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Short Communication

Scales, operculum and otoliths: key to a comprehensive understanding of age determination of Crucian carp, *Carassius carassius* (L.)

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ABSTRACT

The study was undertaken with an objective to determine the age of Crucian carp, *Carassius carassius* from Dal Lake using scales, operculum and otolith. As age determination in fishes is considered to be essential for fisheries management and understanding life history of different fish species. After being appropriately prepared fish scales, operculum and otoliths were examined under the microscope to establish the age of fish by counting the number of annuli, or year markings. Fish were between 1+ to 2+ years old, with length ranging from 121 to 131 mm and 132 to 169 mm respectively. Significant positive correlations were observed between total length and total weight (R^2=0.8128); and between total length and fish age (R^2=0.4547). These relationships indicate a proportional increase in weight with length and an age-dependent size increase, respectively. In the present study growth rings or annuli on scales, operculum and otolith were found visible and clearly distinguishable in *C. carassius* so it has reached an agreement with other studies that these structures are appropriate for age determination.

Key words: Age determination, Dal lake, scales.

The Crucian carp, *Carassius carassius* is a freshwater demersal fish species of the family Cyprinidae, locally referred as “Gang gad” in Kashmir (Kullander *et al*., 1999). It inhabits in lakes, ponds, and slow-moving rivers across Europe and portions of Asia (Lelek, 1987), and over the years, they have been brought to many other areas. This fish was accidentally introduced to Dal Lake in 1956-1958 along with common carp, since then, it has effectively adapted to the lake’s changing environmental conditions (Holopainen and Hyvarinain, 1985). This deep-bodied, laterally compressed species typically exhibits a golden-green shine in juveniles, which darkens with age to deep bronze, with green upper flanks and golden bellies (Smartt, 2007). Adults have red or orange fins and can reach a maximum length of 64 cm. The Crucian carp ranks sixth among cultured freshwater fish globally (FAO, 2017) Carp culture in Kashmir region is based only on three exotic carp fish species *viz*: Silver carp, Grass carp and Common carp (Gohar *et. al,* 2016). The *C. carassius* can be brought to culture conditions and can contribute to enhance production (Mattoo *et. al,* 2025). It also plays a significant role in commercial fishery, aquaculture, and is also favoured as both a game and aquarium fish (Fish-Base, 2004). Age and growth studies are essential components of modern fishery biological research. Determination of age in fish is essential for

fisheries management and understanding life history of different fish species (Hilborn and Walters, 2013). It entails determining fish age, which can provide important details about its growth rate, age at maturity, lifespan and population dynamics. Fish age may be ascertained using various calcified structures (hard parts), including scales, operculum, vertebrae, spines, fin rays and otoliths (Casselman, 1983). These features are occasionally combined for comparative analysis (Khan and Khan 2009; Villizzi and Walker 1999). In order to determine an individual's age, annual (or daily) increments are commonly counted under the assumption that these were formed on an equivalent temporal interval. This assumption is verified through validation, which is equivalent to determining the accuracy of an age estimate (Campana and Thorrold, 2001). Reliable age estimation in fish supports effective stock assessment and informed fisheries management decisions. Accurate age estimation is expected to play an essential step for age-based assessment of fish populations and sustainable resource management. Considering the importance of above-mentioned factors, the present study was carried out with the objective to determine the age of *C. carassius* using scales, opercular bones and otolith.

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MATERIALS AND METHODS

The research work was carried out in Fisheries Resource Management (FRM) Laboratory, Faculty of Fisheries, SKUAST-K, Rangil, Ganderbal. Following steps were taken to meet the objective of the study.

Collection of fish specimens: A total number of 50 specimens of *C. carassius* were collected from Dal Lake with the help of local fishers. The collected fish samples were transported to the FRM Laboratory in insulated boxes containing ice packs. They were then cleaned under running tap water and further examinations were carried as follows.

General morphometrics: Total length of the individual fish sample was measured to the nearest 0.01 millimeter using Geometric scale and the total weight was measured using electronic balance upto the nearest 0.5 gram.

Scale preparation and reading techniques: Total 50 fishes were examined. For this purpose, 5-6 scales were removed from the lateral line near the tip of the pectoral fin using forceps. The scales were washed, cleaned and studied as dry mounts, after removing the extraneous matter and mucus. This was done by washing them with tap water and rubbing in between the fingertips. To make scales clearer and softer they were dipped in a weak solution of one per cent potassium hydroxide (KOH) for about 5 10 minutes, then washed with tap water 2-3 times and gently rub with fingertips to remove mucus and dust (Ujjania, 2012). Scales were mounted between two glass slides so that they won't get curled and studied with the help of microscope (4x) (Tandon and Johal, 1996; Bhatt and Jahan, 2015).

