Internat. Rev. Hydrobiol.	**87**	2002	5–6	661–674
IV. Chemical and Biochemical Composition of Sturgeon Products				

ARNE LUDWIG[1]*, LUTZ DEBUS[2] and INGO JENNECKENS[3]

[1]Institut für Gewässerökologie und Binnenfischerei, Abteilung für Biologie und Ökologie der Fische, 12561 Berlin, Germany; e-mail: Ludwig@izw-berlin.de
[2]Institut für Zoologie, Universität Rostock, Germany
[3]Institut für Tierzucht und Genetik, Universität Göttingen, Germany

A Molecular Approach to Control the International Trade in Black Caviar

key words: species identification, sturgeon, cytochrome *b*, RFLP, mtDNA, conservation

Abstract

The aim of this study was to develop and establish a molecular method for species identification of sturgeon products, esp. black caviar, used in the international trade. Sequences of the entire cytochrome *b* (cyt*b*) gene from 858 fish specimens were used for discriminating between 22 sturgeon species, and potential species-specific restriction sites were determined. No single restriction endonuclease can be used for the differentiation of all species. Depending on the species, from one to four different enzymes are necessary for species identification. Overall, using seven different restriction endonucleases, 17 acipenseriform species can be separated on the mtDNA level on the basis of characteristic species-specific restriction patterns. Three species of the genus *Scaphirhynchus* (*S. albus*, *S. platorhynchus* and *S. suttkusi*), as well as *Acipenser gueldenstaedti* and *A. persicus,* were not differentiated. Our approach provides an opportunity to identify and control the trade in sturgeon products outside of the three main caviar producing species, *A. gueldenstaedti*, *A. stellatus,* and *Huso huso*. Besides the trade, the method is important for the management and conservation programs. The necessity to combine nuclear and mtDNA markers for more precise identification is also discussed. The following hybrids were observed using mitochondrial and nuclear markers: one *A. gueldenstaedti*/*A. stellatus* hybrid, one *A. gueldenstaedti*/*Acipenser ruthenus* hybrid, five hybrids between *A. gueldenstaedti* or *A. persicus* and *A. nudiventris*.

1. Introduction

The families Polyodontidae (paddlefishes) and Acipenseridae (sturgeons) of the order Acipenseriformes include the most valuable freshwater fish species worldwide. These fishes produce black caviar. The overexploitation for black caviar and worsening environmental conditions resulted in a highly threatened status of all acipenseriform species (BIRSTEIN *et al.*, 1997a; BIRSTEIN, 2000). Some of them are close to extinction, for example, *Acipenser sturio, A. dabryanus*, or *Psephurus gladius*. Since April 1, 1998, all sturgeon and paddlefish species, as well as their parts and derivatives in the international trade, have been under the provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, Annex I). Twenty-three acipenseriform species are listed in the CITES Appendix I (*A. brevirostrum* and *A. sturio*) or Appendix II (all other species). The listing in Appendix II means that the international commercial trade in these species or products made of these species is authorized only with the export or import permits and re-export certificates (for details of the trade and on the control of the trade see RAYMAKERS, 2002).

* Present address: Institut für Zoo- und Wildtierforschung, Fachgebiet Evolutionsgenetik, PF 601103, 10252 Berlin, Germany.

 1434-2944/02/5-611-0661 $ 17.50+.50/0

Previous analyses of the trade in caviar of the three main caviar-producing species, the Russian sturgeon *A. gueldenstaedti*, stellate sturgeon *A. stellatus*, and beluga *Huso huso*, with the trade names "osetra," "sevruga" and "beluga," respectively, demonstrated a high level of mislabeled caviar lots occurring on the market. In 1998, about 7% of caviar in the trade was without species declaration (RAYMAKERS, 2002). The same year WOLF *et al.* (1999) observed about 13% of wrongly declared caviar imported to Switzerland. In 1995–96, BIRSTEIN *et al.* (1998, 1999) found up to 32% mislabeled caviar lots on the New York market. However, an additional analysis of the mislabeled samples from New York showed that the number of these lots was slightly overestimated (BIRSTEIN *et al.*, 2000). The confusion with some of the mislabeled osetra caviar lots was due to the previously unknown fact that there are cytochrome-*b* haplotypes within *A. gueldenstaedti* that have a very high similarity to the sequences of the Siberian sturgeon, *A. baeri* (JENNECKENS, 1999; BIRSTEIN *et al.*, 2000; JENNECKENS *et al.*, 2000).

Historically, species identification in the trade was based on a comparison of the size and color of caviar eggs. However, these characteristics are highly unreliable. Normally, sturgeons spawn several times during their life, and the size of eggs depends on the species and the age of females. The recent overfishing resulted in a decline in the number of females belonging to the eldest age classes used for caviar production, and, therefore, in a decrease in the size of eggs to some extent.

The development of molecular DNA methods, including the PCR technique, has offered new opportunities for species identification. Previously published analytical DNA approaches were based on species-specific PCR amplifications (DESALLE and BIRSTEIN, 1996; BIRSTEIN *et al.*, 1998, 1999) and on the PCR-restriction fragment length polymorphism or PCR-RFLP (WOLF *et al.*, 1999) of the mitochondrial (mt) cytochrome *b* (cyt*b*) gene. Studies of several mt genes showed only a few nucleotide differences between sturgeon species (BIRSTEIN and DESALLE, 1998; BIRSTEIN *et al.*, 1998; LUDWIG and KIRSCHBAUM, 1998; LUDWIG *et al.*, 2000a; 2001). The species-specific PCR approach was focused exclusively on finding diagnostic nucleotides for the identification of three commercial species, *A. stellatus*, *A. gueldenstaedti* and *H. huso* (DESALLE and BIRSTEIN, 1996; BIRSTEIN, 1999). Using the PCR-RFLP approach, WOLF *et al.* (1999) investigated 21 specimens of 10 species. However, an accurate and doubtless identification of acipenseriform species needs to include all important taxa within the group to avoid misidentification due to the potential possibility of the intraspecific variation overlap between closely related species. Taking into consideration the limitations of the previous studies, in this study we used the PCR-RFLP approach, but we based our method on the entire cyt*b* gene. We created a database for the cyt*b* gene sequences from 22 sturgeon species and searched it for the species-specific restriction sites. After that we determined experimentally the restriction patterns characterizing each species.

2. Materials and Methods

2.1. Acipenseriform Species Investigated

The names of acipenseriform species and the number of individuals for each species we used in this study are shown in Table 1. DNA was extracted from different tissue samples fixed in alcohol (whole blood, fin clips, liver, eggs, and sperm) and from fresh caviar eggs.

