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12. Observations on Morphological Color Changes in Pontic Shad (*Alosa Immaculata,* Bennet 1835) during Spawning Migration in the Danube

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Abstract: The Pontic shad (*Alosa immaculata* Bennett 1835) is a Black Sea member of the Clupeidae family that migrates into the Danube via the principal Danube Delta branches and spawns in the upstream reaches of the river. Spawning migration starts as early as February and can last well into the summer, with most commercial capture occurring during peak migration in April-May. The coloration of the fish varies in both color and shade, with clearly distinguishable dark-headed and light-headed individuals depending on the fishing location. Here we draw parallels between shad head coloration and turbidity of the Danube, its delta and Black Sea waters. Further studies focused on morphological color change, as well as targeted fish sampling with corresponding turbidity measurements are needed to test this hypothesis and quantify coloration and color-change mechanisms. Ultimately, head coloration might be a useful indicator of where an individual was captured.
Keywords: shad, spawning migration, turbidity, Danube River

INTRODUCTION

The Pontic shad (*Alosa immaculata*, Bennet 1835) is an anadromous member of the Clupeidae family that leads a pelagic life in the Black Sea and migrates into large rivers of the Black Sea drainage basin to spawn (namely, the Danube, Dnieper, Don and Bug rivers). In this study we investigate phenomena observed in the population that migrates and spawns in the Danube River, namely in the shads appearing in the Danube Delta and Lower Danube up to the Iron Gates II dam at rkm 864. However, most fish are believed to spawn in the Danube sector between rkm 180 and rkm 500 (Navodaru & Waldman, 2003)

The earliest migrants start as early as February-March when water temperatures reach 5-6°C, with the peak (largest number of individuals resulting in highest commercial catch) usually occurring in April, at temperatures of 9-13°C (Nastase, Navodaru, Cernisencu, Tiganov, & Popa, 2018). The migration can extend until August. It is a commercial species, one that is highly valued as a seasonal delicacy and tied to local traditions and religious festivities at Easter time. The shad fishery is an important source of livelihood for small scale fishermen in the Danube Delta. As the spawning migration begins in spring, so also does the race to earn an income and capitalize on the river “running silver”, as an old saying goes, made famous by conservation biologist John Waldman (Waldman, 2013).

The Pontic shad used to migrate as far upstream the Bazias area at rkm 1072, which is at the Nera confluence (Bănăduc, Bănăduc, Lenhardt, & Guti, 2014; Teodorescu-Leonte et al., 1957). Two major hydropower dams constructed during the 1960s and 1970s and finally completed in 1984 shortened their historical spawning migration route by some 200 river kilometers. Nevertheless, almost four decades later the Pontic shads continue to arrive at the Iron Gates II dam every spring, and in similar formation to that observed prior to dam construction and known from commercial catch statistics in the former Yugoslavia, nowadays Serbia (Djisalov, 1969).

Fish move to the coastal areas of the Black Sea in late winter and early spring. They gather at the mouths of the Danube Delta branches, feeding and waiting for the right conditions, the combined effect of external and internal cues, to start off on their spawning migration up the Danube Delta branches and then further upstream into the Danube. The conditions that trigger the start of migration are temperature and water discharge, as well as internal cues of the individuals such as physiological state and energy reserves. Visual cues play a fundamental role in many fish behaviors such as predator avoidance and feeding, but also in directed movement (Lucas & Baras, 2001). It may also be possible that social interaction via conspecific visual communication plays a role in coordinating the start of migration waves (Leclercq, Taylor, & Migaud, 2010) as it has been shown for salmon that migratory movements are synchronized through social interaction, and not only external environmental cues (Berdahl, Westley, & Quinn, 2017).

Water turbidity and fish coloration

The turbidity of the water in Danube varies along the length of the river, depending on the amount and type of suspended sediments in the water. The main tributaries of the Danube contribute significantly to its sediment content. However, damming of tributaries as well as of the Danube itself (Iron Gates I and II dams) has altered the amount of sediments delivered to and carried by the Danube. Therefore, the sector downstream of the Iron Gates II dam is characterized by relatively clear water until rkm 637 (the confluence with Iskar) Turbidity further increases after the confluences of the Siret and Prut rivers (rkm 155 and rkm 134, respectively), due to the larger amounts of sediments they contribute, with turbidity steadily increasing downstream to the Black Sea (see Figures 2 and 3).

