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Does hardness of food affect the development of pharyngeal teeth of the black carp, *Mylopharyngodon piceus* (Pisces: Cyprinidae)?

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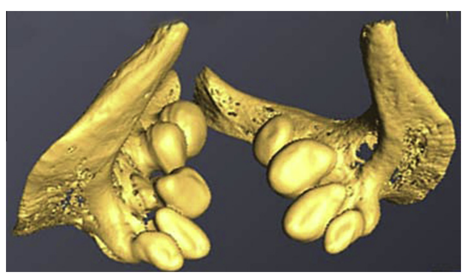
HIGHLIGHTS

- Pharyngeal bones and teeth of black carp fed different diet were examined by CT scans.
- Total volume, surface area and weight were greater in fish fed hard diet.
- The crushing mill of black carp shows adaptive modification with the food sources.

GRAPHICAL ABSTRACT

Three dimensional computed tomography (CT) scan of black carp pharyngeal bones.



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ABSTRACT

We investigated whether food type influences development of the pharyngeal crushing apparatus of black carp (*Mylopharyngodon piceus*, Cyprinidae). Fish fed a hard diet had average standard lengths and weights larger than those fed a soft diet; these observations in part could be related to differences in nutritional value of the two food types. The lower pharyngeal bones, which bear molariform teeth were examined using three dimensional computed tomography (CT) scans. After adjusting for differences in the standard length of the fish, the total volume and exterior surface areas of the pharyngeal teeth were greater in fish fed hard diets than in those fed soft diets.

Total weights of the pharyngeal arches were less in the fish fed a soft diet than in those fed a hard diet. These results indicated that food type affects development of the pharyngeal crushing mill of black carp and therefore if black carp are produced for snail control, a hard diet should be provided from an as early time as possible in the production cycle.

1. Introduction

Phenotypic plasticity of morphology and behavior permits organisms to adapt to changing biotic and abiotic environments during their life span (Norton et al., 1995; Day and McPhail, 1996; Stauffer and Gray, 2004). A number of studies have examined the interactions among diet, feeding performance, and morphological changes (Wimberger, 1991, 1992, 1994; Slootweg et al., 1993; Huysseune, 1995; Mittelbach et al., 1999; Bouton et al., 2002; Grubich, 2003; Hulsey et al., 2005, 2008; Binning et al., 2010). Huysseune (1995) compared two phenotypes of

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Astatoreochromis alluaudi (Cichlidae) and found that teeth of wild-caught snail-eating specimens have a larger size than specimens raised on soft food, while specimens fed a soft diet have more and smaller teeth. Binning et al. (2010), however, suggested that the differences of pharyngeal jaw traits of *A. alluaudi*, which are useful for crushing and manipulating prey in the buccal cavity, were not associated with either the fish's diet or the abundance of prey. Hence, jaw morphology is impacted by environmental factors that vary across populations (e.g., food type and food availability during evelopment). Once molariform teeth are formed in cichlids this trait does not disappear (Binning et al., 2010). Mittelbach et al. (1999) mentioned that diet had a strong effect on the morphology of pumkinseed sunfish (*Lepomis gibbosus*). Thus, muscle mass of fish fed a diet of mixed hard and soft-bodied prey increased by about 230% compared to fish fed only soft-bodied prey. The feeding performance of *Herichthys minckleyi* contributes to the maintenance of molariform and papilliform pharyngeal morphologies (Hulsey et al., 2005). The papilliform teeth shredded plant much better than molariform teeth, while only fish with molariform teeth were able to crush snails; thus, food source is a main determinant of the phenotypic diversity of *H. minckleyi*.