Opercular bone preparation and reading techniques: Fish specimens taken for scales were additionally used for collection of operculum. The operculum was taken from fish sample with the help of surgical scissors. It was then washed and then boiled in water for few minutes till the tissue softens (Phelps *et al*., 2007). The tissue was gently cleaned by hand. The tissue free operculum was placed in 50 per cent hydrogen peroxide (H_2O_2) for 15 minutes. After 15 minutes the operculum was removed and washed with clear water. The cleaned opercular bones were dried at room temperature for three days and the rings were observed under the microscope at 4x magnification (Phelps *et al*., 2007). Rings were also visible when the operculum was placed on dark background (Shamim *et al*., 2021).

Otolith preparation and reading techniques: The same specimens were further used to collect otoliths. The sagittal otoliths were removed from the left side of the otic capsules by opening the otic bulla. They were then washed and cleaned. The cleaned otoliths were placed in hydrogen peroxide (H_2O_2) for 15 minutes and then they were again washed and cleaned for further process. The otoliths were examined under the digital microscope at 4x. The otoliths with annual rings were

ground with sandpaper to make the annuli more identifiable for age reading (Tandon and Johal, 1996).

RESULTS AND DISCUSSION

Fish age estimation is one of the most important components of fisheries resource study (Younger and Weiss, 1975; Casselman, 1983). Age is the basis for analyzing fish growth, sexual maturity, and changes in population structures (Gallagher and Nolan 1999). The comparison of age estimation techniques using various calcified structures has been extensively examined in numerous fish species to determine which structure provides the most reliable results (Khan and Khan 2009). One of the major challenges in age and growth estimation is choosing the right structure and technique to age fish, while comparisons between scales and otoliths are quite common (Abecasis *et al*., 2006). During the present study hard body parts such as scales, operculum and otolith were used to determine the age of *C. carassius*. Observations on age determination of *C. carassius* is given in Table 1, Fig. 1 and its graphical representations are shown in Fig. 2 to 3. From the results obtained it was found the total length of the fishes varied from 121-169 mm. Age of fish ranged from 1+ to 2+ years, with length group from 121-131 mm and 132-169 mm respectively (Table 1).

Table 1: Length- wise age distribution of *C. carassius*

Age of Fish	Length (mm)	Mean (length)	Frequency (N= 50)
1^+	121- 131	127	22
2^+	132-169	140.28	28

