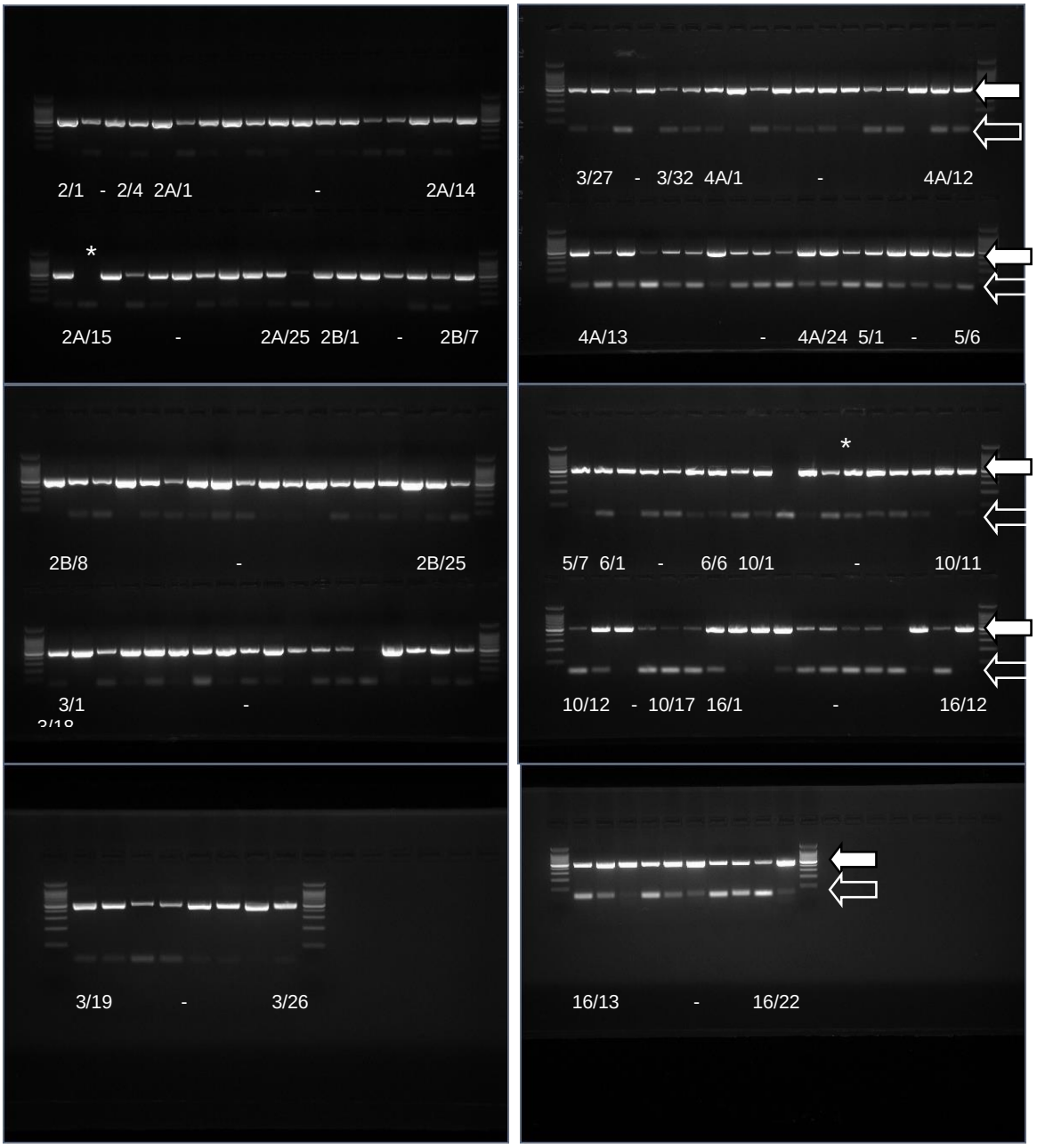

Figure 1: *Alosa*-specific PCR amplicons generated for eggs samples collected from the River Tywi. Individual eggs are numbered based on the site number followed by the egg number. The solid arrow indicates the product expected when using the alocytbf1/ alocytbr1 primer set and the open arrow indicates the primer dimers * indicates the eggs where no *Alosa*-specific signal was not obtained.

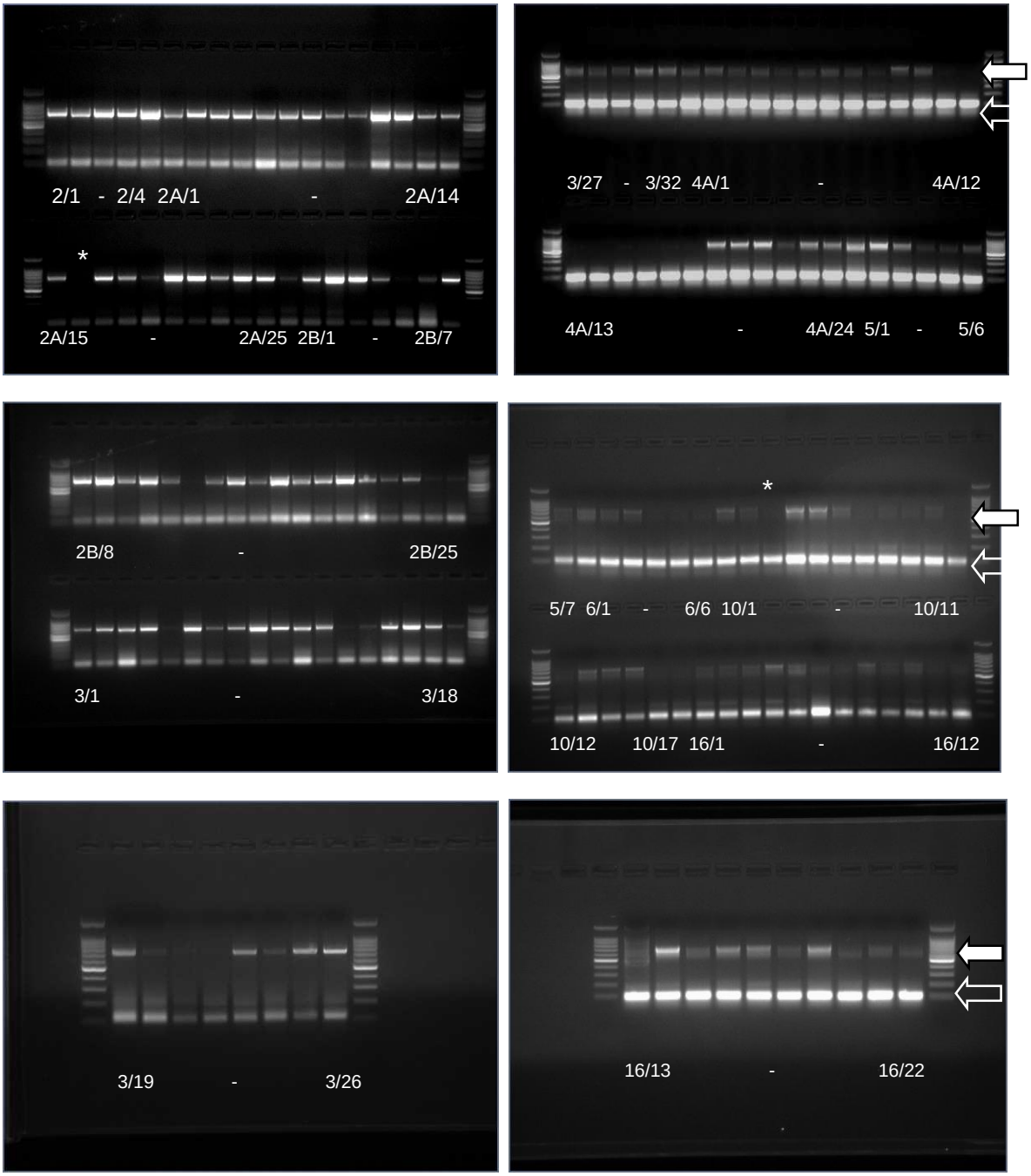

Figure 2: COI generic PCR amplicons generated for eggs samples collected from the River Tywi. Individual eggs are numbered based on the site number followed by the egg number, and are the same as those given in Figure1. The solid arrow indicates the product expected when using the C_FishF1t1 / C_FishR1t1 primer cocktail and the open arrow indicates the primer dimers * indicates the eggs where a PCR signal was not obtained.