2.2. Amplification of the Entire Cytb Gene

DNA was isolated from all tissues using the QIAamp Blood or Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturers instructions. Amplifications were performed in 100 µl volumes containing: 50 ng genomic DNA, 1 U Taq polymerase (MBI-Fermentas, Vilnius, Lithuania), 5 pmoles of

Table 1. Samples of acipenseriform species used in this study.

Species	Common name	n	Geographic origin of sampled species	Name of collector
Family Polyodontidae				
Genus *Polyodon*				
P. spathula	North American paddlefish	2	Aquaculture (Mississippi R.)	L. DEBUS, I. JENNECKENS
Family Acipenseridae				
Subfamily Acipenserinae				
Genus *Acipenser*				
A. baeri	Siberian sturgeon	126	Lena R., Siberia, Aquaculture	I. JENNECKENS, P. WILLIOT
A. brevirostrum	Shortnose sturgeon	6	Aquaculture	B. MAY
A. fulvescens	Lake sturgeon	31	Menominee R. and Wolfe R., USA; Lake Winnebago	L. DEBUS, B. MAY
A. gueldenstaedti	Russian sturgeon or osetra*	98	Caspian Sea; Black Sea; Sea of Azov	E. N. ARTYUKHIN, M. CHEBANOV, L. DEBUS, N. PATRICHE, M. POURKAZEMI
A. medirostris	Green sturgeon	37	Columbia R., USA	B. MAY
A. mikadoi	Sakhalin sturgeon	5	Tumnin R., Russia	E. N. ARTYUKHIN
A. naccarii	Adriatic sturgeon	18	Buna R., Albania	T. GULYAS
A. nudiventris	Ship sturgeon	15	Caspian Sea; Volga R., Russia	L. DEBUS
A. oxyrhinchus	Atlantic sturgeon	90	Delaware R.; Hudson R.; St. John R.	J. GESSNER, B. MAY
A. persicus	Persian sturgeon	17	Caspian Sea., Iran; Volga R.	L. DEBUS, M. POURKAZEMI
A. ruthenus	Sterlet	156	Volga River system, Kuban R. and Ob R., Russia; Danube R., Romania	E. N. ARTYUKHIN, M. CHEBANOV, L. DEBUS, N. PATRICHE
A. schrencki	Amur sturgeon	3	Amur River, Siberia	V. SVIRSKY
A. sinensis	Chinese sturgeon	5	Yangtze River, China	S.-M. ZHANG
A. stellatus	Stellate sturgeon or sevruga*	93	Caspian Sea; Black Sea; Sea of Azov	E. N. ARTYUKHIN, M. CHEBANOV, N. PATRICHE, L. DEBUS
A. sturio	European sturgeon	44	Gironde R., France; North Sea	J. GESSNER, P. WILLIOT
A. transmontanus	White sturgeon	14	Kootenai R., USA	B. MAY, Acc. nos.: AF184107, X14944
Genus *Huso*				
H. huso	Great sturgeon or beluga*	74	Caspian Sea; Black Sea	E. N. ARTYUKHIN, L. DEBUS, D. MIREA
H. dauricus	kaluga* or eastern beluga*	2	Amur River, Siberia	V. SVIRSKY
Subfamily Scaphirhynchinae				
Genus *Scaphirhynchus*				
S. albus	Pallid sturgeon	4	Atchafalaya R., USA	L. DEBUS, B. MAY
S. platorhynchus	Shovelnose sturgeon	15	Mississippi R. and Missouri R., USA	B. MAY
S. suttkusi	Alabama sturgeon	3		GenBank

* Trade name of caviar

each primer, 0.10 mM Tris-HCl (pH 8.8 at 25 °C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 µg/µl bovine serum albumin (BSA), 0.08% (v/v) Nonidet P40 and 100 µM of each dNTP. The entire cyt*b* gene was amplified using primers L14735 (WOLF *et al.*, 1999) and cyt-*b*.rev1 (5´-CTTCGGTTTACAAGACCG-3´) (JENNECKENS, 1999). Amplifications were accomplished at 30 cycles of the following steps: 60 sec at 94 °C, 30 sec at 60 °C, 90 sec at 72 °C and a 5 min final extension at 72 °C.

2.3. The First Step: the Identification of Species-Specific Sites

To identify interspecific nucleotide variations between species, amplified fragments from one specimen per species were sequenced. The PCR product was excised from agarose gel and extracted using the QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). Direct sequencing was performed in both directions. Sequencing of the PCR product was done using the following conditions: 50 ng DNA, 5 pmoles primer and 2 µl BigDye RR Terminator Cycle Sequencing Kit (ABI, USA). The total reaction volume of 10 µl was overlaid by one drop of mineral oil. Amplifications were performed in a thermocycler programmed for 25 cycles, each consisting of 30 sec at 96 °C, 1 min at 50 °C, and 4 min at 60 °C. Beside the primers L14735 and cyt*b*.rev1 mentioned above, we also used four additional sequencing primers (LUDWIG *et al.*, 2000a).

The generated sequences from all specimens were subjected to WEBCUTTER V. 2.0 (http://firstmarket.com/firstmarket/cutter/cut2.html) to identify potential restriction sites of endonucleases that could be used for the differentiation and identification of each species. The endonucleases and their restriction sites are given in Table 2.

2.4. The Second Step: the Restriction Analysis

The second step included the amplification of the entire cyt*b* gene from a few additional specimens and purification of the products using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany). PCR fragments were diluted in 50 µl of the recommended buffer. Than ten microlitres of the extracted PCR-fragment were digested in a final volume of 20 µl with 10 units of the appropriate restriction enzyme (determined in the previous step with WEBCUTTER) in a final concentration of 1× reaction buffer (New England BioLabs). For some species, we performed digestion with different restriction endonucleases separately to obtain species-specific restriction patterns that were detected after electrophoresis in 1.5% (w/v) agarose gels by ethidium bromide staining. In the case of intraspecific variability of restriction patterns**,** we did not use the enzyme for species identification. Additional specimens were sequenced and subjected again to WEBCUTTER to identify diagnostic restriction sites for the

Table 2. Endonucleases diagnostic for species identification and their restriction sites.

Enzymes	Distributor	Catalog number	Restriction sites
Mse I	New England BioLabs	#R0525	5´ ... T^T A A ... 3´ 3´ ... A A T^T ... 5´
*Bsa*J I	New England BioLabs	#R0536	5´ ... C^C N N G G ... 3´ 3´ ... G G N N C^C ... 5´
Bsr I	New England BioLabs	# R0527	5´ ... A C T G G N^ ... 3´ 3´ ... T G A C^C N ... 5´
Taq I	New England BioLabs	#R0149	5´ ... T^C G A ... 3´ 3´ ... A G C^T ... 5´
Ssp I	New England BioLabs	#R0132	5´ ... A A T^A T T ... 3´ 3´ ... T T A^T A A ... 5´
Bsl I	New England BioLabs	#R0555	5´ ... C C N N N N N^N N G G ... 3´ 3´ ... G G N N^N N N N N C C ... 5´
Rsa I	New England BioLabs	#R0167	5´ ... G T^A C ... 3´ 3´ ... C A^T G ... 5´

identification of the species. Besides specimens given in Table 1, we also used the cyt*b* gene sequences of *A. transmontanus*, *Scaphirhynchus albus*, *S. platorhynchus* and *S. suttkusi* from GenBank (accession numbers AF184107, X14944, U55994, U56983–56988, respectively).