Black carp, a cyprinid fish, is well known as a molluscivore (Nico et al., 2005). Cyprinids constitute the largest fish family and many species are diagnosed based on the morphology and position of pharyngeal teeth (Sibbing, 1982). The pharyngeal apparatus consists of a modified fifth pharyngeal arch that bears teeth and a dorsal chewing pad against which the teeth operate. The size and shape of the arch and teeth can be used to predict the diet of the species. Fry, fingerling and small juvenile black carp feed almost entirely on small invertebrates such as zooplankton, benthic organisms, and aquatic insects while large juvenile and adult black carp feed almost exclusively on mollusks (Nico et al., 2005). The initial teeth in black carp and cypriniformes in general are recurved and conical, and are replaced by differential forms (Yue and Nakajima, 1995). Replacement teeth in teleosts may develop in the soft tissue outside the bone to which they will attach ("extraosseous") or in sockets within the bone ("intraosseous") beneath their functional predecessor; cyprinid replacement teeth are extraosseous (Trapani, 2001; Zheng and Liu, 2011). The larval and juvenile teeth are replaced into the adult molariform teeth through seven stages (Nakajima and Yue, 1995). Adult pharyngeal teeth in *Mylopharyngodon piceus* are molariform and the dental formula is 4–5 as a rule, and the dentition is asymmetrical (Yue and Nakajima, 1995; He et al., 2013). Black carp continue to replace the pharyngeal teeth throughout their life span (Yue and Nakajima, 1995) and the new teeth are found as replacement teeth in tissue next to the pharyngeal bone (Pasco-Viel et al., 2010). The switch of feeding habit of black carp is associated with the development of molariform teeth on the lower pharyngeal bone, which creates a superior crushing ability (Liu et al., 1990; Nakajima and Yue, 1995).

The purpose of this study was to compare the morphology of pharyngeal teeth of black carp fed on either soft food or hard food (snails). Although changes could also be anticipated to occur in the dorsal part of the crushing mill such as size and hardness of the chewing pad and possibly in the cranial bones supporting the chewing pad, we believed that these changes would be more difficult to document.

2. Material and methodology

2.1. Black carp

Fifty specimens of black carp with weight ranging from 30 to 35 g (fork length 100–110 mm) were bought from one farm in Tu Son, Bac Ninh, Vietnam. At this stage, molariform teeth were already present, but they may still develop differently depending on food type. The fish were initially maintained in cement tanks (1.2 × 3 × 1.4 m W × L × H) for 1 week. Water level was kept at 1.2 m, and constantly aerated and re-circulated through a filter system at a rate of 0.5 m^3/h. The filter contained a net (mesh size 0.5 × 0.5 mm), and water passed through a layer of foam rubber and a layer of sand before being returned to the tank (Hung et al., 2013). Food pellets, supplied by MINH TAM Company (http://minhtamgroup.com.vn) were round with diameter ranging between 2.5 and 3.0 mm. Black carp were fed at the rate of 5% of body weight per day, which is the amount suggested by MINH TAM company and is also close to the satiation level mentioned in Nico et al. (2005).

2.2. Experimental design

Ten enclosures (1 m × 2 m × 1.7 m, W × L × H) were made of nylon net with mesh size of 1 mm^2 at the bottom and 4 mm^2 around the sides to permit water circulation. Enclosures were placed in a pond (20 × 30 × 1.6 m) at Research Institute for Aquaculture No. 1 (RIA1), Tu Son. The enclosures were fixed to a bamboo pole in each corner and in each corner of the enclosure a brick was placed to ensure that the enclosure would always be extended. Water level was maintained at 0.8 m inside enclosures. Five specimens of black carp were released into each enclosure. Five enclosures were randomly selected to be fed commercial fish food (soft food) and the other groups were supplied with snails (hard food). The amount of food supplied for soft food group was 15 g per day, while hard food group was supplied 30 g per day with various snail species (primarily *Melanoides tuberculata*, *Angulyagra polyzonata* and *Sinotaia aeruginosa*). The shell height of snails ranged between 18 mm and 35 mm.

The experiment was continued for 23 weeks (from 14th August, 2010 to 23rd February, 2011). At the end of the experiment, all surviving specimens of black carp were collected and measured. The fish were anaestisized and subsequently euthanized before fixed in a 4% formalin solution for one week. After 1 week, the fish were washed in water and transferred to 70% ethanol. Subsequently, the pharyngeal apparatus and the chewing pad of each fish were carefully removed. The skull of each black carp and all teeth were preserved separately in 70% ethanol solution.