When freshly caught and still covered in scales, Pontic shad appear silvery-white, with a slightly darker hue along the dorsal side of the body. They have deciduous scales (FishBase System Glossary) and lose them easily and quickly after they die. In commercial catches individuals are very often almost completely bare of scales by the time they reach buyers, or in this case, researchers. The color of the bare skin in the upper (dorsal) half of the body can vary greatly, from shades of gray, to blue, green, yellow and mauve, as well as a mix of those colors. According to (Kolarov, 1991) “coloration varies from greenish yellow to blue-green dorsally and silvery white laterally.” Additionally, pinkish coloration may be observed and is assumed to have developed as a result of prolonged stay in waters with red algae *Phyllophora rubens*, found in the north-west areas of the Black Sea which comprise part of the Pontic shads’ feeding area. (Kolarov, 1991)

However, the head of the fish - which is the primary area of interest here - is not covered in scales and displays as lighter (i.e. translucent) or darker (i.e. pigmented) starting from the tip and along the dorsal length of the head. (Figure 1). Grigore Antipa, one of the earliest and most dedicated researchers of the Danube Delta ichthyofauna, (Antipa, 1905, 1909) described several variants of *Alosa pontica* (an earlier synonym for the species name), where head color was listed as one of the main identifying characters. According to Antipa, there is the “black-headed” variety (*Alosa pontica* var. *nigrescens*), and a “white-headed” variety (*Alosa pontica* var.*danubii*) of the Pontic shad migrating into the Danube Delta (Antipa, 1909; Niculescu-Duvăz, 1959).
Interestingly, Antipa observed that the black-headed shads appear on the Romanian Black Sea coast, near the mouth of the Danube, always a few days later than the other (white-headed) variety, tend to remain in the delta for a very short time, and then “disappear suddenly”. According to Antipa, it is not known where they go. Therefore, we can appreciate that the difference in head color of shad individuals was meticulously noted by one of the most renowned and observant investigators of the Danube Delta’s ichthyofauna.

Leclercq et al., 2010 describe different types of “proximate morphological color changes” in teleosts, defined as “morphological modulations of a given life-stage skin color in response to occurring variations in biotic and abiotic factors”. Of the four categories of environmental factors they describe, one is “surrounding luminosity and background adaptation” categorized as a secondary factor of proximate morphological color change. In the case of shads migrating from clearer (e.g. sea) to more turbid waters (e.g. mouth and delta main branches) and subsequently, later in their migration back into less turbid waters (e.g. upstream of the Prut confluence, or in the Danube sector downstream of the Iron Gates II dam), this may be the most likely environmental factor affecting color change – as a predator evasion tactic (Gusen, 2010). Chemical and physical factors in an environment can cause changes in appearance of organisms. Water clarity can affect pigmentation with melanophores expanding in clear water. (Sigler & Sigler, 1990)

Conservation and management measures for Pontic shads in the Danube

The conservation measures currently in place in Romania for the Pontic shad rely mostly on set periods of total prohibition of shad fishing. The prohibition periods are set at the beginning of each year by the State Fisheries Agency and follow a “sliding” schedule. The shad fishing ban starts earliest in the most downstream reaches and moves progressively upstream, with the duration of prohibition increasing in upstream sectors. The exact prohibition dates during 2016-2019 differed slightly from year to year, but adhered to the same general pattern: In the first sector, the Danube Delta (Black Sea to Marine Mile 43), fishing is prohibited for 10 or 12 days in April (sometimes ending in early May). Prohibition in the second sector (Mm 43 – rkm 238) lasts 15-21 days in April-May, most often overlapping for one week with the prohibition in the first sector. In the third sector, which extends almost to the Iron Gates II dam (rkm 238 – rkm 845.6) it lasts 30 days in May-June, again, overlapping somewhat with the prohibition period in the second sector. Exact prohibition dates for each sector are announced at the beginning of each fishing season.

The different sectors of the river are characterized by different levels of turbidity, usually related to the Danube tributaries that introduce suspended solids into the Danube, thus affecting local and downstream turbidity levels. In addition, damming of the Danube River (Iron Gates I and II dams) and its tributaries also has a significant effect on the amounts of suspended solids that end up in the Danube, its delta and the Black Sea.

This paper aims to investigate the potential for using the coloration of Pontic shads during spawning migration and turbidity levels of the aquatic habitats along their migration route to infer possible capture areas of individuals.