3. Results

3.1. The Results of Sequencing

The amplification products obtained using flanking tRNA primers L14735 and cyt*b*.rev1 consisted of 1221 bp. Each copy included the entire cyt*b* gene, a 34 bp-long fragment (including the primer sequence) of the $tRNA^{Glu}$ gene, and a 46 bp-long fragment of the $tRNA^{Thr}$ gene (including the primer sequence). The structure of the PCR product is given in detail in LUDWIG *et al.* (2001).

3.2. Finding Diagnostic Restriction Sites and the Restriction Analysis

The cyt*b* gene sequences were subjected to WEBCUTTER V. 2.0 to identify the potential species-specific nucleotide changes. Such changes can result in obtaining or loosing diagnostic restriction sites and, therefore, in different restriction patterns characterizing the investigated species. Because of a possibility of intraspecific nucleotide variation, several rounds of restriction screening, *i.e.* amplification, enzyme digestion and the analysis of the RFLP patterns were performed using additional specimens for each species. For example, based on the sequence data for *A. baeri,* this species can be distinguished from *A. gueldenstaedti*, *A. naccarii* and *A. persicus* due to the A → G transition, which occurred in the diagnostic nucleotide position 916 of the cyt*b* gene (Table 3). This non-silent point mutation (resulting in an amino acid exchange) created the palindromic sequence AGTACT that is recognized by *Sca* I (#R0138, New England BioLabs). The other three species had the sequence AATACT, an uncleavable motif for this restriction endonuclease.

However, among 126 individuals of *A. baeri* studied, two fish were not identified using this enzyme. They showed the same restriction pattern as obtained for *A. gueldenstaedti*, *A. naccarii*, and *A. persicus*. Sequence analyses of amplification products from both specimens revealed a silent A → G transition, which occurred in the nucleotide position 918 within the cyt*b* gene (haplotype Aba2, Table 3). This mutation resulted in a loss of the *Sca* I restriction site due to the change of the hexanucleotide sequence AGTACT into AGTGCT. We concluded that *Sca* I was unsuitable for the identification of *A. baeri* and we did not use it for this species. Through the similar procedure we found that *Bsr* I should be used for the differentiation of *A. baeri* from the species complex of closely related species *A. gueldenstaedti*, *A. naccarii*, and *A. persicus* (see Fig. 1).

Our experiments showed that it was not possible to identify all studied species using one restriction endonuclease. Depending on a species, from one to four different enzymes are necessary for the identification. Overall, we were able to identify most of the acipenseriform species on the basis of characteristic species-specific restriction patterns using seven different restriction endonucleases (Table 2).

We were able to identify *A. oxyrhinchus*, *A. ruthenus*, *A. stellatus*, *A. sturio*, *H. huso*, three species belonging to the genus *Scaphirhynchus,* and *Polyodon spathula* by characteristic *Mse* I restriction band patterns. Also, by using this enzyme we could differentiate two species groups from each other: (1) *A. baeri*, *A. brevirostrum*, *A. gueldenstaedti*, *A. fulvescens*, *A. naccarii*, *A. nudiventris,* and *A. persicus*, and (2) *A. medirostris*, *A. mikadoi*, *A. transmontanus*, *A. schrencki*, *A. sinensis,* and *H. dauricus*.

Table 3. Haplotypes (HP) of *Acipenser gueldenstaedti* (Agu1–4), *A. persicus* (Ape1–3), *A. naccarii* (Ana Buna River), *A. baeri* (Aba Lena River), *A. baeri*-haplotypes H4–6 (Volga River).

HP	Nucleotide position in bp of the entire cyt*b*																												
	195	196	306	318	342	345	393	429	483	498	562	648	654	685	690	693	705	735	831	840	891	893	898	912	916	918	1053	1056	1058	1125
Agu 1	T	G	A	G	T	C	T	A	A	T	G	T	A	C	G	G	G	C	C	T	T	T	G	A	A	A	A	G	T	A
Agu 2	T	G	A	A	T	C	T	A	A	T	G	T	A	C	G	G	G	C	C	T	T	T	G	A	A	A	A	G	T	A
Agu 3	C	G	A	A	T	C	C	G	A	C	G	T	A	C	G	G	G	C	C	C	T	T	G	A	A	A	G	G	T	A
Agu 4	T	G	A	A	C	C	T	A	G	T	G	T	A	C	G	G	A	C	C	T	C	C	G	A	A	A	A	G	T	A
Ape 1	T	G	A	A	T	C	T	A	A	C	G	T	A	C	G	G	G	C	C	T	T	T	G	A	A	A	A	A	C	A
Ape 2	T	G	A	A	T	C	T	A	A	C	G	T	A	C	G	G	G	C	C	T	T	T	G	A	A	A	A	A	T	A
Ape 3	T	G	A	A	T	C	T	A	A	C	G	T	T	C	G	G	G	C	T	T	T	T	G	A	A	A	A	G	T	A
AnaB	T	G	A	A	T	T	T	A	A	T	G	T	A	C	G	G	G	C	C	T	T	T	G	A	A	A	A	G	T	A
AbaH4	T	A	G	A	C	C	T	A	A	C	G	C	A	T	A	A	G	T	C	T	T	T	A	G	G	A	A	G	T	A
H5	T	A	G	A	C	C	T	A	A	C	G	C	A	T	A	A	G	T	C	T	T	T	A	G	G	G	A	G	T	A
H6	T	A	G	A	C	C	T	A	A	C	G	C	A	T	A	A	G	T	C	T	T	T	A	G	G	A	A	G	T	G