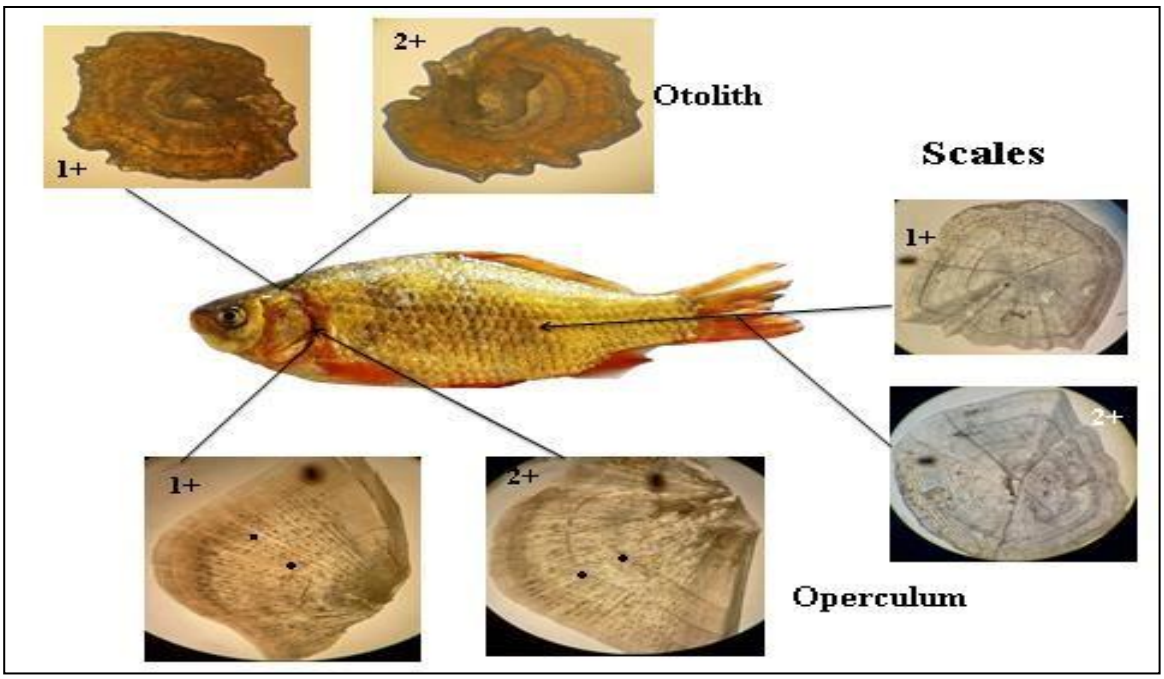


Fig. 1: Pictorial representation of the structures used for ageing in *C. carassius*

A significant correlation (R^2=0.8128) was observed between total length and total weight of *C. carassius*. Similar results were reported by (Ujjania, 2012; Nargis, 2006; Muhammad *et* al., 2020; Bhatt and Bhanu, 2020; Shamim *et al*., 2021) in their studies on carps and other species. The Crucian carp had ctenoid scales, growth of a scale starts at the center known as the focus, and expands outward, especially towards the scale's front edge. Fin ridges develop in a circular pattern around this focus as the scale grows. Each year, new circular growth lines, or circuli, are added.

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This scale growth mirrors the fish's own growth, and by counting the annuli, or annual growth rings, the fish's age can be determined.

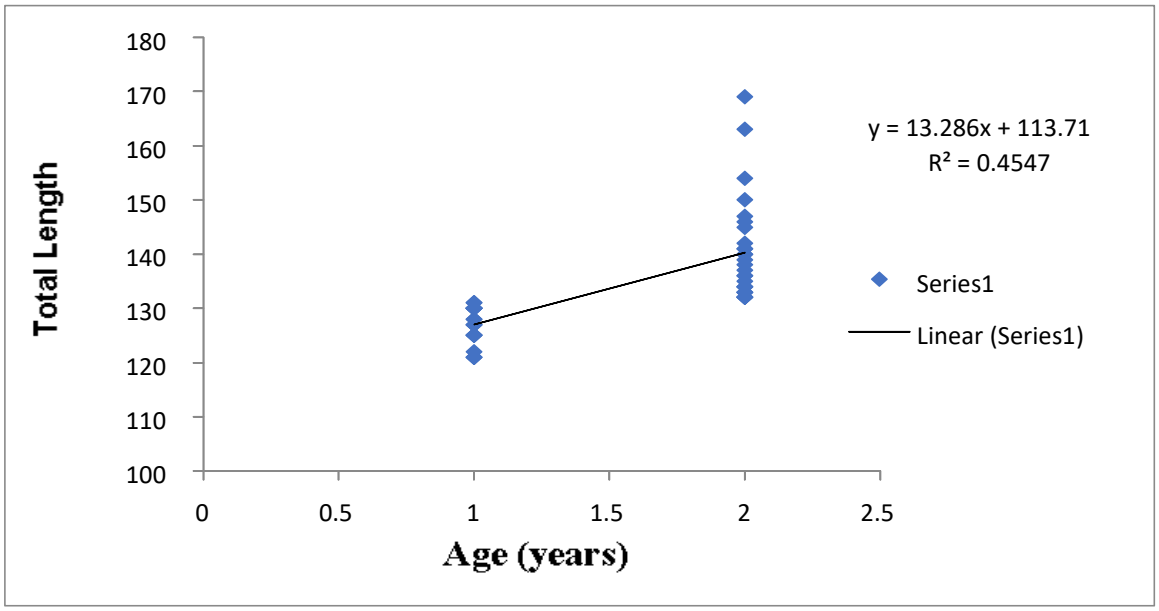


Fig 2: Scatter plot showing relationship between total length and age (years) of *C. carassius*

