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Identification of caviar from increasing global aquaculture production – Dietary capric acid as a labelling tool for CITES implementation in caviar trade

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ABSTRACT

Sturgeon (Acipenseridae) have been subjected to intense fisheries pressure due to their highly-priced eggs. Despite the fact that all 27 species are highly endangered and have been listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), regulating international trade, illegal catches still continue to threaten wild populations. In trade, the product is suspected to be camouflaged as caviar from aquaculture origin. Thus, tools to discriminate wild and aquaculture products have to be developed to enforce CITES regulation and thereby allow for the development of the aquaculture industry. Here, the feasibility to use food additives, namely fatty acids and vitamin E, is assessed for labelling caviar from aquaculture origin. Farmed female sterlet (Acipenser ruthenus) were fed on a diet supplemented with alpha-tocopherol, caprylic and capric acid at 15, 4 and 20 mg/g respectively. Marker concentrations in the caviar were determined from biopsies sampled via micro-incisions over a period of 90 days (0 d, 10 d, 60 d and 90 d) and compared to a control fed on a diet supplemented with 39 mg/g sunflower oil. To address the question whether the effectiveness in using the markers is dependent on strict timing with regard to gonad development and maturation, maturing (early vitellogenesis, 4 year old fish) and mature (late vitellogenesis, stage IV-V) females were studied. The latter represent fish that were ready to be used for caviar production. Neither alpha-tocopherol nor caprylic acid accumulated in the caviar and concentrations quantified by GC–MS were below the detection limit (<0.005 mg/g). In contrast, capric acid was detected as early as 10 d after the onset of the feeding trial at concentrations between 0.01-0.03 mg/g in maturing as well as mature ovaries. Capric acid would therefore provide a cheap and easy-to-assess tool for labelling caviar from aquaculture, thereby allowing its utilization as an identification system under CITES as recently requested recently by the IUCN Sturgeon Specialist Group.

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1. Introduction

Most species of sturgeon and paddlefish (Acipenseriformes) have been heavily exploited due to their valuable caviar. Prices as high as US\$1000/kg for farmed Siberian sturgeon and even exceeding U\$2500/kg for selected products from wild origin are observed on the markets (Wuertz et al., 2007a,b). Since habitat loss, pollution and overfishing have severely reduced the stocks of all species commercially exploited, all of the 27 recognized species have been listed on the annex to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1997 thereby regulating international trade of all products. Trade control under the CITES regulations is carried out by the identification of safe catch quotas and permits for export based upon sound population assessment according

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to species and country. Despite the attempted control over caviar trade, illegal fishing continues to threaten many populations and illicit trade of mislabelled caviar is rarely uncovered (Wuertz et al., 2007a,b; Raymakers, 2006; Ludwig, 2006). Today, species discrimination is successfully performed by molecular genetic techniques (Ludwig, 2006; DeSalle and Birstein, 1996), but methods for reliable source identification are still lacking which offers potential to circumvent the control of black marketing of illegal caviar (Gessner et al., 2008).

Decreasing caviar supply from wild populations currently is met by a constant increase in aquaculture production of caviar. This tendency was illustrated most impressively by a sharp rise from 2004 to 2007, exceeding the production from wild fish for the first time in 2006 (Fig. 1). Due to the availability of the aquaculture produced caviar on a large scale, the camouflage of illegal wild product becomes easier. Therefore, the need to discriminate aquaculture from wild origin is important for the conservation of wild stocks as well as for the future development of sturgeon aquaculture. In order to fulfill the obligations originating from CITES and allow for aquaculture



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Fig. 1. International caviar trade of wild caught and aquaculture sturgeon as reported to the CITES Secretariat. Data were obtained from the UNEP-WCMC CITES trade database (checked for congruency with the export quotas reported to the CITES Secretariat). Bronzi (pers. commun) considering average retail prices of 5 US\$/kg meat and 1000 US \$/kg caviar.

development, effective identification of the source (farmed vs. wild) is essential (Gessner et al., 2008; Ludwig, 2007). Although morphologic parameters were addressed (Debus et al., 2002), chemical and biochemical markers were identified as most promising for their potential use as source identification system (Gessner et al., 2008; Wirth et al., 2000). In a first comparison of caviar originating from wild caught and intensively farmed fish, among several parameters including fatty acid composition of triglycerides (TG) and phospholipids (PL), as well as concentrations of metal ions and chlorinated hydrocarbons, fatty acid composition of TGs revealed significant differences, suggesting the potential use as markers upon oral administration (Gessner et al., 2008). From a preliminary screening of caviar (Table 1), we tested the use of three biochemical markers, alpha-tocopherol, capric and caprylic acid, normally present in the caviar at low concentration and administered here with the food in order to validate their feasibility and suitability as labelling tool for caviar originating from aquaculture production. Since sturgeon gonads consist of a portion of adipose and a portion of gametogenic tissue until adipose tissue is completely resorbed in late vitellogenic females, accumulation was assumed to be stage-dependent. Consequently, marker accumulation was studied in first maturing female sterlet *Acipenser ruthenus* as well as in females in late vitellogenesis. Late vitellogenic follicles will be referred to as caviar, although not salted here.