5.4. Sequence analysis

Sequence analysis was undertaken on 15 of the sequences generated using the COI generic primer cocktail to determine the origin of the PCR signal. The samples chosen were those that produced a weak signal when using the *Alosa*-specific primer set, but also generated a signal when using the generic primer sets. In addition, sample 5/7, an egg considered too small to be shad in origin based on taxonomic examination by the sampling team, was also sequenced

The intensity of the products generated by the PCR assays were generally weak which made sequencing difficult, however, it was possible to obtain a consensus sequence for each of the products of between 277 and 652 nucleotides in length (Appendix B).

Alignment of the sequences revealed two distinct sequences that shared 98.56% nucleotide identity (Figure 3), and phylogenetic analysis confirmed the two distinct lineages (Figure 4). BLAST analysis showed that 20% of the COI gene sequences (2a/13 4a/16 and 5/7) were *A. fallax* in origin and the remaining sequences (2a/10, 2a/11 and 18; 3/31; 4a/9. 4a/14 and 4a/22; 10/15, 10/16 and 10/17; 16/7 and 16/8) were *A. alosa* in origin (Appendix C)

```
#KJ204646_Alosa_fallax      CTTCTCGGAG ATGATCAGAT CTATAACGTC ATCGTTACGG CGCACGCCTT CGTAATAATC TTCTTCATAG
#KJ128407_Alosa_alosa       .......... .......... .......... .......... .......... .......... ..........
#2a/10                      .......... .......... .......... .......... .......... .......... ..........
#2a/11                      .......... .......... .......... .......... .......... .......... ..........
#2a/13                      .......... .......... .......... .......... .......... .......... ..........
#2a/18                      .......... .......... .......... .......... .......... .......... ..........
#3/31                       .......... .......... .......... .......... .......... .......... ..........
#4/9                        .......... .......... .......... .......... .......... .......... ..........
#4/14                       .......... .......... .......... .......... .......... .......... ..........
#4/16                       .......... .......... .......... .......... .......... .......... ..........
#4/22                       .......... .......... .......... .......... .......... .......... ..........
#5/7                        .......... .......... .......... .......... .......... .......... ..........
#10/15                      .......... .......... .......... .......... .......... .......... ..........
#10/16                      .......... .......... .......... .......... .......... .......... ..........
#10/17                      .......... .......... .......... .......... .......... .......... ..........
#16/7                       .......... .......... .......... .......... .......... .......... ..........
#16/8                       .......... .......... .......... .......... .......... .......... ..........
#KC015147_Alosa_sapidissima .......... .......... ...C...... .......... .......... .......... ..........
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```
#KJ204646_Alosa_fallax      TAATGCCAAT TCTAATTGGC GGCTTTGGGA ATTGACTAGT CCCCCTTATG ATCGGGGCAC CAGACATGGC
#KJ128407_Alosa_alosa       .......... .......... .......... .......... .......... .......... ..........
#2a/10                      .......... .......... .......... .......... .......... .......... ..........
#2a/11                      .......... .......... .......... .......... .......... .......... ..........
#2a/13                      .......... .......... .......... .......... .......... .......... ..........
#2a/18                      .......... .......... .......... .......... .......... .......... ..........
#3/31                       .......... .......... .......... .......... .......... .......... ..........
#4/9                        .......... .......... .......... .......... .......... .......... ..........
#4/14                       .......... .......... .......... .......... .......... .......... ..........
#4/16                       .......... .......... .......... .......... .......... .......... ..........
#4/22                       .......... .......... .......... .......... .......... .......... ..........
#5/7                        .......... .......... .......... .......... .......... .......... ..........
#10/15                      .......... .......... .......... .......... .......... .......... ..........
#10/16                      .......... .......... .......... .......... .......... .......... ..........
#10/17                      .......... .......... .......... .......... .......... .......... ..........
#16/7                       .......... .......... .......... .......... .......... .......... ..........
#16/8                       .......... .......... .......... .......... .......... .......... ..........
#KC015147_Alosa_sapidissima .......... .......... .......... .......G.. .......... .......... ..........
```

```
#KJ204646_Alosa_fallax      ATTCCCACGA ATGAACAACA TGAGCTTCTG GCTACTTCCA CCCTCATTCC TCCTCCTCCT TGCCTCCTCC
#KJ128407_Alosa_alosa       .......... .......... .......... A........G .......... .......... ..........
#2a/10                      .......... .......... .......... A........G .......... .......... ..........
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#2a/11                    .......... .......... .......... A.........G .......... .......... ..........
#2a/13                    .......... .......... .......... .......... .......... .......... ..........
#2a/18                    .......... .......... .......... A.........G .......... .......... ..........
#3/31                     .......... .......... .......... A.........G .......... .......... ..........
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#4/14                     .......... .......... .......... A.........G .......... .......... ..........
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#4/22                     .......... .......... .......... A.........G .......... .......Y.. ..........
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#10/15                    .......... .......... .......... A.........G .......... .......... ..........
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#10/17                    .......... .......... .......... A.........G .......... .......... ..........
#16/7                     .......... .......... .......... A.........G .......... .......... ..........
#16/8                     .......... .......... .......... A.........G .......... .......... ..........
#KC015147_Alosa_sapidissima .......... .......... .......... A......... .......... .......... ..........


#KJ204646_Alosa_fallax     GGGGTTGAAG CCGGGGCAGG GACCGGGTGA AC
#KJ128407_Alosa_alosa      .......... .......... A......... ..
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#2a/13                    .......... .......... .......... ..
#2a/18                    .......... .......... A......... ..
#3/31                     .......... .......... A......... ..
#4/9                      .......... .......... A......... ..
#4/14                     .......... .......... A......... ..
#4/16                     .......... .......... .......... ..
#4/22                     .......... .......... A......... ..
#5/7                      .......... .......... .......... ..
#10/15                    .......... .......... A......... ..
#10/16                    .......... .......... A......... ..
#10/17                    .......... .......... A......... ..
#16/7                     .......... .......... A......... ..
#16/8                     .......... .......... A......... ..
#KC015147_Alosa_sapidissima ..A.....G. .......... A......... ..
```

Figure 3: Alignment of the partial COI gene sequence (242 nucleotides) generated for putative shad eggs from the River Tywi with sequences from *Alosa alosa*, *Alosa fallax* and the American shad, *Alosa sapidissima*. Eggs chosen for COI sequencing had produced weak products when screening using the *Alosa* -specific PCR assay. Positions of nucleotide variation are indicated using the appropriate IUB code.

