

## REPRODUCIBILITY OF AGE DETERMINATION BY SCALE AND VERTEBRA IN PONTIC SHAD (*ALOSA PONTICA* EICHWALD, 1838), FROM THE DANUBE

ŽELJKA VIŠNJIĆ-JEFTIĆ<sup>1</sup>, MIRJANA LENHARDT<sup>2</sup>, I. NAVODARU<sup>3</sup>,  
A. HEGEDIŠ<sup>1</sup>, Z. GAČIĆ<sup>1</sup>, and M. NIKČEVIĆ<sup>1</sup>

<sup>1</sup>Center for Multidisciplinary Studies, University of Belgrade, 11000 Belgrade, Serbia

<sup>2</sup>Siniša Stanković Institute for Biological Research, 11060 Belgrade, Serbia

<sup>3</sup>Danube Delta National Institute, 820112 Tulcea, Romania

**Abstract** — Specimens of Pontic shad (*Alosa pontica* Eichwald, 1838) were collected during April and May of 2006 in the Danube River (rkm 863) just downstream of the Iron Gate (Djerdap) II dam. Twenty-eight scales and 30 vertebrae were prepared and given to five interpreters with fish aging experience of from 3 to 30 years and with shad aging experience of from 0 to 12 years. Interpreters with experience in shad age determination showed lower values for the index of average percent error (IAPE) and an index of coefficient of variation (ICV). In regard to within-interpreter reproducibility, no significant differences were found between scale and vertebrae as structure for age determination. The most experienced reader showed the lowest value for IAPE, while the least experienced reader showed the highest value for IAPE. The results of this work indicate that experience in age determination by particular structure had more impact on age determination precision than specificity of structure, scale or vertebra. More work is needed on adopting standard protocols that must include some sort of common interaction between responsible age readers.

**Key words:** Aging precision, fish, average percent error, coefficient of variation, *Alosa pontica*, Serbia

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### INTRODUCTION

Estimates of fish age provide important demographic parameters to analyze and assess fish populations (Maceina and Sammons, 2006). Accurate age information is critical to the biological understanding and management of most fish species (Hurley et al., 2004; LaBay and Lauer, 2006). Mistakes in fish age determination can have negative effects on fish stock management.

Fish can be aged using a number of structures which produce periodic growth increment, including scales, vertebrae, fin rays, cleithra, opercula, and otoliths (Campana, 2001). Two important considerations when selecting a structure for aging a sample of fish are whether the structure yields accurate estimates of fish age and whether the structure can be obtained without killing the specimens (Brenden et al., 2006).

Unfortunately, the process of estimating fish age incorporates a procedural error associated with the structure being examined and an interpretational error due to the element of subjectivity inherent in all age estimations (Campana, 2001). For this reason, methods of age validation and estimation of aging precision have been developed.

Pontic shad (*Alosa pontica* Eichwald, 1838) is an economically important fish species which is highly appreciated by a certain number of consumers in the Lower Danube Region because it is the Christian custom of local people to eat Pontic shad during Lent (Ciolac and Patriche, 2004). Pontic shad stocks in the Black Sea and Danube River have decreased mainly due to overfishing and pollution (Navodaru, 1996; Navodaru and Waldman, 2003). Accurate age determination of migrants is necessary for better management of Pontic shad stocks shared among Ukraine, Romania, Bulgaria, and Serbia. Studies

on age determination of migrating specimens are important for solving common problems in fishery management.

Age determination of Pontic shad has been performed mainly with scales in Romania (Teodorescu-Leonte et al., 1957; Cautis and Teodorescu-Leonte, 1964), Ukraine (Pavlov, 1953), Bulgaria (Ivanov and Beverton, 1985; Kolarov, 1991), and Turkey (Erguden et al., 2007). Yilmaz and Polat (2002) investigated the most accurate structure and method for determining the age of Pontic shad inhabiting the Black Sea. Among five bony structures (scales, vertebrae, otoliths, opercles, subopercles) which they investigated, vertebrae were approved as giving the most accurate estimates.

In the past, isolated individuals of Pontic shad migrated as far as Budapest, at Danube rkm 1650 (Banarescu, 1964). Nowadays, it migrates for spawning in the Danube River to rkm 864 (the Djerdap II dam). Specimens analyzed in this work were caught just downstream from that dam. The present paper evaluates the reproducibility of age determinations by different individuals and compares the skill level of a group of experienced shad agers relative to inexperienced agers. Also, we wanted to assess the relative ease of determining shad age from a particular structure, scale or vertebra.