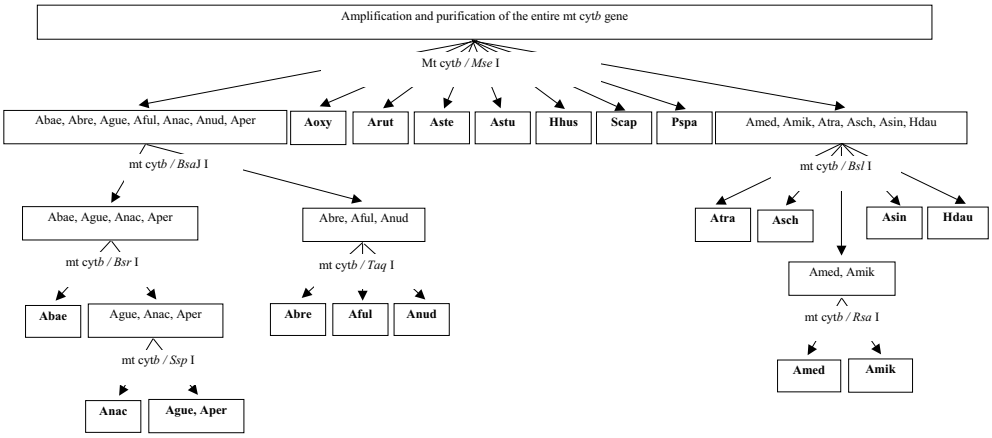

Figure 1. Protocol of species-identification using PCR-RFLP analyses (*Acipenser baeri* – Abae, *A. brevirostrum* – Abre, *A. fulvescens* – Aful, *A. gueldenstaedti* – Ague, *A. medirostris* – Amed, *A. mikadoi* – Amik, *A. naccarii* – Anac, *A. nudiventris* – Anud, *A. oxyrhinchus* – Aoxy, *A. persicus* – Aper, *A. ruthenus* – Arut, *A. schrencki* – Asch, *A. sinensis* – Asin, *A. stellatus* – Aste, *A. sturio* – Astu, *A. transmontanus* – Atra, *Huso huso* – Hhus, *H. dauricus* – Hdau, genus *Scaphirhynchus* – Scap and *Polyodon spathula* – Pspa). The names of finally identified species are shown in bold

Three species of the genus *Scaphirhynchus*, *S. albus*, *S. platorhynchus*, and *S. suttkusi,* showed no species-specific nucleotides in their cyt*b* genes (Table 4). Therefore, they could not be differentiated using our approach. The same was true in the case of *A. gueldenstaedti* and *A. persicus* (Table 3).

We separated the complex of species consisting of *A. baeri*, *A. gueldenstaedti*, *A. naccarii*, and *A. persicus*, and the complex of *A. brevirostrum*, *A. fulvescens*, and *A. nudiventris*, by using *Bsa*J I. After this *A. baeri* can be identified by using *Bsr* I, and *A. naccarii*, by using *Ssp* I. The application of *Taq* I produced species-specific characteristic restriction band patterns for *A. brevirostrum*, *A. fulvescens*, and *A. nudiventris*, and the application of *Bsl* I revealed the species-specific restriction band patterns for *A. transmontanus*, *A. schrencki*, *A. sinensis*, and *H. dauricus*. Finally, we used *Rsa* I to discriminate *A. medirostris* from *A. mikadoi.*

Table 4. Haplotypes (HP) of *Scaphirhynchus albus* (Sal 1–4), *S. platorhynchus* (Spl SD2, H2, H1, SD1) and *S. suttkusi* (Ssu A 1–3).

HP	Nucleotide position in cyt*b*						
	87	111	136	303	597	891	1125
Sal 1–2	A	A	T	C	C	C	A
Sal 3–4	G	A	T	T	T	T	A
Spl SD2	G	A	T	T	T	T	G
Spl H2	G	A	T	C	T	T	A
Spl H1	G	G	C	T	T	T	A
Spl SD1	G	A	T	T	T	T	A
Ssu A1–3	G	A	T	T	T	T	A

Table 5. The length of restriction fragments of amplification products subjected to different enzymes for 17 acipenseriform species. The total length of amplification products – including entire cyt*b* and additional 80 bp of related tRNA genes – before restriction was 1222 bp in *Acipenser medirostris*, *A. mikadoi*, and *A. nudiventris*, and 1221 bp in all other species studied.

Species	Restriction enzyme						
	Mse I	*Bsl* I	*Rsa* I	*Bsa*J I	*Bsr* I	*Taq* I	*Ssp* I
Genus *Polyodon*							
P. spathula	9, 66, 177, 413, 556						
Genus *Acipenser*							
A. baeri	9, 58, 66, 84, 414, 590			142, 207, 233, 639	81, 111, 112, 951		
A. brevirostrum	9, 58, 66, 84, 414, 590			142, 440, 639		no RS	
A. fulvescens	9, 58, 66, 84, 414, 590			142, 440, 639		213, 1008	
A. guelden-staedti	9, 58, 66, 84, 414, 590			142, 207, 233, 639	81, 112, 1062		275, 284, 662
A. medirostris	9, 58, 67, 84, 345, 659	69, 81, 130, 175, 327, 440	39, 55, 90, 112, 185, 319, 422				
A. mikadoi	9, 58, 67, 84, 345, 659	69, 81, 130, 175, 327, 440	39, 90, 112, 185, 319, 477				
A. naccarii	9, 58, 66, 84, 414, 590			142, 207, 233, 639	81, 112, 1062		99, 275, 284, 563
A. nudiventris	9, 58, 67, 84, 414, 590			143, 440, 639		158, 1064	
A. oxyrhinchus	66, 104, 222, 339, 570						
A. persicus	9, 58, 66, 84, 414, 590			142, 207, 233, 639	289, 932		275, 284, 662
A. ruthenus	9, 42, 58, 66, 191, 399, 456						
A. schrencki	9, 58, 66, 84, 345, 659	68, 88, 130, 441, 494					
A. sinensis	9, 58, 66, 84, 345, 659	88, 109, 430, 494					
A. stellatus	9, 58, 66, 84, 1004						
A. sturio	95, 222, 909						
A. trans-montanus	9, 58, 66, 84, 345, 659	88, 130, 494, 509					
Genus *Huso*							
H. huso	9, 58, 66, 84, 95, 414, 495						
H. dauricus	9, 58, 66, 84, 345, 659	88, 130, 175, 319, 509					
Genus *Scaphirhynchus*	9, 58, 66, 84, 95, 909						

A summarizing overview of different restriction endonucleases for sturgeon species identification is presented in Figure 1. The length of DNA fragments obtained by enzyme digestion for each species is given in Table 5.

4. Discussion

Black caviar is one of the most valuable products derived from aquatic organisms. The three sturgeon species *A. gueldenstaedti*, *A. stellatus*, and *H. huso* have been traditionally used for caviar production. So far, this delicacy originates mainly from sturgeons caught in the Caspian Sea and its tributaries, esp. the Volga River. Also, the Sea of Azov and the Black Sea and their tributaries are important, but production of black caviar in these areas is lower than in the Caspian Sea basin (TAYLOR, 1997). Recently, fish farms started to raise in captivity two other sturgeon species, *A. baeri* and *A. transmontanus*, with the goal to produce caviar from aquacultured sturgeons (HJUL, 1996; ANONYMOUS, 1997; NEGRONI, 1999).