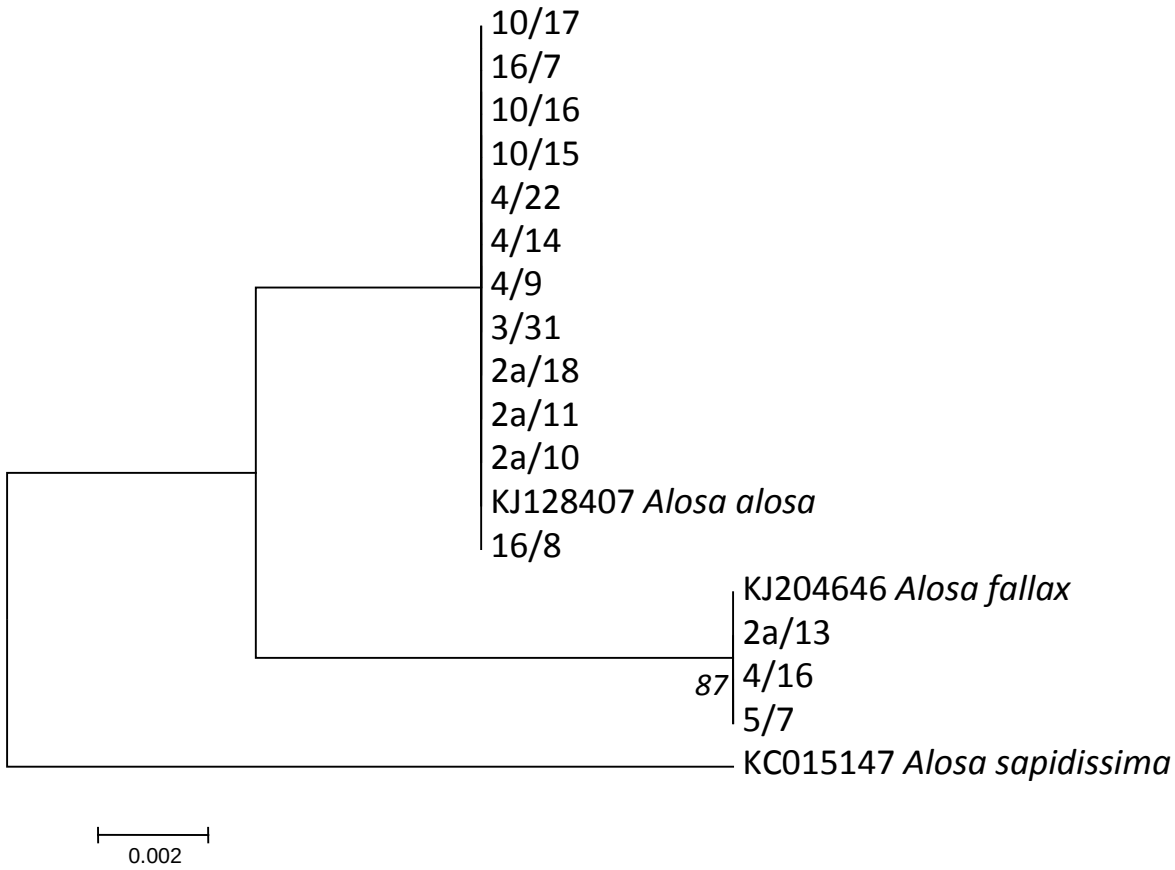


Figure 4: Phylogenetic relationships between the partial COI gene sequences obtained for the putative *Alosa* spp. eggs collected on the River Tywi. The tree was generated using neighbour-joining distance methods and bootstrap values >70 % are shown at the branch points. Partial COI sequence from the American shad *Alosa sapidissima* was used as an out-group. The scale is the number of nucleotide substitutions per nucleotide

6. Discussion

This study was undertaken as part of a regular quality assurance of the shad egg sampling programme in Welsh rivers (Wye, Usk and Tywi), using a genetic approach to determine if a representative sample of eggs were correctly identified as shad. Similar studies suggest that although a high proportion of eggs are correctly identified, but there is nevertheless a risk that some material is misidentified (Hardouin *et al.* 2013).

In this current study, none of the eggs collected from the river Tywi were identified as a non-target species. A high proportion (98.8%) eggs were verified as being shad, and the remainder did not yield DNA of sufficient quality to identify using molecular methods. This result is substantially better than the 85% accuracy achieved in 2013, and shows a significant increase in the reliability of the taxonomic identification in the field. Based on this level of accuracy for the taxonomic identification, none of the 9 sites surveyed on the R.Tywi would have been erroneously recorded as supporting the spawning of shad, if using field identification alone. Shad spawning was confirmed at Nantgaredig Bridge, where all samples collected in 2013 had proved to be minnow (Hardouin *et al*. 2013). The confirmation of successful spawning at Llandeilo Bridge sample is potentially significant as although there are numerous records of shad spawning upstream of this location (Garrett *et al*. 2013), this represents the upstream record of shad spawning that has been confirmed using molecular methods.

Some of the eggs identified as shad based on the use of *Alosa*-spp.-specific Cyt b primers set (Alexandrino *et al.* 2006) produced relative low amplification signals. To eliminate the possibility of poor primer specificity and potential cross reactivity of the primers with the mitochrondrial (Cyt b) gene target sequence from other unrelated species, these eggs were also confirmed as shad by the amplification and sequence analysis of a second mitochondrial gene (COI) sequence generated using a universal primer set (Ivanova et al. 2007). Two eggs that could not be identified genetically using the *Alosa* spp.-specific PCR assay also failed to generate an amplification product with the COI primer set, and therefore, failure to generate products was most likely due to a poor DNA yield or DNA quality rather than the egg originating from a different species

Of the 15 eggs selected for COI sequencing all were shown to be shad in origin, and 20% of the eggs were assigned to *A. fallax* and 80% assigned to *A. alosa* based on the sequence alignments. These results are consistent with previous studies which estimated the percentage of *A. alosa* mtDNA in the Tywi shad populations as 71% and 72% respectively (Alexandrino & Faria 2004; Hardouin *et al*. 2013). Analysis of nuclear DNA sequences would be required to determine if these eggs represent the progeny of a hybridisation between *Alosa fallax* and *Alosa alosa*. to resolve this issue.

Overall, identification of the species origin of the eggs sampled from the R. Tywi using a combination of Cyt b and COI primers (Alexandrino et al. 2006, Ivanova *et al*. 2007) was successful. However, the amplification signals generated when using the M13 tagged primers taken from Ivanova *et al.* (2007) were generally poor, with a very obvious primer dimer formation (Figure 2). If this type of quality assurance is done

regularly it would benefit significantly from a re-evaluation of the suitability of this primer set. The poor quality of the amplification data was most likely due to a combination of the high melting temperatures (Tm) for these primers (Tm = 74.0°C - 85.9°C) and the low annealing temperature (48°C) used in the PCR assay. It the future, it would be advised that the Tm of the primers is reduced by removing the M13 tags, and/or the annealing temperature in the assay is increased. Since the 3' termini of the primers start at the same position on the COI gene the same cocktail of truncated primers could also be used to sequence the products without adversely affecting the quality of the sequence data.

7. Conclusions

- 162 eggs were collected from 19 known and potential shad spawning sites on the Tywi. Of these, 160 were confirmed as shad eggs and two yielded poor quality DNA that could not be identified. These results indicate that samplers were correctly identifying shad eggs.
- Shad spawning was confirmed from Nantgaredig.

8. Acknowledgements

I am extremely grateful to Dr. Tristan Hatton-Ellis for helping to improve the report. Heather Garrett, Leila Thornton and Alex Harding co-ordinated and led fieldwork. Other samplers were Mark Bishop, Jill Howells, Hilary Foster, Meryl Tandy, Iestyn Evans, Julie Gething, Chloe Jennings, James Moon, Richard West, Emma Keenan, Ali Baird, Nicola Broadbrige, Paul Hyatt and Kerry Rogers.

9. References

Aprahamian MW and Aprahamian CD (1990). Status of the genus *Alosa* in the British Isles: past and present. *Journal of Fish Biology*, 37A, 257–258.

Aprahamian MW, Aprahamian CD, Baglinière J-L, Sabatié R, Alexandrino P. 2003. Alosa alosa *and* Alosa fallax *spp. Literature Review and Bibliography*. R&D Technical Report W1-014/TR. Environment Agency, Bristol.

Alexandrino P, Faria R. 2004. *Population Genetic Structure of Shad in the UK*. Report to CCW. Bangor.

Alexandrino P, Faria R, Linhares D, Castro F, Le Corre M, Sabatie R , Baglinière JL, Weiss S (2006). Interspecific differentiation and intraspecific substructure in two closely related clupeids with extensive hybridization, *Alosa alosa* and *Alosa fallax*. *Journal of Fish Biology*, 69 (supplement b), 242–259.

Garrett H., Thomas Rh, Hatton-Ellis TW. 2013. *Afon Tywi SAC shad egg survey 2012*. CCW Staff Science Report No. 12/8/4. Countryside Council for Wales, Bangor.

Hardouin EA, Stuart S, Andreou D. 2013. *Monitoring Allis and Twaite Shad: quality assurance and species identification using molecular techniques.* NRW Evidence Report No: 1, 41pp, Natural Resources Wales, Bangor.

Hillman RJ. 2003. *The Distribution, Biology and Ecology of Shad in South-West England*. R&D Technical Report W1-047/TR. Environment Agency, Bristol.