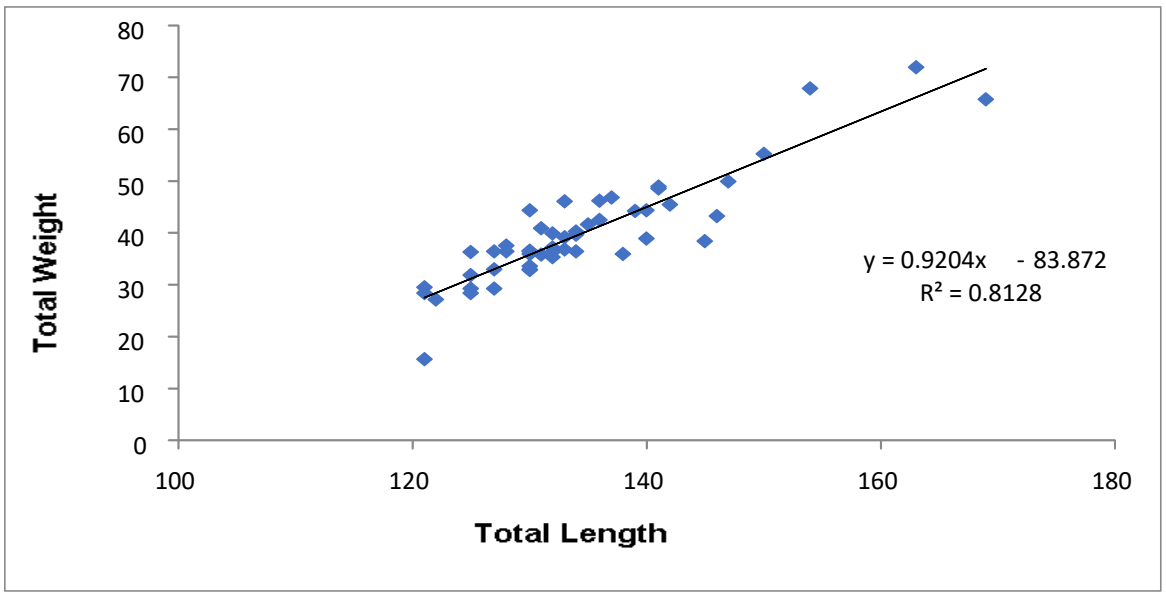


Fig 3: Scatter plot showing relationship between total length and total weight of *C. carassius*

The initial full circulus of each year's growth serves as a yearly marker (Fred, leaflet 488). Scales with growth marks have been shown to be a better structure for ageing Black prochilodus, *Prochilodus nigricans* (Louberis and Panfilli, 1992). In addition to showing a clear and sharp annuli, scales are easy to collect and prepare, and they are non-destructive to fish. As per Nargis (2006) for cyprinid fishes, use of opercular bones for age determination is quite easy and simple and provides better results. The age rings on the opercular bones can also be seen with the naked eye and can also be more clearly observed under the compound microscope.

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Opercular bones have been found to be the effective structures for determining the age of several important freshwater fish species, including *Cyprinus carpio* (McConnell, 1952; Shamim *et al*., 2021), *Esox Lucius* (Frost and Kipling, 1959), *Labeo senegalensis* (Blake & Black, 1978), *Catla catla* (Khan & Khan 2009), *Perca fluviatilis* (Shafi & Maitland, 1971), and *Hypophthalmichthys molitrix* (Khan *et al*., 2015). In the present study sagittal otoliths were used, which was largest and more easily readable of the three types of otoliths (sagittal, lapillus, and asteriscus), making them effective for determining fish age accurately.

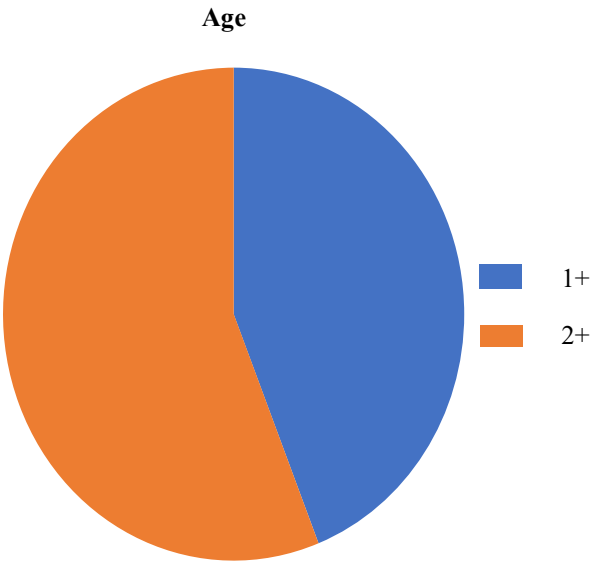


Fig 4: Pie chart depicting age distribution of *C. carassius* of studied length groups