Due to the drastic decline in wild sturgeon populations caused by anthropogenic factors, the caviar production from wild sturgeons decreased during the 1990s. Unfortunately, the continuing high demand for caviar on the international market resulted in an exploitation of other endangered sturgeon species. Additionally, the price for caviar from different species differs considerably. Lots of mislabeled caviar has already been found on the market (DE SALLE and BIRSTEIN, 1996; COHEN, 1997; BIRSTEIN *et al*., 1998, 1999; WOLF *et al*., 1999). Therefore, the identification of species origin of black caviar is an essential prerequisite for the protection of caviar trade and consumers against deceits, as well as for conservation programs.

4.1. Advantages and Disadvantages of Different Caviar Identification Techniques

In the 1980s, isoelectric focusing (IEF) of caviar proteins was introduced as a technique for species identification of black caviar (REHBEIN, 1985; KEYVANFAR *et al*., 1988; CHEN *et al*., 1996). These studies included the three commercial sturgeon species and up to three additional sturgeon species.

In contrast to morphological and organoleptic characteristics previously used by traders, the results of IEF analyses were objective and other persons than well-trained caviar experts could identify the species origin of caviar. However, the application of IEF had several disadvantages. First, a comparison of roe from practically all extant sturgeon and paddlefish species should have been carried out to ensure species-specific patterns of proteins on electropherograms. Second, the inclusion of reference samples is necessary on the same gel. Third, the method allowed researchers to examine only big quantities of eggs. However, a possibility exists that a caviar sample can contain a mixture of eggs from different sturgeon species. Therefore, a method of species identification should be sensitive enough to work with a single caviar egg.

In the 1990s, mtDNA-based techniques were developed. One method relied on interspecific conserved nucleotide differences (diagnostic nucleotides) within the cyt*b* gene sequences (DESALLE and BIRSTEIN, 1996; BIRSTEIN *et al.*, 1998). For each species, one or two sets of two primers created for diagnostic nucleotides within the cyt*b* gene were used. In these studies, the occurrence of an amplified PCR-product obtained with a species-specific primer pair showed the species origin of black caviar. The method was developed to identify single eggs only of the most important caviar producers, *H. huso*, *A. gueldenstaedti* and *A. stellatus* (DESALLE and BIRSTEIN, 1996). It was critical for the support of the CITES listing of sturgeons (BIRSTEIN, 1999).

Another method, the so-called PCR-SSCP, was based on visualized interspecific nucleotide differences within an amplified part of the cyt*b* gene by using native polyacrylamide gel electrophoresis (REHBEIN, 1997). The main disadvantage of the PCR-SSCP method was that the intraspecific nucleotide variation (Table 3) causes a variety of banding patterns for individuals of the same species.

We based our approach on the third, the PCR-RFLP method originally introduced for sturgeon identification by WOLF *et al.* (1999). In general, the PCR-RFLP method has many advantages: one universal primer pair is needed and no reference specimens are necessary after establishing the specific restriction patterns. However, WOLF *et al.* (1999) used a limited number of species and specimens. In the meantime, BIRSTEIN *et al.* (1998, 2000) showed that single specimens do not represent the entire intraspecific variability. Furthermore, other sturgeon species not investigated by WOLF *et al.* (1999) could potentially exhibit a restriction pattern indistinguishable from that they analyzed. Therefore, misidentification can occur if to use this method without an additional research.

To overcome these limitations, we generated and analyzed the entire cyt*b* gene sequences from 858 specimens representing 22 acipenseriform species (Table 1). Due to the extremely threatened status of the remaining extant acipenseriform species *A. dabryanus* (family Acipenseridae, subfamily Acipenserinae), *Pseudoscaphirhynchus fedtschenkoi*, *P. hermanni*, *P. kaufmanni* (family Acipenseridae, subfamily Scaphirhynchinae), and *Psephurus gladius* (family Polyodontidae, genus *Psephurus*) (BIRSTEIN *et al.*, 1997a), no tissue samples could be obtained from individuals of these taxa. However, their threatened status makes practically impossible to meet a caviar sample from these species in the international caviar trade. Except for the species pair *A. gueldenstaedti*/*A. persicus* and the three *Scaphirhynchus* species, we found species-specific conserved nucleotide differences suitable for the identification of 17 acipenseriform species (Table 5; Fig. 1), which are the most important for caviar production.

4.2. Intra- and Interspecific Variation

Although the cyt*b* gene is rather conservative, the intraspecific variation was found within it in some fishes (MEYER, 1993). During the search for restriction sites, we observed the intraspecific nucleotide variation within this gene in sturgeons. The existence of several haplotypes was discovered as a non-homogenous banding pattern, and in such cases we did not use the particular enzyme for further analysis. For instance, variable nucleotide positions were observed within the entire cyt*b* gene in four closely species, *A. baeri*, *A. gueldenstaedti*, *A. naccarii* and *A. persicus* (Table 3). In most cases the diagnostic, *i.e.*, conserved nucleotide differences were useful for a PCR-RFLP-based discrimination of species.

However, we cannot exclude the possibility of single haplotypes unrecognized so far which might occur at a low frequency in wild populations. The ongoing process of population decline in all sturgeon species reduce the genetic variability within each population and lead to a fixation of single haplotypes as demonstrated for *A. sturio* (LUDWIG *et al.*, 2000b). In our study, new intraspecific nucleotide changes sometimes resulted in obtaining or loss of at least one restriction site. This caused a creation of a restriction pattern, which differed from that of individuals of the same or other species.

As mentioned above, we could not discriminate *A. gueldenstaedti* from *A. persicus* using our PCR-RFLP approach, as well as the three *Scaphirhynchus* species from each other, due to the lack of interspecific nucleotide differences (Table 3, 4). The similarity within each of the two groups might be due to recent speciation events, which have not changed the slowly evolving cyt*b* gene yet. However, within a part of the D-loop, which is the fastest evolving mtDNA region (MEYER, 1993), CAMPTON *et al.* (2000) observed no fixed nucleotide substitutions in *S. albus* and *S. platorhynchus*, while *S. suttkusi* differed from the other

two species by a single base-pair substitution. The authors concluded that the low genetic divergence reflected a level of isolated populations or subspecies rather than a species-specific level. Similarly, our results and the studies of BISCHOF and SZALANSKI (2000) did not support the species separation within the genus *Scaphirhynchus*.