MATERIAL AND METHODS

Samples were collected every year from 2016 to 2019 during spawning migration. Sampling locations were selected according to the availability of fish (obtained from commercial fishermen, fish markets or fish collection stations) or the possibility of experimental/research fishing (Figure 1). Fish were collected as early as the beginning of March and as late as the beginning of July, but not during all those months of all years.

Adult fish were captured by experimental fishing performed by researchers in the field and were also obtained from commercial sources that provided a sufficient level of certainty of the fishing location (fish collection stations, fish markets, directly from fishermen). In Romania, during all three years, fish were collected at the mouths of the three branches of the Danube Delta (St. George, Sulina and Chilia) and upstream of the Danube Delta at rkm 123 (near the town of Isaccea). In Serbia, sampling was conducted only during 2016 at rkm 860, which is downstream of the Iron Gates II dam near the town of Prahovo. A total of 604 specimens were collected, processed and now constitute a baseline collection for multi-disciplinary investigations into the migratory behavior of Pontic shads in the Lower Danube River.

Samples were either processed immediately or frozen at - 20°C and processed later in the laboratory. All fish were measured to the nearest 1 millimeter, for total length (TL), standard length (SL), and fork length (FL). All fish were weighed to the nearest 1 gram. Sex was determined by dissection and stomach content was inspected for presence/absence of food and intestinal parasites.

The majority of fish were photographed as soon as they were obtained, but some were only photographed after freezing and thawing. This was not considered to be a matter for concern, as it appears that coloration of the head was not altered by the freezing/thawing process. Head color was assessed only qualitatively, with a value of “dark” or light” being assigned to each specimen. Observations regarding coloration were made on site, as well as in the laboratory. However, photographs (see Figures 4a – 4f), taken for purposes other than color observation, were made under varying light conditions and with different backgrounds. In hindsight, it is advisable to have a standard background and controlled lighting in order to have comparable images for color-related observations.

Considering the fact that coloration of fish was not the primary data target of the sampling, turbidity was not measured on site. Therefore, a literature search was conducted to obtain general information on water turbidity in the Danube sectors of interest, namely the Black Sea coastal areas at the Danube Delta, within the Danube Delta main branches and upstream to the Prut river confluence, as well as downstream of the Iron Gates II dam (Figure 2, Figure 3).

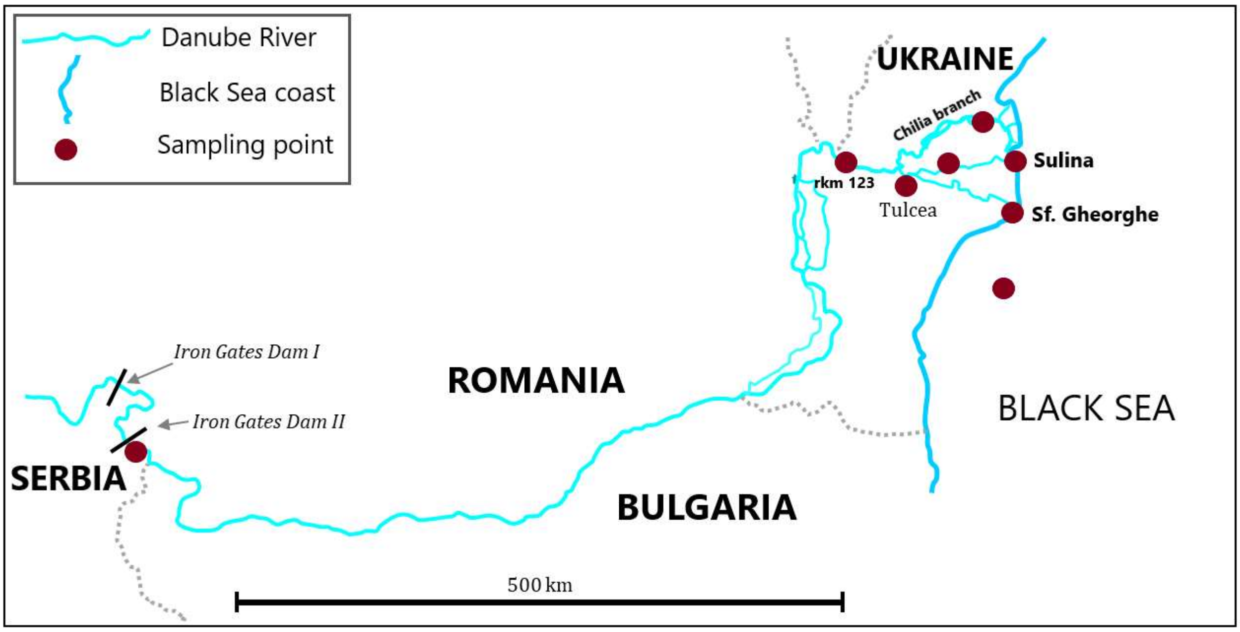

Figure 1. Sampling locations during spawning migrations of 2016 - 2019.