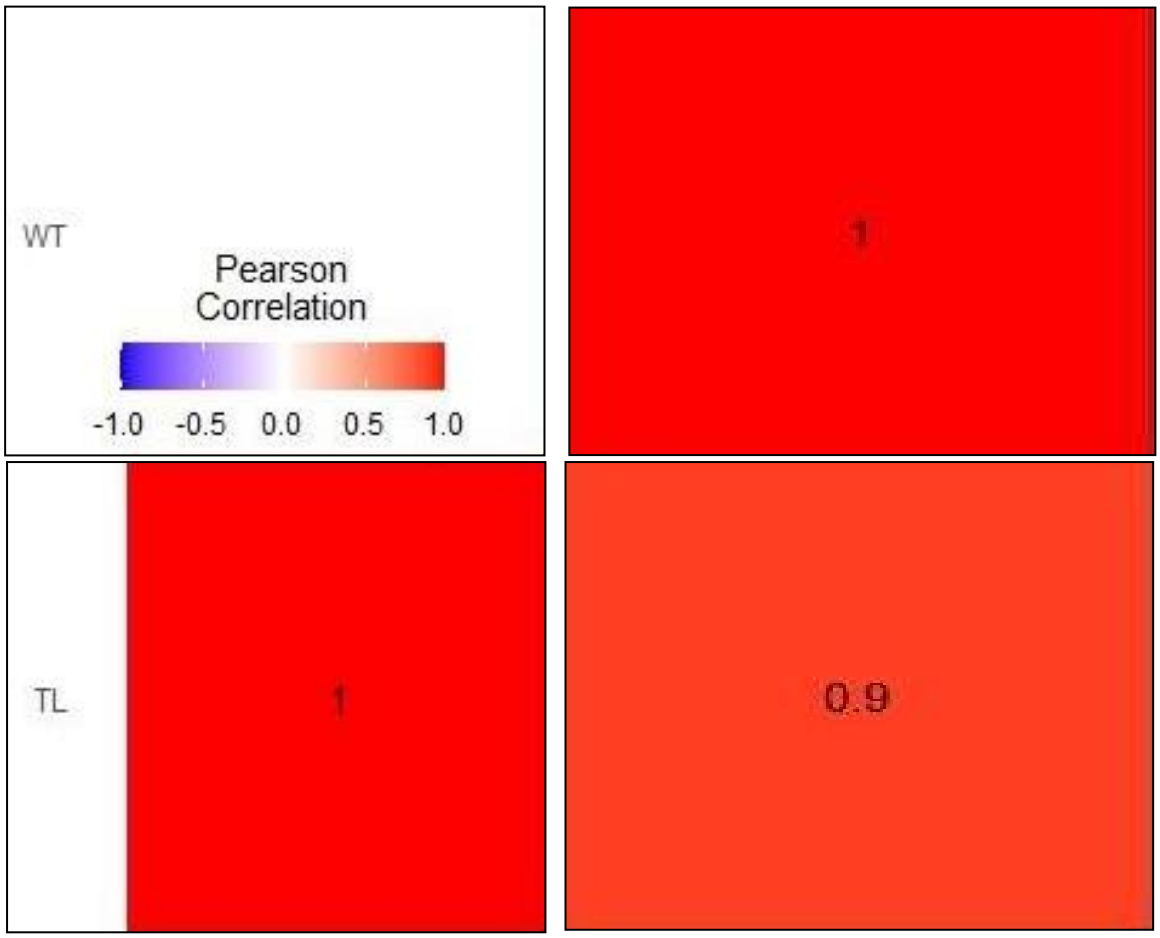


Fig 5: Pearson correlation between total length and total weight of *C. carassius*

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As per Morales-Nin (1992) sometimes, the interpretation in otolith is complicated as the annuli were not easily available due to the presence of false rings they are typically formed during crucial stages of fish's life, such as when it reaches sexual maturity. Additionally research comparing various calcified structures for age determination in different fish species demonstrates that although otoliths can provide accurate results, they are more challenging to handle and interpret than scales particularly in freshwater species like carp (Luo *et al.,*2016). In case of Crucian carp and related species, opercular bones and scales are still preferred for their reliability (Mahe *et al.,* 2016).

CONCLUSION

Fish ranged in age from 1+ to 2+ years, with lengths between 121-131 mm and 132-169 mm respectively. A significant correlation (R^2=0.8128) and (R^2=0.4547) was observed between total length and total weight; total length and age of the fish respectively. These relationships indicate a proportional increase in weight with length and an age-dependent size increase, respectively. Scales, opercular bones and otolith were found to be the appropriate structure for ageing of *C. carassius* as the growth rings or annuli were visible and easily distinguishable. Using scales is the non-destructive method of age determination and can also be also used for live fishes.

CONFLICT OF INTEREST

All the authors affirm that there is no conflict of interest among them. All research activities comply with relevant legal, institutional and ethical standards.

AUTHOR CONTRIBUTION

All the authors contributed equally in this research article.

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