The validity of *A. persicus* as a distinct species is discussed likewise controversial in the literature (BIRSTEIN and BEMIS, 1997). *Acipenser persicus* was described as a separate species by BORODIN (1897), but it was later considered as a sub-species of *A. gueldenstaedti, A. gueldenstaedti persicus* by BERG (1948). VLASENKO *et al.* (1989) raised it to the species rank again. In our study, *A. persicus* had no species-characteristic diagnostic nucleotide substitution in the cyt*b* gene. This finding is supported by previous data (JENNECKENS, 1999; LUDWIG *et al*., 2000). BIRSTEIN (1999) and BIRSTEIN *et al.* (2000) also did not find differences between these species while comparing partial sequences of the cyt*b* and ND5 genes, and of the D-loop. They concluded that *A. persicus* should not be considered as a separate species, but as a subspecies *A. gueldenstaedti persicus.*

Evidently, a critical re-evaluation of the species classification of *A. persicus* is needed. Both *A. gueldenstaedti* and *A. persicus* exhibit striking morphological similarities, and live in the overlapping areas of the Caspian Sea. Arising questions like 'How many species are there within the genus *Acipenser*?' (BIRSTEIN and BEMIS, 1997) manifest problems the sturgeon biologists are confronted with. In the future, the development of nuclear DNA markers may help to make a clear conclusion on the taxonomic status of *A. persicus.*

Caviar from both of these species is traded under the same name "osetra." However, trading laws were changed in 2001: an exact species declaration is necessary now (SPROTTE, personal communication, 2001). Evidently, this formal change is not supported by the current molecular data, and therefore, discrimination between *A. gueldenstaedti* and *A. persicus* using interspecific substitutions is still not possible.

4.3. Conservation Aspects

Identification of black caviar is important not only from the economical point of view. The accurate species identification is an essential prerequisite for fishery management and conservation programs. Possibly, the identification of mislabeled black caviar lots can help indirectly to protect endangered sturgeon species from being used for caviar production. If such mislabeled lots are constantly excluded from the market, this will probably result in a decrease in poaching.

Due to the drastic decline of sturgeon stocks, sturgeon hatcheries were built in the Caspian Sea and Sea of Azov basin in the 1960s–70s (BARANNIKOVA, 1988; KHODOREVSKAYA *et al.*, 1997). However, the reproduction in captivity might cause a lot of problems: inbreeding, a loss of genetic variability, swamping of indigenous gene pools by introgression of homogenous stocks, or outbreeding depression (LARGIADER and SCHOLL, 1995). Furthermore, deliberate transfers of sturgeon species into the new habitat can have a negative ecological impact on the native flora and fauna, such as the transfer of parasites to which the native sturgeon species are not adapted (SOKOLOV and VASIL'EV, 1989).

Unfortunately, the history of sturgeon management is a story of ongoing stock-mixings and introductions of non-native stocks and/or species (see for example population history of *A. oxyrhinchus*, WALDMAN, 2000). However, interspecific hybridization, esp. the release or even the escape of artificially produced hybrids, can result in a loss of genetic variability or locally adapted gene complexes. Consequently, the interspecific hybridization has become an increasingly important issue in conservation biology. Although the mtDNA markers display maternal haplotypes only, they can be used as a preliminary characteristic of the stock structure and genetic diversity.

4.4. The Problem of Hybridization

MtDNA is known to be inherited maternally, and, therefore, the mtDNA-based methods can identify only the maternal species origin of eggs used for caviar. However, the interspecific hybridization between sturgeon species occurs naturally at a low frequency (HOLCIK, 1989; BIRSTEIN *et al*., 1997b). Besides, intensive artificial hybridization efforts have been carried out in the former Soviet Union (AREFJEV, 1997). A high level of viability and fertility characterizes several artificially obtained types of sturgeon hybrids. Therefore, potentially caviar can be produced from some of the hybrids. For the identification of hybrids, besides mtDNA-methods, it is necessary to have nuclear DNA markers to identify the parental genome.

So far, only one nuclear marker was available for the identification of one of the black caviar producers, *A. stellatus* (JENNECKENS *et al.*, 2001). Additional studies of seven microsatellite loci showed a diagnostic band pattern for different numbers of ploidy found in different species of sturgeons (LUDWIG *et al*., 2001). Hybrids between species of such different ploidy levels can be identified easily using microsatellites. For instance, by combining nuclear (microsatellites) and mitochondrial (cyt*b*) markers (JENNECKENS, 1999; JENNECKENS *et al.*, 2001; LUDWIG *et al.*, 2001), we were able to identify the following natural hybrids: one *A. gueldenstaedti*/*A. stellatus* hybrid, one *A. gueldenstaedti*/*A. ruthenus* hybrid, as well as five hybrids between *A. gueldenstaedti* or *A. persicus* and *A. nudiventris*. Using the cyt*b* sequences, in all these cases we identified *A. gueldenstaedti* or *A. persicus* as the maternal species. All specimens had triploid band patterns at several microsatellite loci. The *A. gueldenstaedti*/*A. stellatus* and the *A. gueldenstaedti*/*A. ruthenus* hybrids came from the Volga River, and the *A. nudiventris*-hybrids were caught in the Iranian waters of the Caspian Sea near the mouth of the Safid Rud River.

Additionally, eleven individuals of thirty-four sturgeons caught in the Volga River, which were classified morphologically as *A. gueldenstaedti,* had identical or a very similar RFLP-band pattern to *A. baeri* from the Lena River, Siberia. The sequence analysis supported this finding (BIRSTEIN *et al.*, 2000; JENNECKENS *et al.*, 2000). The reasons for this high level of similarity between haplotypes of both species were discussed in BIRSTEIN *et al.* (2000) and JENNECKENS *et al.* (2000).

Despite the potential problem of hybridization, at present the mtDNA markers are the best tools for caviar species identification. Future studies should be focused on the development of nuclear markers. The combination of the mtDNA and nuclear markers will allow researchers to obtain the maximum accuracy in the discrimination of acipenseriform species.

5. Acknowledgement

The authors thank numerous colleagues, staff members and professional fishermen from Albania, Canada, France, Georgia, Germany, Iran, Netherlands, Romania, Russia, and USA game fish agencies for logistic support, obtaining specimens, and permitting catches during protected spawning periods (P. ANDERS, E. N. ARTYUKHIN, V. BIRSTEIN, H. BOLIG, J. FLETCHER, J. GESSNER, O. GOEMANN, R. GROSS, T. GULYAS, H. KINCAID, F. KIRSCHBAUM, S. LAPAN, M. LEPAGE, B. MAY, N. PATRICHE, M. POURKAZEMI, E. ROCHARD, V. SVIRSKY, C. TRACY, P. WILLIOT and S.-M. ZHANG). Special thanks to V. BIRSTEIN and his colleagues for their previous work on sturgeon conservation. These studies were an essential prerequisite for our work and each conservation program. Our studies were possible only with grants from the DFG (KI 189/11-1 and ME 712/12) and the Federal Agency for Nature Conservation and the Ministry of Environment, Nature Conservation and Nuclear Safety, Germany (FKZ-No. 808 05 078).