## MATERIAL AND METHODS

Scales and vertebrae were obtained from Pontic shad caught by fishermen during April and May of 2006 in the Danube River just downstream from the Djerdap II dam at rkm 863. Specimens were caught using a drifting pelagic gillnet with mesh size of 32.5 mm. A total of 189 specimens were caught, from which 30 specimens were chosen randomly for scale and vertebra collection. Scales were collected from the area below the dorsal fin and above the lateral line and stored dry in labeled envelopes. Dissected vertebrae (from the 4<sup>th</sup> to 10<sup>th</sup>) were placed in boiling distilled water for 2-3 minutes, cleaned of flesh and fat, and stored dry in labeled envelopes.

The experiment involved five interpreters: three experienced shad readers from Romania (R1, R2,

R3) and two interpreters from Serbia (S1, S2) with experience on other fish species, but not with shad. At the time of the experiment, reader R1 had 12 years of experience in Pontic shad age determination by scale, while readers R2 and R3 had 15 and 5 years of such experience, respectively. Readers S1 and S2 had no experience in Pontic shad age determination, but 30 and 3 years of experience, respectively, in determining the age of other fish. For all readers, aging was done with vertebrae for the first time. Twenty-eight scale and 30 vertebra preparations from shad were given to the interpreters. They were asked to complete their counts in time appropriate for them, independently of one another. They had no information about length or weight of the fish. The second part of the experiment was identical and was performed 3 months after the first part.

Within-interpreter reproducibility and between-interpreter precision were measured by the average percent error (APE) and coefficient of variation (CV) as indicated by Campana (2001). When averaged across many fish, they become the index of average percent error (IAPE) and index of coefficient of variation (ICV). Within-interpreter reproducibility was based on the two (first and second) replicate counts by all interpreters, and between-interpreter reproducibility was based on the first count by interpreters.

The relative ease of determining age by a particular structure, scale or vertebra, was established on the basis of within-interpreter precision. The sign test was used to ascertain whether a statistical difference exists between structures for within-interpreter reproducibility.

## RESULTS

Interpreters with experience in shad age determination (R1, R2, R3) showed lower values of IAPE and ICV using both scales and vertebrae than interpreters with no experience in shad age determination (S1, S2) (Table I). The IAPE varied between 7.6 and 10.2 (mean = 8.9), while ICV varied between 9.8 and 14.4 (mean = 12.1) (Table I).

Age values estimated inexperienced interpreters

**Table 1.** Mean values for the IAPE and ICV and the number of preparations for scale and vertebra readings by experienced interpreters (R1, R2, R3) and inexperienced interpreters (S1, S2).

| interpreter | scale |      |    | vertebra |      |    |
|-------------|-------|------|----|----------|------|----|
|             | IAPE  | ICV  | n  | IAPE     | ICV  | n  |
| R1, R2, R3  | 7.6   | 9.8  | 28 | 9.5      | 12.4 | 30 |
| S1, S2      | 8.4   | 11.9 | 28 | 10.2     | 14.4 | 30 |

**Table 2.** Values for the IAPE and ICV and the number of preparations for scale and vertebra readings for two replicate counts by all interpreters.

| interpreter | scale |      |    | vertebra |      |    |
|-------------|-------|------|----|----------|------|----|
|             | IAPE  | ICV  | n  | IAPE     | ICV  | n  |
| R1          | 5.5   | 7.8  | 28 | 8.1      | 11.4 | 30 |
| R2          | 8.3   | 11.8 | 28 | 7.9      | 11.2 | 30 |
| R3          | 6.5   | 9.1  | 28 | 6.3      | 8.9  | 30 |
| S1          | 9.7   | 13.7 | 28 | 9.5      | 13.5 | 30 |
| S2          | 8.1   | 11.5 | 28 | 6.8      | 9.7  | 30 |

using scales were in all specimens higher, the average value of the difference between the two groups being 1.7 years, i.e., inexperienced readers overestimated fish age. The same was true using vertebrae, where the average value of the difference between the two groups was 1.5, with higher values for each particular fish age being obtained by the inexperienced group.

Values of IAPE and ICV relating to within-interpreter precision are presented in Table II. The sign test for IAPE indicated no significant differences between structures with respect to within-interpreter reproducibility ( $Z = 0.894$ ,  $P = 0.371$ ).

The reader most experienced in Pontic shad age determination (R1) showed the lowest value of IAPE (5.5), while the least experienced reader (S1) had the highest value of IAPE (9.7) (Table II).