2. Materials and methods

2.1. Animals, sampling, histology

For each diet, five female sterlets of two different age classes (4a, 8a) representing maturing (early vitellogenesis) and mature (late vitellogenesis, stage IV-V) fish were kept under a natural photoperiod at the facilities of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries. Tanks (2.4 m³) were part of a recirculation system (water turnover 1.5 volumes/h). Basic water parameters were accessed daily $(O_2 7.8 \pm 0.5/8.1 \pm 0.48 \text{ mg/L}, \text{pH} 7.25 \pm 0.71/7, 24 \pm 0.72, 22.09 \pm 0.60/$ 22.2 ± 0.61 °C), nitrate and ammonia twice a week (NO₃⁻-N 100-300 mg/L, NH₄⁺N>0.01 mg/L). Fish were fed at 2% of fish biomass per day using a commercial trout feed (Trouvit Classic 1P, Skretting, 47% crude protein, 14% total fat, digestible energy 18.5 MJ/kg). Makers and, to provide comparable texture and fat content for the control group, sunflower oil were sprayed to the feed with a plastic liner in a climate chamber at 4 °C avoiding light exposure. The final concentration was adjusted to 1.5% (alpha-tocopherol, Sigma Aldrich), 0.4% (caprylic acid, Sigma Aldrich) and 2% (capric acid, Sigma Aldrich) or 3.9% (sunflower oil); capric acid was melted in a water bath at 32 °C prior to application.

Individuals were tagged with PIT tags (Trovan®, Germany), implanted below the second dorsal scute. For the selection of females and for successive sampling over the experimental period, fish from a 4 and 8 year age class were biopsied under narcosis (70 ppm MS-222) as described in Wuertz et al. (2006). The experiment was conducted in compliance with the institutional guidelines for the care and use of animals and the national legislation (TierSchG). Samples were taken on 0 d, 10 d, 60 d and 90 d of the experimental feeding trial. The samples were frozen at -70 °C and were stored in complete darkness. Unlike most fishes, the gonads of sturgeon, dependant of the developmental stage, comprise a germinal and a prominent adipose portion. Consequently, in maturing fish, ovarian tissue had to be separated from adipose tissue. For histological examination, samples were preserved in Bouin's fixative for 12 h, dehydrated, embedded in paraffin, cut to 5-8 µm and hematoxylin-eosin stained and staged according to the system from Le Menn and Pelissero (1991), as modified by Wuertz (2005) for sterlet. Nomenclature of cellular and extracellular layers follows the system presented by Debus et al. (2008).

Table 1

Concentration of caprylic and capric acid in the TG and PL fraction of commercial wild and farmed caviar, gonad fat and feed, considering the main commercial sturgeon species (for details on samples see Gessner et al. 2008).

Species	Source	No.	Caprylic acid [mg/g]						Capric acid [mg/g]					
			Eggs		Gonad fat		Feed		Eggs		Gonad fat		Feed	
			TG	PL	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL
H. huso	Wild	10	bdl	bdl					bdl	bdl				
A. güldenstädtii	Wild	12	bdl	bdl					bdl	bdl				
A. stellatus	Wild	5	bdl	bdl					bdl	bdl				
A. stellatus	Farmed	4	bdl	bdl					bdl	bdl				
A. baerii ^a	Farmed	6	bdl	bdl	bdl	bdl	bdl ^b	bdl ^b	bdl	bdl	bdl	bdl	bdl ^b	bdl ^b
A. baerii ^c	Farmed	12	bdl	bdl	bdl	bdl	bdl ^d	bdl ^d	bdl	bdl	bdl	bdl	bdl ^d	bdl ^d
P. spathula	Farmed	2	bdl	bdl					bdl	bdl				
P.spathula	Wild	2	bdl	bdl					bdl	bdl				

bdl – below detection limit (0.005 mg/g).

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