Hillman RJ, Cowx IG, Harvey J. 2003.*Monitoring the Allis and Twaite Shad.* Conserving Natura 2000 Rivers Monitoring Series No. 3. English Nature, Peterborough.

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, doi: 10.1111/j.1471-8286.2007.01748.x

Maitland PS, Hatton-Ellis TW. 2003. *Ecology of the Allis and Twaite Shad:* Alosa alosa *and* Alosa fallax. Conserving Natura 2000 Rivers, Life in UK Rivers Ecology Series No. 3. English Nature, Peterborough.

Noble RAA, Nunn AD, Harvey JP, Cowx IG. 2007. *Shad monitoring and assessment of conservation condition in the Wye, Usk and Tywi SACs*. CCW Environmental Monitoring Report No. 40. Countryside Council for Wales, Bangor.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24, 1596-1599.

Thomas, Rh., and C. Dyson. 2012a. *River Wye Shad Egg Survey 2011*. CCW Staff Science Report No. 11/8/4. Countryside Council for Wales, Bangor.

Thomas, Rh., and C. Dyson. 2012b. *River Usk Shad Egg Survey 2011*. CCW Staff Science Report 11/8/3. Countryside Council for Wales, Bangor.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F,Higgins DG (1997) The CLUSTAL_X windows interface:flexible strategies for multiple sequence alignmentaided by quality analysis tools. *Nucleic Acids Research*, 25, 4876−4882

10. Appendices

10.1. Appendix A: Sampling procedures for Shad genetic work

10.1.1 Egg collection and storage for genetic work analysis

Cefas will provide clean, 15 prelabelled sample pots, parafilm and marker pens for each river and instructions for the dispatch of the egg samples to the laboratory. Sample pots will contain 95% ethanol to enable samples to be fixed and stored. In addition to normal kick sampling equipment, sampling teams will need to bring the following:

- Forceps
- Disposable PVC gloves
- Coolbag with icepacks or other means of keeping samples cool.
- PPE required for handling 95% ethanol as determined by your risk assessment.

All suspected shad eggs collected from each site should be placed carefully in the pots using a clean pair of forceps, taking care not to burst the egg. Transfer of excessive quantities of water with the eggs should also be avoided as this will dilute the fixative. Eggs that appear to be close to hatching should not be collected as these are likely to hatch in the alcohol and thereby pose an increased risk of contamination; a new pot should be used for each sampling site.

Each pot should contain eggs from one site only. It is recommended to collect no more than 30 eggs in a single pot, and use multiple pots for the same sample site if required. Cefas will separate individual eggs in the laboratory.
When sampling is completed, the lids of the individual sample tubes should be sealed using the parafilm provided. This will reduce the risk of ethanol leakage and/or evaporation.

Gloves should be worn when handling eggs in order to avoid cross-contamination. The usual health and safety measures associated with handling ethanol in the field should be observed. There is no need to clean forceps between eggs unless they are obviously dirty, in which case they may be rinsed with alcohol.

Once placed in the sample tubes the eggs will need to be placed in a cool bag and transferred to a refrigerator at approximately 5°C as soon as possible after collection in the field. The sample tubes will then need to be shipped to the Cefas Weymouth laboratory by a reliable courier with the temperature maintained at approximately 5°C. NRW will pay for the costs of the courier from point of collection to point of delivery.

Sample containers should be sent to:-

Dr David Stone
Cefas Weymouth Laboratory,
Barrack Road, The Nothe, Weymouth, Dorset. DT4 8UB

10.1.2 CCW Shad Egg Monitoring Protocol

The shad monitoring protocol is slightly different to that in the LIFE+ monitoring methodology publication. Only shad eggs should be recorded. Any 'suspect' eggs can be noted but recorded as unknown eggs.

The protocol is as follows;

1. A field sheet should be filled in for each site with NGR and data collected as cited below.

2. 10 kick samples should be undertaken and the number of shad eggs in each kick counted and recorded on the field sheet. Each kick sample lasts for 15 seconds.

3. If more than 10 shad eggs have been recorded at the end of 10 kicks then the survey is complete.

4. However, if 10 or less shad eggs are found then kick surveys should continue (with number of shad eggs recorded for each kick on the survey form) until over 10 shad eggs are recorded up to a maximum of 25 kick samples.

5. If 10 or less shad eggs have been recorded the survey should be terminated after 25 kick samples.

6. Presence of other SAC species (e.g. bullheads or lampreys) and invasive species (e.g. knotweed) should be recorded on the survey form.

7. Photos should be taken of up and downstream at each site

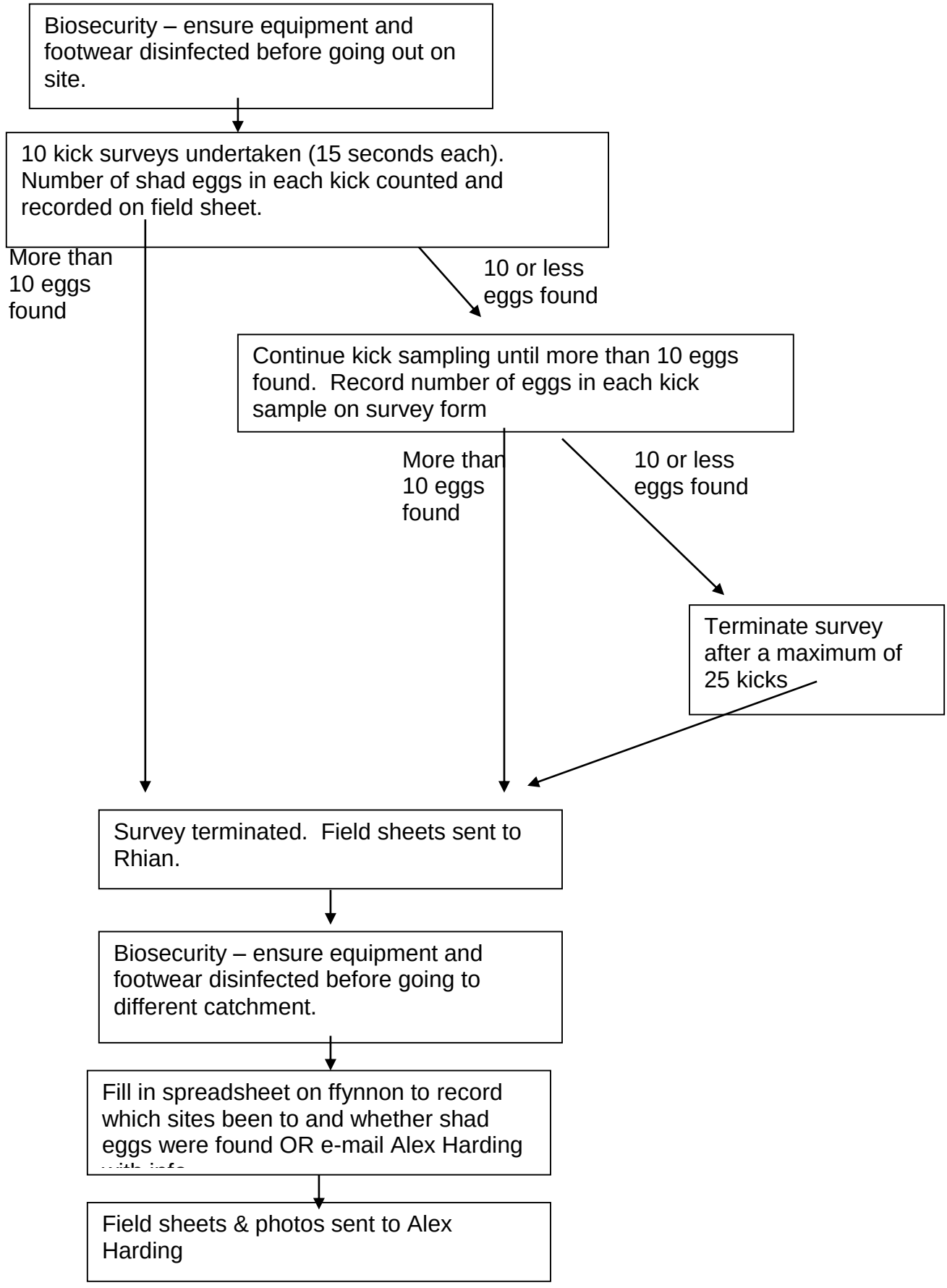
8. All survey forms (electronic or paper copy) should be sent to Alex Harding. If a paper copy is sent in the post then a copy should also be retained with the surveyor in case it gets lost in the post.