## DISCUSSION

There is no *a priori* value of precision which can be designated as a target level for aging studies, since precision is highly influenced by the species and the nature of the structure, not just the age reader (Campana, 2001). Data relating to American shad scales showed that ICV for repeatability between the first and second age estimate for the same fish

varied between 3.79 and 11.08 (mean = 6.9) for 13 biologists whose length of experience in aging shad ranged from 2 to 25 years (McBride et al., 2005). In our study, ICV for scales varied between 7.8 and 13.7 (mean = 10.8) for readers with experience in Pontic shad age determination ranging from 0 to 12 years, which is in accordance with the American findings.

Although a standard measure of reproducibility in fish age and growth studies probably cannot be defined, values of IAPE below 10% can be taken as an acceptable level of precision (Vilizzi et al., 1998). In the present study, IAPE ranged from 7.6 to 10.2% for between-interpreter reproducibility and from 5.5 to 9.7% for within interpreter reproducibility, so it is on an acceptable level of precision. Our data showed lower values of IAPE for the experienced group and the lowest value of IAPE for the most experienced reader. This work and the results on American shad demonstrated that precision can be improved with further training of the readers. This is in accordance with the finding of Power et al. (2006) that more experienced age readers had greater levels of precision.

The results obtained by Yilmaz and Polat (2002), namely that vertebrae can be approved as giving more accurate estimates in age determination than

scales, are contrary to our results (no significant differences between structures), which can be explained in terms of reader experience. Unfortunately, there are no data on reader experience in their work.

The differences between groups experienced and unexperienced in Pontic shad age estimation, with overestimates of Pontic shad age in the inexperienced group (by 1.7 year for scales and 1.5 years for vertebrae) can be attributed to false rings. Thus, the necessity of having information about length for age from sources apart from scales or other structures for age determination is very clear (Mann and Steinmetz, 1985). New methods involving nuclear microscopy of otoliths, where sudden elevations of the Sr: Ca ratio are interpreted as seaward migration events and the physical location of these elevations on the otolith are related to the age and size at which the fish first emigrated from fresh water (Limburg et al., 2003), could be one more useful tool in Pontic shad age validation.

Migratory species are the most affected by the construction of dams and other obstacles blocking the route to the spawning ground. Combined with severe pollution and intensive fishing, their presence inevitably leads to the future extinction of individual species. The indicated combination of factors has been the cause of decline of sturgeons, shad, and other migratory fish (Maitland, 1986; Navodaru, 1998; Limburg, 2001).

The main conclusions of this work are that no statistically significant difference exists between reproducibility of age determination by scales and vertebrae in Pontic shad, and that training of readers could produce better reproducibility. However, for good management of such species, validation of age determination needs to be carried out as was done for American shad (McBride et al., 2005).

In light of the results obtained in this work and because preparation of vertebrae for reading takes more time than for scales, scales should be used as a valid structure for age estimation in Pontic shad. More work is needed on adopting standard protocols, which must include some sort of common interaction between responsible age readers. In this

way, a precise and practical method will be developed that can be used routinely to age sufficiently large fish samples (Appelberg et al., 2005).

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## РЕПРОДУЦИБИЛНОСТ СТАРОСНЕ ДЕТЕРМИНАЦИЈЕ ПОМОЋУ КРЉУШТИ И ПРШЉЕНОВА КОД *ALOSA PONTICA* EICHWALD, 1838 ИЗ ДУНАВА

ЖЕЉКА ВИШЊИЋ-ЈЕФТИЋ<sup>1</sup>, МИРЈАНА ЛЕНХАРТ<sup>2</sup>, И. НАВОДАРУ<sup>3</sup>,  
А. ХЕГЕДИШ<sup>1</sup>, З. ГАЧИЋ<sup>1</sup> и М. НИКЧЕВИЋ<sup>1</sup>

<sup>1</sup>Центар за мултидисциплинарне студије, Универзитет у Београду, 11000 Београд, Србија

<sup>2</sup>Институт за биолошка истраживања "Синиша Станковић", 11060 Београд, Србија

<sup>3</sup>Danube Delta National Institute, 820112 Tulcea, Romania

У овој студији анализирана је применљивост старосне детерминације помоћу крљушти и пршљенова код *Alosa pontica* Eichwald, 1838 из Дунава. Неопход-

но је више студија о сачињавању стандардних протокола који би били евалуирани од стране компетентних стручњака.