6. References

ANONYMOUS, 1997: California joint venture project will produce sturgeon caviar. – Fish Farm. Int. **24**: 6p.

AREFJEV, V. A., 1997: Sturgeons hybrids: natural reality and practical prospects. – Aquacult. Mag. **7/8**: 52–58.

BARANNIKOVA, I. A., 1988: Review of sturgeon farming in the Soviet Union. – J. Ichthyol. **27**: 62–71.

BERG, L. S., 1948: The Freshwater Fishes of the USSR and Adjacent Countries, Vol. 1. Akademia Nauk USSR, Moscow and Leningrad. Part 1 (in Russian, English translation published by Israel Program for Scientific Translations, Jerusalem. 505 pp.).

BIRSTEIN, V. J., 1999: New perspectives for monitoring migratory animals – improving knowledge for conservation. – Env. Policy and Law **29**: 235–238.

BIRSTEIN, V. J., 2000: Sturgeons and paddlefishes. – *In*: READING, R. P. and B. MILLER (eds): Endangered Animals. A Reference Guide to Conflicting Issues, Greenwood Press, Westpoint, pp. 269–278.

BIRSTEIN, V. J. and W. E. BEMIS, 1997: How many species are there within the genus *Acipenser*? – *In*: BIRSTEIN, V. J., J. R. WALDMAN and W. E. BEMIS (eds.): Sturgeon Biodiversity and Conservation, Kluwer Academic Publishing, Dordrecht, pp. 157–163.

BIRSTEIN, V. J., W. E. BEMIS and J. R. WALDMAN, 1997a: The threatened status of acipenseriform fishes: A summary. – *In*: BIRSTEIN, V. J., J. R. WALDMAN and W. E. BEMIS (eds.): Sturgeon Biodiversity and Conservation, Kluwer Academic Publishing, Dordrecht, pp. 437–444.

BIRSTEIN, V. J., R. HANNER and R. DESALLE, 1997b: Phylogeny of Acipenseriformes cytogenetic and molecular approaches. – *In*: BIRSTEIN, V. J., J. R. WALDMAN and W. E. BEMIS (eds.): Sturgeon Biodiversity and Conservation, Kluwer Academic Publishing, Dordrecht, pp. 127–155.

BIRSTEIN, V. J. and R. DESALLE, 1998: Molecular phylogeny of Acipenserinae. – Mol. Phylogen. Evol. **9**: 141–155.

BIRSTEIN, V. J., P. DOUKAKIS, B. SORKIN, and R. DESALLE, 1998: Population aggregation analysis of three caviar-producing species of sturgeons and implications for the species identification of black caviar. – Cons. Biol. **12**: 766–775.

BIRSTEIN, V. J., P. DOUKAKIS, and R. DESALLE, 1999: Molecular phylogeny of Acipenserinae and black caviar species identification. – J. Appl. Ichthyol. **15**: 12–16.

BIRSTEIN, V. J., P. DOUKAKIS, and R. DESALLE, 2000: Polyphyly of mtDNA lineages in the Russian sturgeon, *Acipenser gueldenstaedti*: forensic and evolutionary implications. – Cons. Gen. **1**: 81–88.

BISCHOF, R. and A. L. SZALANSKI, 2000: Mitochondrial DNA variation in pallid and shovelnose sturgeons, *Scaphirhynchus* (Pisces: Acipenseridae) – Tran. Neb. Aca. Sci. **26**: 19–21.

BORODIN, N., 1897: A report about a summer 1895 zoological expedition on the board of the kruizer Uralets in the northern part of the Caspian Sea. – Vestnik Rybopromyshlennosti, Rubovodstva i Rybolovstva **12**: 1–31.

CAMPTON, D. E., A. L. BASS, A. CHAPMAN, and B. W. BOWEN, 2000: Genetic distinction of pallid, shovelnose, and Alabama sturgeon: emerging species and the US Endangered Species Act. – Cons. Genet **1**: 17–32.

CHEN, I.-C., F. A. CHAPMAN, C. I. WEI, and S. F. O'KEEFE, 1996: Preliminary studies on SDS-PAGE and isoelectric focusing identification of sturgeon sources of caviar. – J. Food Sci. **61**: 533–359.

COHEN, A., 1997: Sturgeon poaching and black caviar market: a case study. – *In*: BIRSTEIN, V. J., J. R. WALDMAN and W. E. BEMIS (eds.): Sturgeon Biodiversity and Conservation, Kluwer Academic Publishing, Dordrecht, pp. 1–38.

DESALLE, R. and V. J. BIRSTEIN, 1996: PCR identification of black caviar. – Nature **381**: 197–198.

HOLCIK, J., 1989: General introduction to fishes. Acipenseriformes. – *In*: J. HOLCIK (ed.): The freshwater fishes of Europe (Vol. 1, Part II), Aula-Verlag, Wiesbaden. 1–469 pp.

HJUL, P., 1996: French farms are to market fish of kings. – Seaf. Int. **5**: 60–61.

JENNECKENS, I., 1999: Investigations on biochemical and molecular markers on their suitability in the identification of sturgeons and their hybrids. PhD-thesis, Universität Göttingen.

JENNECKENS, I., J. N. MEYER, L. DEBUS, C. PITRA, and A. LUDWIG, 2000: Evidence of mitochondrial DNA clones of Siberian sturgeon, *Acipenser baeri*, within Russian sturgeon, *Acipenser gueldenstaedti*, caught in the River Volga. – Ecol. Let. **3**: 503–508.

JENNECKENS, I., J.-N. MEYER, G. HÖRSTGEN-SCHWARK, B. MAY, L. DEBUS, H. WEDEKIND, and A. LUDWIG, 2001: A fixed allele at microsatellite locus LS-39 exhibiting species-specificity for the black caviar producer *Acipenser stellatus*. – J. Appl. Ichthyol. **17**: 39–42.

KEYVANFAR, A., D. ROCHU and J. M. FINE, 1988: Comparative study of sturgeon oocyte soluble proteins by isoelectric focusing. – Comp. Biochem. Physiol. B **90**: 393–396.

KHODOREVSKAYA, R. P., G. F. DOVGOPOL, O. L. ZHURAVLEVA and A. D. VLASENKO, 1997: Present status of commercial stocks of sturgeons in the Caspian Sea basin. – *In:* BIRSTEIN, V. J., J. R. WALDMAN and W. E. BEMIS (eds.) Sturgeon Biodiversity and Conservation, Kluwer Academic Publishing, Dordrecht, pp. 209–219.