In addition;

- Photos should be taken of any eggs for which there is uncertainty over whether they are shad eggs. This uncertainty should be recorded on the survey form.
- Biosecurity – equipment and wellington boots/waders should be completely air dried or dipped in Virkon S Aquatic when moving between catchments.

The protocol is shown in flow chart below:

Figure 5: shad egg survey protocol



10.2. Appendix B: Cyt Bb gene sequences

10.2.1 Input file for the partial Cytb sequences obtained for the shad eggs collected on the River Tywi in 2014

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>KJ204646_*Alosa fallax*
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CTCCGGGGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGC
CCACGCCGGAGCATCCGTCAACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCA
>10/17
TTAATCCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCATCG
TTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATT
GACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTG
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GAACAGTCTATCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGAGCATCCGTCGACCTAACTATCTTC
TCTCTTCATCTAGCAGGTATTTCATCAATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGA
AACCCCCTGCAATCTCACAATATCAAACGCCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCC
TTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACAA
CCTTCTTTGACCCGGCAGGGGGAGGGGACCC
>16/7
ATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAG
ATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCA
ATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCC
ACGAATGAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGT
TGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGCCCACGCCGG
AGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCAATTCTTGGGGCCATTAA
TTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACGCCCCTATTTGTGTG
ATCCGTACTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCT
CCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATAC
CAACACCTATTCTGATTCTTCGGTCACCCTGAAGTGTCAT
>16/8
ACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCC
TTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTT
ATGATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGACTACTTCCGCCCT
CATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCC
GCCTTTGGCAGGCAATCTTGCCCACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGC
AGGTATTTCATCAATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAAT
CTCACAATATCAAACACCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCTCTCACTC
CCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACAA

10.3. Appendix C: BLAST results

10.3.1 Blast results for partial COI gene sequence for the egg sample 2a/13

```
BLASTN 2.2.30+
Reference: Zheng Zhang, Scott Schwartz, Lukas Wagner, and
Webb Miller (2000), "A greedy algorithm for aligning DNA
sequences", J Comput Biol 2000; 7(1-2):203-14.


Reference for database indexing: Aleksandr Morgulis, George
Coulouris, Yan Raytselis, Thomas L. Madden, Richa Agarwala,
Alejandro A. Schaffer (2008), "Database Indexing for
Production MegaBLAST Searches", Bioinformatics 24:1757-1764.


RID: 61MXSX5U013


Database: Nucleotide collection (nt)
29,089,105 sequences; 82,820,087,835 total letters
Query=2a/13
Length=625


                                                                  Score     E
Sequences producing significant alignments:                      (Bits)  Value

gb|KJ204646.1|  Alosa fallax voucher MT01883 cytochrome oxidas...   1155   0.0
gb|KJ128409.1|  Alosa fallax voucher NRM:57055 cytochrome oxid...   1155   0.0
gb|KJ128408.1|  Alosa fallax voucher NRM:52513 cytochrome oxid...   1155   0.0
dbj|AP009131.1|  Alosa alosa mitochondrial DNA, complete genome     1155   0.0
gb|KC500181.1|  Alosa alosa voucher TR212EK cytochrome oxidase...   1149   0.0
gb|KC500180.1|  Alosa alosa voucher TR211EK cytochrome oxidase...   1149   0.0
gb|KJ552649.1|  Alosa agone isolate Ex04E10 cytochrome oxidase...   1144   0.0
gb|KJ552379.1|  Alosa agone isolate Ex04F2 cytochrome oxidase ...   1144   0.0
gb|KC500174.1|  Alosa alosa voucher TR202EK cytochrome oxidase...   1144   0.0
gb|KC500173.1|  Alosa alosa voucher TR201EK cytochrome oxidase...   1144   0.0

ALIGNMENTS
>gb|KJ204646.1| Alosa fallax voucher MT01883 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KJ204647.1| Alosa fallax voucher MT01882 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KJ204649.1| Alosa fallax voucher MT02896 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KJ204650.1| Alosa fallax voucher MT01885 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KJ204651.1| Alosa fallax voucher MT01884 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
Length=652

 Score =  1155 bits (625),  Expect = 0.0
 Identities = 625/625 (100%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

Query  1    AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  60
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Sbjct  28   AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  87

Query  61   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  88   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  147

Query  121  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  148  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  207

Query  181  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
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Sbjct  208  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  267

Query  241  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  268  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  327

Query  301  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  328  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  387

Query  361  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  388  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  447

Query  421  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  448  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  507

Query  481  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  508  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  567

Query  541  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  568  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  627

Query  601  GGACCCAATTTTATACCAACACCTA  625
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Sbjct  628  GGACCCAATTTTATACCAACACCTA  652


>gb|KJ128409.1| Alosa fallax voucher NRM:57055 cytochrome oxidase subunit I (COI)
gene, partial cds; mitochondrial
Length=648

 Score =  1155 bits (625),  Expect = 0.0
 Identities = 625/625 (100%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

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Sbjct  21   AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  80

Query  61   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
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Sbjct  81   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  140

Query  121  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  141  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  200

Query  181  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  201  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  260

Query  241  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  261  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  320

Query  301  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  321  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  380

Query  361  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  381  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  440

Query  421  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  441  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  500

Query  481  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  501  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  560

Query  541  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
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Sbjct  561  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  620

Query  601  GGACCCAATTTTATACCAACACCTA  625
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Sbjct  621  GGACCCAATTTTATACCAACACCTA  645


>gb|KJ128408.1| Alosa fallax voucher NRM:52513 cytochrome oxidase subunit I (COI)
gene, partial cds; mitochondrial
Length=648

 Score =  1155 bits (625),  Expect = 0.0
 Identities = 625/625 (100%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

Query  1    AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21   AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  80

Query  61   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  81   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  140

Query  121  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  141  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  200

Query  181  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  201  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  260

Query  241  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  261  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  320

Query  301  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  321  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  380

Query  361  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
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Sbjct  381  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  440

Query  421  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  441  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  500

Query  481  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  501  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  560

Query  541  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  561  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  620

Query  601  GGACCCAATTTTATACCAACACCTA  625
            |||||||||||||||||||||||||
Sbjct  621  GGACCCAATTTTATACCAACACCTA  645


>dbj|AP009131.1| Alosa alosa mitochondrial DNA, complete genome
Length=16698

 Score =  1155 bits (625),  Expect = 0.0
 Identities = 625/625 (100%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5557  AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  5616

Query  61    ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5617  ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  5676

Query  121   CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
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Sbjct  5677  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  5736

Query  181   GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5737  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  5796

Query  241   ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
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Sbjct  5797  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  5856

Query  301   AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
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Query  361   TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
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Sbjct  5917  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  5976

Query  421   CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
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Query  481   ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
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Sbjct  6037  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  6096

Query  541   TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
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Sbjct  6097  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  6156

Query  601   GGACCCAATTTTATACCAACACCTA  625
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>gb|KC500181.1| Alosa alosa voucher TR212EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500182.1| Alosa alosa voucher TR213EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500183.1| Alosa alosa voucher TR214EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500184.1| Alosa alosa voucher TR215EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500185.1| Alosa alosa voucher TR216EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500186.1| Alosa alosa voucher TR219EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500188.1| Alosa alosa voucher TR217EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500189.1| Alosa alosa voucher TR218EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
Length=654

 Score =  1149 bits (622),  Expect = 0.0
 Identities = 624/625 (99%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

Query  1     AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  60
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Sbjct  27    AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  86

Query  61    ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
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Sbjct  87    ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  146

Query  121   CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
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Sbjct  147   CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  206

Query  181   GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
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Sbjct  207   GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  266

Query  241   ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
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Sbjct  267   ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  326
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Query  301  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
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Query  361  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
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Sbjct  387  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  446

Query  421  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
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Sbjct  447  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  506

Query  481  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
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Sbjct  507  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  566

Query  541  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
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Sbjct  567  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  626

Query  601  GGACCCAATTTTATACCAACACCTA  625
            |||||||||||||||||||||||||
Sbjct  627  GGACCCAATTTTATACCAACACCTA  651


>gb|KC500180.1| Alosa alosa voucher TR211EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
Length=654

 Score =  1149 bits (622),  Expect = 0.0
 Identities = 624/625 (99%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

Query  1    AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  60
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Sbjct  27   AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  86

Query  61   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  87   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  146

Query  121  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  147  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  206

Query  181  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  207  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  266

Query  241  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  267  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAAGCCGGGGCAGGGACCGGGTG  326

Query  301  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  327  AACAGTCTACCCACCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  386

Query  361  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  387  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  446

Query  421  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  447  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  506

Query  481  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  507  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  566

Query  541  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  567  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  626

Query  601  GGACCCAATTTTATACCAACACCTA  625
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Sbjct  627  GGACCCAATTTTATACCAACACCTA  651
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>gb|KJ552649.1| Alosa agone isolate Ex04E10 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
Length=652

 Score =  1144 bits (619),  Expect = 0.0
 Identities = 623/625 (99%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

Query  1    AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28   AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  87

Query  61   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  88   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  147

Query  121  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  148  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  207

Query  181  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  208  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  267

Query  241  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  268  GCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  327

Query  301  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
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Sbjct  328  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  387

Query  361  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
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Sbjct  388  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  447

Query  421  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
            |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  448  CACAATCATTAATATAAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  507

Query  481  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
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Sbjct  508  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  567

Query  541  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  568  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  627

Query  601  GGACCCAATTTTATACCAACACCTA  625
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Sbjct  628  GGACCCAATTTTATACCAACACCTA  652


>gb|KJ552379.1| Alosa agone isolate Ex04F2 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552455.1| Alosa agone isolate Ex04E11 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552682.1| Alosa agone isolate Ex04E8 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552733.1| Alosa agone isolate Ex04E12 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
Length=652

 Score =  1144 bits (619),  Expect = 0.0
 Identities = 623/625 (99%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

Query  1    AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28   AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  87

Query  61   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  88   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  147

Query  121  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  148  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  207

Query  181  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  208  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  267

Query  241  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  268  GCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAAGCCGGGGCAGGGACCGGGTG  327

Query  301  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  328  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  387

Query  361  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  388  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  447

Query  421  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  448  CACAATCATTAATATAAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  507

Query  481  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  508  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  567

Query  541  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  568  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  627

Query  601  GGACCCAATTTTATACCAACACCTA  625
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Sbjct  628  GGACCCAATTTTATACCAACACCTA  652


>gb|KC500174.1| Alosa alosa voucher TR202EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500175.1| Alosa alosa voucher TR206EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500176.1| Alosa alosa voucher TR207EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500177.1| Alosa alosa voucher TR208EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500178.1| Alosa alosa voucher TR209EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500179.1| Alosa alosa voucher TR210EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500187.1| Alosa alosa voucher TR203EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500190.1| Alosa alosa voucher TR204EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500191.1| Alosa alosa voucher TR205EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500192.1| Alosa alosa voucher TR200EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
Length=654

 Score =  1144 bits (619),  Expect = 0.0
 Identities = 623/625 (99%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

Query  1    AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  60
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Sbjct  27   AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  86

Query  61   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  87   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  146

Query  121  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  147  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  206

Query  181  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  207  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  266
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Query  241  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
            ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  267  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGAGTTGAAGCCGGGGCAGGGACCGGGTG  326

Query  301  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  327  AACAGTCTACCCACCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  386

Query  361  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  387  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  446

Query  421  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  447  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  506

Query  481  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  507  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  566

Query  541  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  567  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  626

Query  601  GGACCCAATTTTATACCAACACCTA  625
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Sbjct  627  GGACCCAATTTTATACCAACACCTA  651


>gb|KC500173.1| Alosa alosa voucher TR201EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
Length=654

 Score =  1144 bits (619),  Expect = 0.0
 Identities = 623/625 (99%), Gaps = 0/625 (0%)
 Strand=Plus/Plus

Query  1    AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  60
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Sbjct  27   AGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGCCAACCCGGGGC  86

Query  61   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  120
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Sbjct  87   ACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCCTTCGTAATAAT  146

Query  121  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  147  CTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTAGTCCCCCTTAT  206

Query  181  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  207  GATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTCTGGCTACTTCC  266

Query  241  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGGTTGAAGCCGGGGCAGGGACCGGGTG  300
            |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  267  ACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGAGTTGAAGCCGGGGCAGGGACCGGGTG  326

Query  301  AACAGTCTACCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  360
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  327  AACAGTCTACCCACCTTTGGCAGGCAATCTTGCCCACGCCGGGGCATCCGTCGACCTGAC  386

Query  361  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  387  TATCTTCTCTCTTCATCTAGCAGGTATTTCATCGATTCTTGGGGCCATTAATTTTATTAC  446

Query  421  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  447  CACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACACCCCTATTTGTGTG  506

Query  481  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  507  ATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTAGCTGCTGGGAT  566

Query  541  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  567  TACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCGGCAGGGGGAGG  626
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Query  601  GGACCCAATTTTATACCAACACCTA  625
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Sbjct  627  GGACCCAATTTTATACCAACACCTA  651


  Database: Nucleotide collection (nt)
    Posted date:  Nov 8, 2014  10:16 PM
  Number of letters in database: 82,820,087,835
  Number of sequences in database:  29,089,105

Lambda      K        H
    1.33    0.621     1.12
Gapped
Lambda      K        H
    1.28    0.460    0.850
Matrix: blastn matrix:1 -2
Gap Penalties: Existence: 0, Extension: 0
Number of Sequences: 29089105
Number of Hits to DB: 0
Number of extensions: 0
Number of successful extensions: 0
Number of sequences better than 10: 40
Number of HSP's better than 10 without gapping: 0
Number of HSP's gapped: 40
Number of HSP's successfully gapped: 40
Length of query: 625
Length of database: 82820087835
Length adjustment: 34
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Effective length of database: 81831058265
Effective search space: 48362155434615
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A: 0
X1: 13 (25.0 bits)
X2: 32 (59.1 bits)
X3: 54 (99.7 bits)
S1: 13 (25.1 bits)
S2: 23 (43.6 bits)
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10.3.2 BLAST results for partial COI gene sequence for egg sample 2a/11

```
BLASTN 2.2.30+
Reference: Zheng Zhang, Scott Schwartz, Lukas Wagner, and
Webb Miller (2000), "A greedy algorithm for aligning DNA
sequences", J Comput Biol 2000; 7(1-2):203-14.


Reference for database indexing: Aleksandr Morgulis, George
Coulouris, Yan Raytselis, Thomas L. Madden, Richa Agarwala,
Alejandro A. Schaffer (2008), "Database Indexing for
Production MegaBLAST Searches", Bioinformatics 24:1757-1764.