RESULTS AND DISCUSSION

The main observations of head coloration were made while manual processing of adult pre-spawner individuals obtained from sampling points that could be categorized as: near or in the Black Sea; within the Danube Delta (on the main branches at some distance from the Black Sea); the "unique" Danube immediately upstream of the delta (rkm 123), and far upstream, at the Iron Gates II dam (rkm 860) which is the longest distance that Pontic shads can migrate up the Danube (Figure 1).
The individuals captured in or near the Black Sea tended to have darker head coloration. A small number of individuals were obtained by winter trawl fishing in the Black Sea in November and December 2016, and had "dark" head coloration (Figure 4a). Furthermore, the samples obtained from commercial fish collection stations (such as those in the towns of Sf. Gheorghe and Sulina, both near the Black Sea coast) sometimes contained a mix of "dark" and "light" headed individuals, with the "dark" individuals comprising the majority (Figure 4b). This can be explained by the fact that fish collection stations usually contain the pooled catches of a larger number of commercial fishermen working within a certain distance from the collection station, and who may be fishing at the mouth of the delta branch and even in the sea or, on the other hand, deeper inland in a main Danube delta branch. Inspection of stomach content often revealed the presence of undigested or semi-digested food, which indicates that the fish were captured in the sea or soon after entering one of the Danube delta branches. This suggests that they had not spent a very long time in the more turbid waters and still retained dark coloration.
When analyzing the coloration of specimens obtained from farther upstream in the delta branches (Maliuc rkm 45 on the Sulina branch, or from Tulcea branch, rkm 70), all fish had the "light" coloration, which presents as translucent, often with a greenish hue. (Figures 4c and 4d, respectively).
Samples captured downstream of the Iron Gates II (rkm 860) all exhibited "dark" head coloration (Figure 4e). A single, spent post-spawner captured in July 2017 at rkm 123 appeared "light-headed" (Figure 4f), which would correspond to it spending an extended amount of time in the more turbid sectors of the river, both during upstream (pre-spawning) and downstream (post-spawning) migration.

By examining water turbidity levels in the three main sectors (Black Sea, the Danube Delta branches, the Danube River), as well as in the areas of river-sea transition, we observed that the areas of low turbidity, or high transparency, corresponded to areas where sampled fish had the "dark" head coloration. These include the Black Sea and coastal areas, as well as the farthest upstream reaches of the Pontic shad's range in the Danube. The "light" head coloration was encountered in samples that came from the Danube Delta farther inland, where the turbidity is higher (Figure 2 and Figure 3). The Danube's higher transparency downstream the Iron Gates II dam was confirmed by the local commercial fishermen assisting the research team, who only started fishing after dusk or nightfall because the high transparency in the absence of rain enabled the fish to avoid their nets. Similarly, recreational fishermen in the same area are able to catch Pontic shad by angling, because clear waters allow the fish to see their lures. Despite the generally accepted notion that Pontic shads stop feeding during spawning migration (Kolarov, 1991; Niculescu-Duvăz, 1959), stomach content of the fish captured by driftnets at rkm 860 revealed that more than half of the fish contained food (mainly insect larvae), which means that they had been feeding just previous to capture and prior to spawning. This may also be explained by clearer visibility allowing them to see prey.

Characteristic turbidity situations for Danube Delta waters and the coastal area in the month of February are presented in Figure 2. This situation was observed during 2008, which is considered a "typical" year with regard to hydrological conditions (Güttler, Niculescu, & Gohin, 2013). Turbidity levels for the entire Danube are presented in Figure 3, with main tributaries in the study area, downstream of the Iron Gates II dam, marked in blue.