LARGIADER, C. R. and A. SCHOLL, 1995: Effects of stocking on the genetic diversity of brown trout populations of the Adriatic and Danubian drainages in Switzerland. – J. Fish Biol. **47**: 209–225.

LUDWIG, A. and F. KIRSCHBAUM, 1998: Comparison of mitochondrial sequences of the Adriatic and the European sturgeon. – J. Fish Biol. **52**: 1289–1291.

LUDWIG, A., B. MAY, L. DEBUS and I. JENNECKENS, 2000a: Heteroplasmy in the mtDNA control region of sturgeon (*Acipenser*, *Huso* and *Scaphirhynchus*). – Genetics **156**: 1933–1947.

LUDWIG, A., I. JENNECKENS, L. DEBUS, A. LUDWIG, J. BECKER and F. KIRSCHBAUM, 2000b: Genetic analyses of archival specimens of the European sturgeon – *A. sturio*. Proceedings of the Symposium on Conservation of the Atlantic Sturgeon *Acipenser sturio* L., 1758 in Europe – Boletin Instituto Espanol de Oceanografia **16**: 181–190.

LUDWIG, A., N. M. BELFIORE, C. PITRA, V. SVIRSKY and I. JENNECKENS, 2001: Genome duplication events and functional reduction of ploidy levels in sturgeon (*Acipenser*, *Huso* and *Scaphirhynchus*). – Genetics **158**: 1203–1215.

MEYER, A., 1993: Evolution of mitochondrial DNA in fishes. – *In*: HOCHACHKA, P. W. and T. P. MOMMSEN (eds.): The biochemistry and molecular biology of fishes (Vol. 2), Elsevier, Amsterdam, pp. 1–38.

NEGRONI, G., 1999: Caviar set to boom – stock scarcity sees price rocket. – Fish Farm Int. **26**: 20.

RAYMAKERS, C., 2001: International trade in sturgeon and paddlefish species – the effect of CITES listing. – International Review of Hydrobiology **87**: 525–537.

REHBEIN, H., 1985: Caviare: proximate composition, amino acid content and identification of fish species. – Z. Lebensm. Unters. Forsch. **180**: 457–462.

REHBEIN, H., 1997: Fischartbestimmung von Caviar durch Protein- und DNA-Analyse. – Inf. Fischwirtsch. **44**: 27–30.

SOKOLOV, L. I. and V. P. VASIL'EV, 1989: *Acipenser nudiventris* LOVETSKY 1828. – *In:* HOLCIK, J. (ed.) The Freshwater Fishes of Europe. General Introduction to Fishes. Acipenseriformes. (Vol. 1, Part II). AULA-Verlag, Wiesbaden, pp. 206–226.

TAYLOR, S., 1997: The historical development of the caviar trade and the caviar industry. – *In:* BIRSTEIN, V. J., A. BAUER and A. KAISER-POHLMANN (eds.) Sturgeon stocks and caviar trade workshop. – Proceedings of a workshop. Gland-Switzerland, IUCN 1997 (Vol. 17), pp. 45–53.

VLASENKO, A. D., A. V. PAVLOV and V. P. VASIL'EV, 1989: *Acipenser persicus* BORODIN, 1897. – *In:* HOLCIK, J. (ed.) The Freshwater Fishes of Europe. General Introduction to Fishes. Acipenseriformes. (Vol. 1, Part II). AULA-Verlag, Wiesbaden, pp. 345–366.

WALDMAN, J. R., 2000: Restoring *Acipenser sturio* L., 1758 in Europe: Lessons from the *Acipenser oxyrhinchus* MITCHILL, 1815 experience in North America. – Proceedings of the Symposium on Conservation of the Atlantic Sturgeon *Acipenser sturio* L., 1758 in Europe – Boletin Instituto Espanol de Oceanografia **16**: 237–244.

WOLF, C., P. HÜBNER and J. LÜTHY, 1999: Differentiation of sturgeon species by PCR-RFLP. – Food Res. Int. **32**: 699–705.

Internat. Rev. Hydrobiol.	**87**	2002	5–6	675

ERRATUM

to **Vol. 87 (4), p. 461** in **RADZIEJEWSKA, T., Responses of Deep-Sea Meiobenthic Communities to Sediment Disturbance Simulating Effects of Polymetallic Nodule Mining**

Figure 2 and its caption should appear as follows:

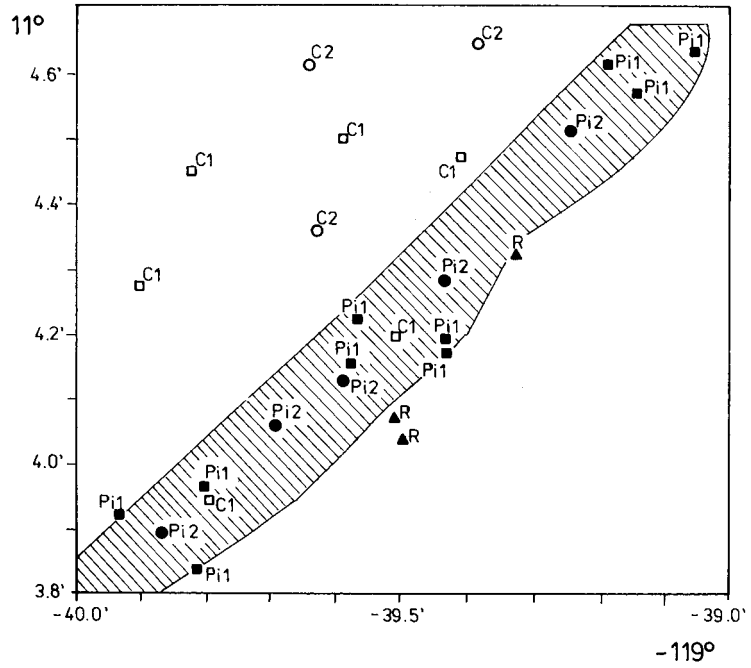

Figure 2. Layout of the IOM BIE test site with Disturber towing zone hatched (modified from KOTLINSKI and STOYANOVA, 1998): multiple corer sampling sites: open squares = intact sites (C1, *Pre-disturbance* stage) (cores MC1–MC6); solid squares = impacted zone sites (Pi1, *Post-disturbance* 1 stage) (cores MC7–MC16); open circles = control sites (C2, *Post-disturbance* 2 stage) (cores MO1–MO3); solid circles = impacted zone sites (Pi2, *Post-disturbance* 2 stage) (cores MO4, MO7, MO8, MO10, MO11); solid triangles = resedimentation area sites (R, *Post-disturbance* 2 stage) (cores MO5, MO6, MO9)

AHCBAU 30 (2-3) 69–164
(2002)
ISSN 0323-4320 · Vol. 30
No. 2-3 · October 2002

Acta hydrochimica et hydrobiologica

Contents