2.3. Scanning procedure

Pharyngeal arches with attached and replacement teeth were mounted in a thin-walled plastic tube in foam and positioned vertically in the scanner to collect transverse slices. Each column contained from 5 to 7 samples. Scans were performed on the OMNI-X HD-600 high resolution X-ray CT scanner (Varian Medical Systems, Lincolnshire, IL) at the Center for Quantitative X-ray Imaging, Pennsylvania State University. The specimens were scanned with energy settings of 180 kV and 0.110 mA. Image data were reconstructed as 1024 × 1024 16-bit grayscale TIFF images with an *x*, *y* pixel size of 0.059 mm and slice thickness and spacing of 0.062 mm. The 16-bit images were converted to 8-bit TIFFs using ImageJ 1.46 to ensure that the gray values were scaled in the same way for all images in each specimen's dataset (Ryan and Walker, 2010; Griffin et al., 2010; Shaw and Ryan, 2012).

The volume of the bone and exterior surface area of each attached tooth was measured from the CT scan data. Each tooth was defined using a combination of automatic and manual histogram-based segmentation methods. Once the individual teeth were segmented, the three-dimensional volumes and surface areas of the crown of the tooth, tooth waist, and the base of tooth

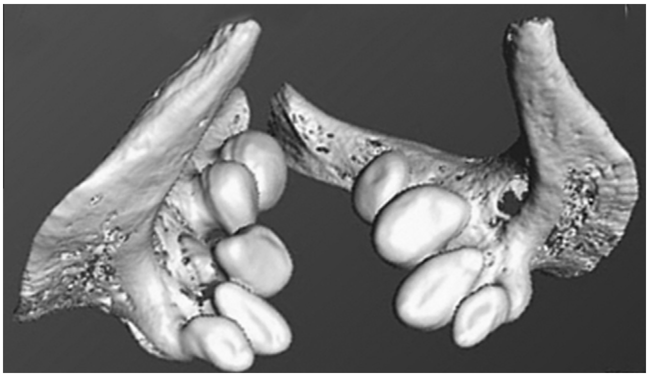

Fig. 1. Three-dimensional CT scan of black carp pharyngeal bones and teeth.

attached to the pharyngeal bone were calculated (Fig. 1). All processing and analysis of the CT data were performed in Avizo 6.3.

2.4. Data analysis

Surface area, volume, and weight of pharyngeal bones and teeth were compared between the two groups using linear regression adjusting for standard length of the fish; all measures were $\log_n$ transformed before the analysis. Differences with p-values < 0.05 were considered significant.

3. Results

Three black carp specimens in the group raised on soft food (12%) and seven specimens in the snail-eating group (28%) died. The standard length and the weight ranges of black carp fed on hard diet were 158–235 mm and 80–244 g and on soft food were 154–205 mm and 74–174 g. Fish fed on hard diet on average were 25.5 mm longer ($p < 0.001$) and on average 63.4 g heavier ($p < 0.001$) than those fed on soft diet. Most of snails released as food for black carp were consumed and enclosures had a lot of shell fragments at the bottom.

The number of replacement teeth of individual black carp specimens ranged from 5 to 9. The average number of replacement teeth of black carp in the snail-eating group was 1.14 times higher compared to the group fed soft food, and this difference was statistically significant ($p < 0.05$).

The value of both the volume (pharyngeal bones plus teeth), dry weight, and the exterior surface area increased with size of black carp (Fig. 2). Hence, comparisons between the two groups were adjusted for standard length. The volume of attached teeth in the group fed on soft food was only 49% of that in the snail-eating group ($p < 0.01$). The slopes of the two regression lines did not differ significantly. Although the surface area increased with fish size, the difference between the two groups of fish was not significant. Dry weight (bone plus teeth) increased with fish size and the slopes of the regression lines for the two groups were not significantly different and the weight of bones/teeth from the group fed on soft diet was only 50.7% ($p < 0.001$) of that of a fish of the same size fed hard diet (Fig. 2c).