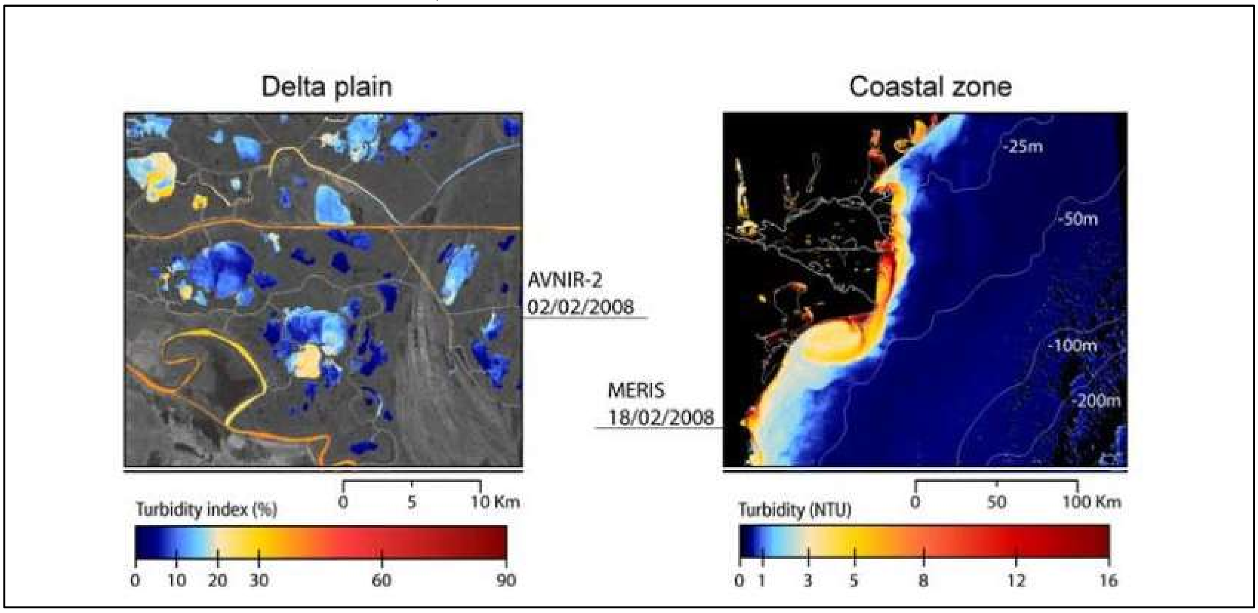

Figure 2: Water turbidity in the Danube Delta plain and coastal zone, measured in February 2008. Adapted from (Güttler et al., 2013)

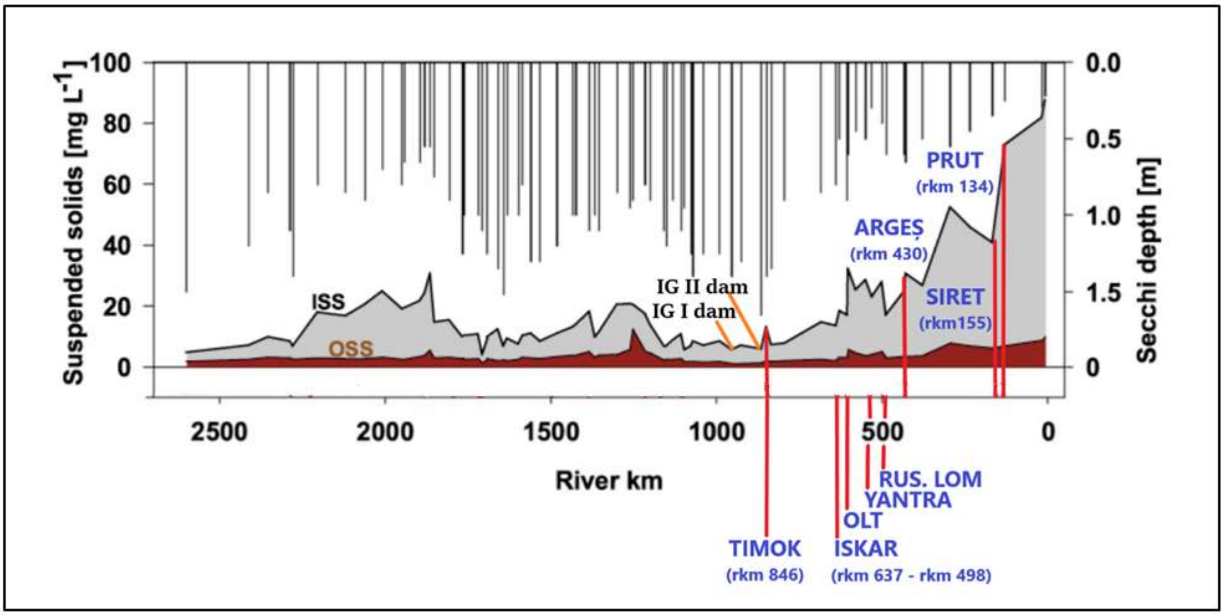

Figure 3. Turbidity presented by Secchi depth (vertical bar from the top) and total suspended solids (TSS) as inorganic and organic part (ISS and OSS respectively) with main Danube tributaries downstream of IG II dam within the study area marked in blue. Adapted from (Liska, Wagner, & Slobodnik, 2008)