RID: 61PYBX3A01R


Database: Nucleotide collection (nt)
29,089,105 sequences; 82,820,087,835 total letters
Query=2a/11
Length=652
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Sequences producing significant alignments:	Score (Bits)	E Value
gb|KJ552458.1| Alosa alosa isolate Ex53B1 cytochrome oxidase ...	1199	0.0
gb|KJ128407.1| Alosa alosa voucher NRM:54753 cytochrome oxida...	1181	0.0
gb|KJ554963.1| Alosa alosa isolate Ex53B3 cytochrome oxidase ...	1177	0.0
gb|KJ552463.1| Alosa vistonica isolate CIBIOSS2 cytochrome ox...	1171	0.0
gb|KJ552478.1| Alosa alosa isolate Ex53B5 cytochrome oxidase ...	1170	0.0
gb|KJ768202.1| Alosa fallax voucher MLFPI252 cytochrome oxida...	1160	0.0
gb|KJ204646.1| Alosa fallax voucher MT01883 cytochrome oxidas...	1155	0.0
gb|KJ552379.1| Alosa agone isolate Ex04F2 cytochrome oxidase ...	1155	0.0
dbj|AP009131.1| Alosa alosa mitochondrial DNA, complete genome	1155	0.0
gb|KC500181.1| Alosa alosa voucher TR212EK cytochrome oxidase...	1147	0.0

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ALIGNMENTS
>gb|KJ552458.1| Alosa alosa isolate Ex53B1 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552509.1| Alosa alosa isolate Ex53B6 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552581.1| Alosa alosa isolate CIBIOSS1 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552690.1| Alosa alosa isolate Ex53B2 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552695.1| Alosa alosa isolate Ex53B4 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
Length=652

 Score =  1199 bits (649),  Expect = 0.0
 Identities = 651/652 (99%), Gaps = 0/652 (0%)
 Strand=Plus/Plus

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Sbjct  1    CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60

Query  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120
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Sbjct  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120

Query  121  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180
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Sbjct  121  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180

Query  181  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240
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Sbjct  181  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240

Query  241  GAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300
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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300

Query  301  GGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGC  360

Query  361  CCACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CCACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420

Query  421  AATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  AATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480

Query  481  ACAATATCAAACGCCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCT  540
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  ACAATATCAAACACCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCT  540

Query  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600

Query  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652


>gb|KJ128407.1| Alosa alosa voucher NRM:54753 cytochrome oxidase subunit I (COI)
gene, partial cds; mitochondrial
Length=648

 Score =  1181 bits (639),  Expect = 0.0
 Identities = 643/645 (99%), Gaps = 0/645 (0%)
 Strand=Plus/Plus

Query  8    CTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCC  67
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCC  60

Query  68   GAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACG  127
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACG  120

Query  128  GCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGG  187
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGG  180

Query  188  AATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAATGAACAAC  247
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAATGAACAAC  240

Query  248  ATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAA  307
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAA  300

Query  308  GCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGCCCACGCC  367
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGCCCACGCC  360

Query  368  GGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCAATTCTT  427
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  GGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCAATTCTT  420

Query  428  GGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTCACAATAT  487
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  GGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTCACAATAT  480

Query  488  CAAACGCCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCTCTCACTC  547
            |||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CAAACACCCCTATTTGTGTGATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTCTCACTC  540

Query  548  CCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTC  607
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  CCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTC  600

Query  608  TTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652
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            ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  TTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  645


>gb|KJ554963.1| Alosa alosa isolate Ex53B3 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
Length=637

 Score =  1177 bits (637),  Expect = 0.0
 Identities = 637/637 (100%), Gaps = 0/637 (0%)
 Strand=Plus/Plus

Query  16   TGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAG  75
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAG  60

Query  76   CCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGC  135
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGC  120

Query  136  CTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACT  195
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACT  180

Query  196  AGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTT  255
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTT  240

Query  256  CTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAAGCCGGGGC  315
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  CTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAAGCCGGGGC  300

Query  316  AGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGAGCATC  375
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGAGCATC  360

Query  376  CGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCAATTCTTGGGGCCAT  435
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCAATTCTTGGGGCCAT  420

Query  436  TAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACGCC  495
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  TAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACGCC  480

Query  496  CCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCT  555
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCT  540

Query  556  AGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCC  615
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  AGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCC  600

Query  616  GGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652
            |||||||||||||||||||||||||||||||||||||
Sbjct  601  GGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  637


>gb|KJ552463.1| Alosa vistonica isolate CIBIOSS2 cytochrome oxidase subunit I
gene, partial cds; mitochondrial
 gb|KJ552592.1| Alosa immaculata isolate Ex51B2 cytochrome oxidase subunit I
gene, partial cds; mitochondrial
Length=652

 Score =  1171 bits (634),  Expect = 0.0
 Identities = 646/652 (99%), Gaps = 0/652 (0%)
 Strand=Plus/Plus

Query  1    CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60

Query  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120

Query  121  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  121  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180

Query  181  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240

Query  241  GAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300

Query  301  GGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGC  360
            ||| ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  301  GGTCGAAGCCGGGGCAGGAACCGGGTGAACAGTCTACCCGCCTTTGGCAGGCAATCTTGC  360

Query  361  CCACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CCACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420

Query  421  AATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  AATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480

Query  481  ACAATATCAAACGCCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCT  540
            |||||||||||| |||||||||||||||||||| ||||| ||||||||||||||||||||
Sbjct  481  ACAATATCAAACACCCCTATTTGTGTGATCCGTGCTTGTGACGGCCGTTCTCCTTCTTCT  540

Query  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTTACAGACCGAAATCTAAATAC  600

Query  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652


>gb|KJ552478.1| Alosa alosa isolate Ex53B5 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
Length=635

 Score =  1170 bits (633),  Expect = 0.0
 Identities = 634/635 (99%), Gaps = 0/635 (0%)
 Strand=Plus/Plus

Query  17   GGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGC  76
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAATCCGAGCCGAACTGAGC  60

Query  77   CAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCC  136
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CAACCCGGGGCACTTYTCGGAGATGATCAGATCTATAACGTCATCGTTACGGCGCACGCC  120

Query  137  TTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTA  196
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGCTTTGGGAATTGACTA  180

Query  197  GTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTC  256
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAATGAACAACATGAGCTTC  240

Query  257  TGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAAGCCGGGGCA  316
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGGGTTGAAGCCGGGGCA  300

Query  317  GGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGAGCATCC  376
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGCCCACGCCGGAGCATCC  360

Query  377  GTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCAATTCTTGGGGCCATT  436
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  GTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCAATTCTTGGGGCCATT  420

Query  437  AATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACGCCC  496
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  AATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTCACAATATCAAACGCCC  480

Query  497  CTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTA  556
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  481  CTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCTCTCACTCCCTGTGCTA  540

Query  557  GCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCG  616
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  GCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACAACCTTCTTTGACCCG  600

Query  617  GCAGGGGGAGGGGACCCAATTTTATACCAACACCT  651
            |||||||||||||||||||||||||||||||||||
Sbjct  601  GCAGGGGGAGGGGACCCAATTTTATACCAACACCT  635


>gb|KJ768202.1| Alosa fallax voucher MLFPI252 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
Length=652

 Score =  1160 bits (628),  Expect = 0.0
 Identities = 644/652 (99%), Gaps = 0/652 (0%)
 Strand=Plus/Plus

Query  1    CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60

Query  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCCT  120

Query  121  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180
            |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CGTTACCGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180

Query  181  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240
            |||||||||||||||||||||||||||||| |||||||||||||||||||||| |||||
Sbjct  181  CTTTGGGAATTGACTAGTCCCCCTTATGATTGGGGCACCAGACATGGCATTCCCGCGAAT  240

Query  241  GAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300

Query  301  GGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGC  360
            |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  301  GGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTACCCGCCTTTGGCAGGCAATCTTGC  360

Query  361  CCACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420
            |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CCACGCCGGGGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420

Query  421  AATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  AATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480

Query  481  ACAATATCAAACGCCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCT  540
            |||||||||||| |||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  481  ACAATATCAAACACCCCTATTTGTGTGATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCT  540

Query  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600

Query  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652


>gb|KJ204646.1| Alosa fallax voucher MT01883 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KJ204647.1| Alosa fallax voucher MT01882 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KJ204649.1| Alosa fallax voucher MT02896 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KJ204650.1| Alosa fallax voucher MT01885 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KJ204651.1| Alosa fallax voucher MT01884 cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
Length=652

 Score =  1155 bits (625),  Expect = 0.0
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 Identities = 643/652 (99%), Gaps = 0/652 (0%)
 Strand=Plus/Plus

Query  1    CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60

Query  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120

Query  121  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180

Query  181  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240

Query  241  GAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300
            |||||||||||||||||| |||||||| ||||||||||||||||||||||||||||||||
Sbjct  241  GAACAACATGAGCTTCTGGCTACTTCCACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300