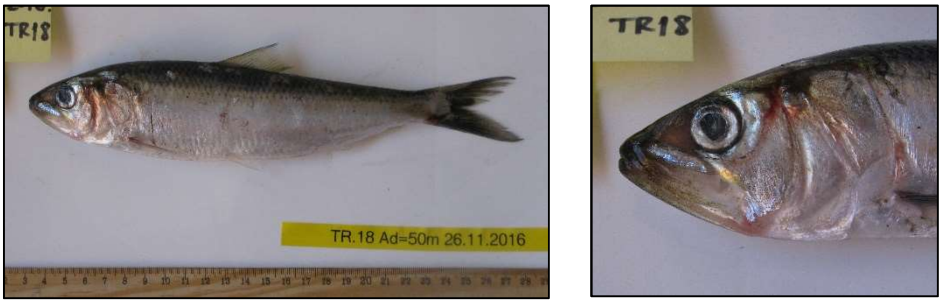

Figure 4a: "Dark head" juvenile specimen from the Black Sea, captured by trawl fishing (November 2016). Whole fish (left), head (right).

Figure 4b: "Dark head" specimen from Black Sea coastal waters, captured near Sulina (April 2018). Whole fish (left), head (middle), head from above (right).

Figure 4c: "Light head" specimen from Danube Delta captured in the Chilia branch, Vylkove (April 2019). Whole fish (left), head (right).

Figure 4d: "Light head" specimen from Danube Delta, captured in Tulcea branch (April 2018). Whole fish (left), head (middle), head from above (right, with flash).

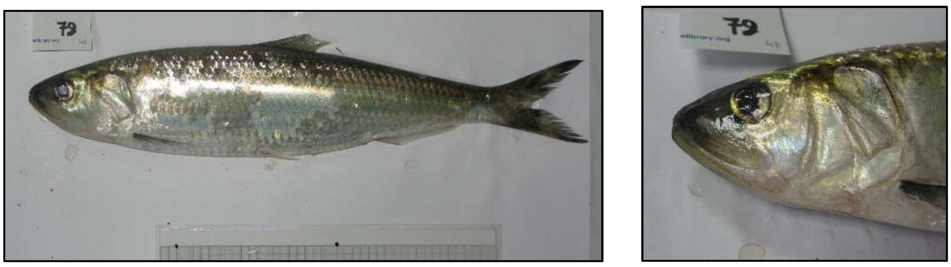

Figure 4e: "Dark head" specimen from the Danube, rkm 860 (April 2016). Whole fish (left), head (right).

Figure 4f. “Light head” specimen from the Danube, rkm 123 (July 2017). Post-spawner female migrating downstream to sea. Side view (right), dorsal view (left).

CONCLUSIONS & RECOMMENDATIONS

This preliminary investigation into the coloration of adult Pontic shads, specifically the coloration of the anterior end and dorsal side of the head, may potentially be used to assign captured individuals to particular broader sectors of their migration route. The findings may be of interest and practical use to the general public as well, as Pontic shad are a spring delicacy in Romania and fish captured in the freshwater sectors of the Danube are most valued and sought out by consumers. However, fish captured in the sea, or at the mouth of the Danube Delta branches may be falsely presented to an unknowing public as coming from the freshwater locations, and thus be sold at a higher price on the market.

More detailed investigation is necessary to precisely describe coloration categories and, if possible, quantify the color change, as well as to understand its adaptive significance. Histological studies are needed to describe the color-change mechanisms and confirm the stability of coloration under different capture and storage conditions (e.g. freezing and thawing, dehydration etc.). Future studies should include precise sampling localization and measurement of turbidity at the sampling point as well as some distance downstream, in order to have data on the turbidity of waters the captured fish spent its most recent days, or weeks, in and which determined its proximal morphological color change.

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