Query  301  GGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGC  360
            |||||||||||||||||| |||||||||||||||| ||||||||||||||||||||||||
Sbjct  301  GGTTGAAGCCGGGGCAGGGACCGGGTGAACAGTCTACCCGCCTTTGGCAGGCAATCTTGC  360

Query  361  CCACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420
            |||||||| ||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  361  CCACGCCGGGGCATCCGTCGACCTGACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420

Query  421  AATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  GATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480

Query  481  ACAATATCAAACGCCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCT  540
            |||||||||||| |||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  481  ACAATATCAAACACCCCTATTTGTGTGATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCT  540

Query  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600

Query  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652
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Sbjct  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652


>gb|KJ552379.1| Alosa agone isolate Ex04F2 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552455.1| Alosa agone isolate Ex04E11 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552682.1| Alosa agone isolate Ex04E8 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
 gb|KJ552733.1| Alosa agone isolate Ex04E12 cytochrome oxidase subunit I gene,
partial cds; mitochondrial
Length=652

 Score =  1155 bits (625),  Expect = 0.0
 Identities = 643/652 (99%), Gaps = 0/652 (0%)
 Strand=Plus/Plus

Query  1    CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60

Query  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120

Query  121  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180

Query  181  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240
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Query  241  GAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300
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Sbjct  241  GAACAACATGAGCTTCTGGCTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300

Query  301  GGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGC  360
            |||||||||||||||||| |||||||||||||||| |||||||||||||||||||||||||
Sbjct  301  GGTTGAAGCCGGGGCAGGGACCGGGTGAACAGTCTACCCGCCTTTGGCAGGCAATCTTGC  360

Query  361  CCACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420
            ||||||||| ||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  361  CCACGCCGGGGCATCCGTCGACCTGACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420

Query  421  AATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480
             |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  421  GATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATAAAACCCCCTGCAATCTC  480

Query  481  ACAATATCAAACGCCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCT  540
            |||||||||||| |||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  481  ACAATATCAAACACCCCTATTTGTGTGATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCT  540

Query  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600

Query  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652


>dbj|AP009131.1| Alosa alosa mitochondrial DNA, complete genome
Length=16698

 Score =  1155 bits (625),  Expect = 0.0
 Identities = 643/652 (99%), Gaps = 0/652 (0%)
 Strand=Plus/Plus

Query  1     CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  60
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Sbjct  5530  CCTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAAT  5589

Query  61    CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  120
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Sbjct  5590  CCGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCAT  5649

Query  121   CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5650  CGTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGG  5709

Query  181   CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5710  CTTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAAT  5769

Query  241   GAACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  300
            |||||||||||||||| |||||||| ||||||||||||||||||||||||||||||||||
Sbjct  5770  GAACAACATGAGCTTCTGGCTACTTCCACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGG  5829

Query  301   GGTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGC  360
            |||||||||||||||||| |||||||||||||||| |||||||||||||||||||||||||
Sbjct  5830  GGTTGAAGCCGGGGCAGGGACCGGGTGAACAGTCTACCCGCCTTTGGCAGGCAATCTTGC  5889

Query  361   CCACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  420
            ||||||||| ||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  5890  CCACGCCGGGGCATCCGTCGACCTGACTATCTTCTCTCTTCATCTAGCAGGTATTTCATC  5949

Query  421   AATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  480
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Sbjct  5950  GATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTC  6009

Query  481   ACAATATCAAACGCCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCT  540
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Sbjct  6010  ACAATATCAAACACCCCTATTTGTGTGATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCT  6069

Query  541   CTCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATAC  600
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Query  601   AACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652
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>gb|KC500181.1| Alosa alosa voucher TR212EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500182.1| Alosa alosa voucher TR213EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500183.1| Alosa alosa voucher TR214EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500184.1| Alosa alosa voucher TR215EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500185.1| Alosa alosa voucher TR216EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500186.1| Alosa alosa voucher TR219EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500188.1| Alosa alosa voucher TR217EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
 gb|KC500189.1| Alosa alosa voucher TR218EK cytochrome oxidase subunit 1 (COI)
gene, partial cds; mitochondrial
Length=654

 Score =  1147 bits (621),  Expect = 0.0
 Identities = 641/651 (98%), Gaps = 0/651 (0%)
 Strand=Plus/Plus

Query  2    CTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAATC  61
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Sbjct  1    CTTTACCTAGTATTTGGTGCCTGAGCAGGGATAGTAGGCACTGCCTTAAGTCTCTTAATC  60

Query  62   CGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCATC  121
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CGAGCCGAACTGAGCCAACCCGGGGCACTTCTCGGAGATGATCAGATCTATAACGTCATC  120

Query  122  GTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGC  181
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GTTACGGCGCACGCCTTCGTAATAATCTTCTTCATAGTAATGCCAATTCTAATTGGCGGC  180

Query  182  TTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAATG  241
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TTTGGGAATTGACTAGTCCCCCTTATGATCGGGGCACCAGACATGGCATTCCCACGAATG  240

Query  242  AACAACATGAGCTTCTGACTACTTCCGCCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGG  301
            |||||||||||||||| |||||||| ||||||||||||||||||||||||||||||||||
Sbjct  241  AACAACATGAGCTTCTGGCTACTTCCACCCTCATTCCTCCTCCTCCTTGCCTCCTCCGGG  300

Query  302  GTTGAAGCCGGGGCAGGAACCGGGTGAACAGTCTATCCGCCTTTGGCAGGCAATCTTGCC  361
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Sbjct  301  GTTGAAGCCGGGGCAGGGACCGGGTGAACAGTCTACCCACCTTTGGCAGGCAATCTTGCC  360

Query  362  CACGCCGGAGCATCCGTCGACCTAACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCA  421
            |||||||| ||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  361  CACGCCGGGGCATCCGTCGACCTGACTATCTTCTCTCTTCATCTAGCAGGTATTTCATCG  420

Query  422  ATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTCA  481
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  ATTCTTGGGGCCATTAATTTTATTACCACAATCATTAATATGAAACCCCCTGCAATCTCA  480

Query  482  CAATATCAAACGCCCCTATTTGTGTGATCCGTACTTGTAACGGCCGTTCTCCTTCTTCTC  541
            |||||||||||| |||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  481  CAATATCAAACACCCCTATTTGTGTGATCCGTGCTTGTAACGGCCGTTCTCCTTCTTCTC  540

Query  542  TCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACA  601
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  TCACTCCCTGTGCTAGCTGCTGGGATTACAATGCTCCTAACAGACCGAAATCTAAATACA  600

Query  602  ACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  652
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  ACCTTCTTTGACCCGGCAGGGGGAGGGGACCCAATTTTATACCAACACCTA  651


  Database: Nucleotide collection (nt)
    Posted date:  Nov 8, 2014  10:16 PM
  Number of letters in database: 1,211,939,210
  Number of sequences in database:  29,086,194
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Gapped
Lambda      K        H
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Gap Penalties: Existence: 0, Extension: 0
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Number of Hits to DB: 0
Number of extensions: 0
Number of successful extensions: 0
Number of sequences better than 10: 40
Number of HSP's better than 10 without gapping: 0
Number of HSP's gapped: 40
Number of HSP's successfully gapped: 40
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Length of database: 82816317834
Length adjustment: 34
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Effective length of database: 81827387238
Effective search space: 50569325313084
Effective search space used: 50569325313084
A: 0
X1: 13 (25.0 bits)
X2: 32 (59.1 bits)
X3: 54 (99.7 bits)
S1: 13 (25.1 bits)
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S2: 23 (43.6 bits

Data Archive Appendix

Data outputs associated with this project are archived as project number 461 media number 1552 on server–based storage at Natural Resources Wales.

The data archive contains:

[A] The final report in Microsoft Word and Adobe PDF formats.

[B] An Excel spreadsheet, River Tywi shad egg sampling locations.xlsx detailing the mtDNA Cyt b PCR results for each egg.

[C] A single FASTA format file, R Tywi COI gene.fas, for the partial COI gene sequences

Metadata for this project is publicly accessible through Natural Resources Wales' Library Catalogue http://194.83.155.90/olibcgi by searching 'Dataset Titles'. The metadata is held as record no 115891

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