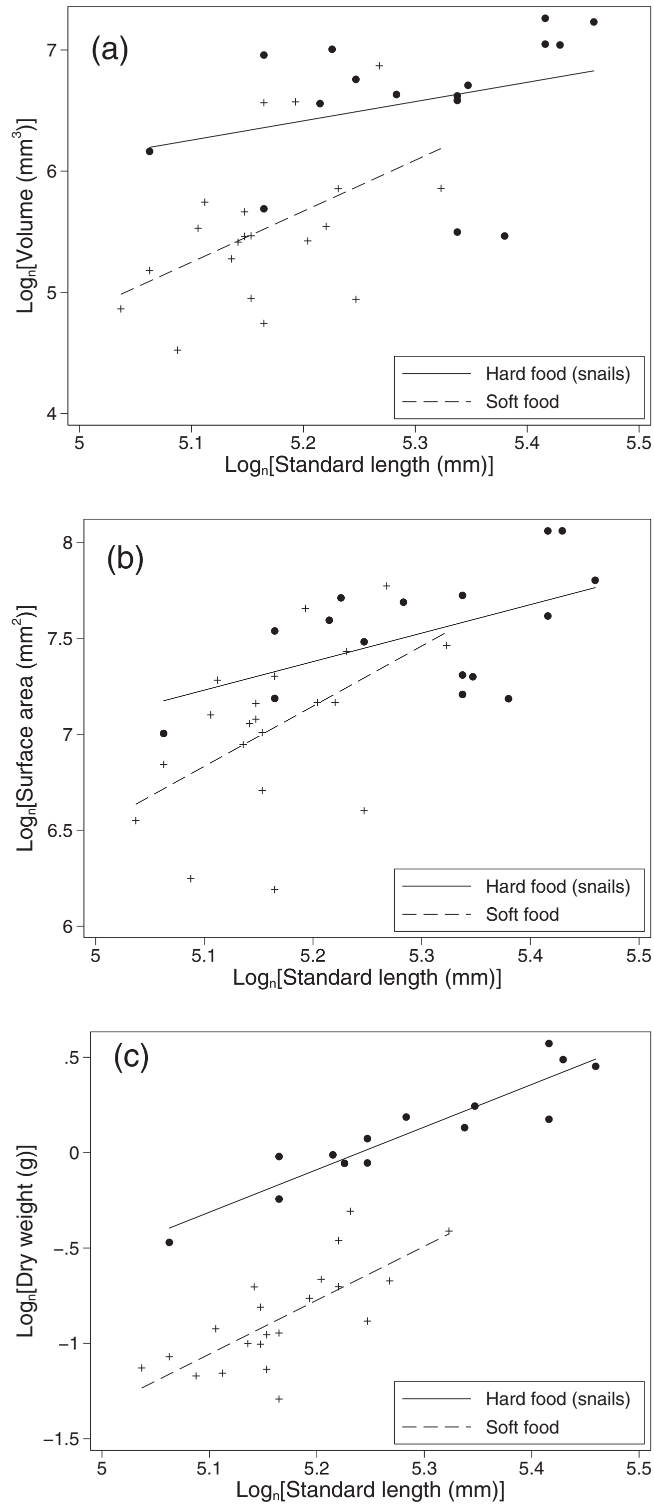

Fig. 2. Volume (a), surface area (b) and dry weight (c) of pharyngeal bone and attached teeth from black carp fed either hard or soft diet relative to standard length of the fishes.

4. Discussion

The results indicate that the feeding regimen not only influenced the grow out of black carp, but also the development of the pharyngeal crushing mill, based on the dry weight of the pharyngeal bones and teeth, and the volume of attached teeth. The black carp fed on snails seemed to grow faster, and the pharyngeal bones and teeth were more developed than the group fed commercial fish food, but mortality was higher. Interestingly, the fish fed on snails had more replacement teeth than those fed on soft food. Thus, we concluded that the black carp is a facultative snail eater and can survive on other diets.

Although we did use the smallest size of black carp available, they already had developed the molariform teeth. Liu et al.

(1990) mentioned the fixed teeth of black carp are present early in its life span (26 days after hatching). The pharyngeal apparatus of black carp 30–330 mm in total length may be capable of masticating and grinding molluscs. Unfortunately, we could not get information about the maintenance conditions prior to our purchase of experimental fish; the fish could well have been from various nurseries. The pharyngeal teeth of black carp are replaced several times in its life cycle (Liu et al., 1990) like other cyprinid fish species, such as big head carp *Aristichthys nobilis* (Nakajima and Yue, 1989), Indian major carp *Cirrhinus mrigala* (Kumari et al., 2009), common carp, *Cyprinus carpio* (Eastman, 1971; Sibbing, 1982), and some other species (Eastman and Underhill, 1973). When the old tooth is thin, it will be absorbed and dropped and subsequently replaced by a new one. In case black carp consume hard food such as snails and bivalves, the teeth may be worn thin faster than when kept on soft food diet. This could be the reason for the increased number of replacement teeth in snail-eating group.

These results indicate that food type affects development of the pharyngeal crushing mill of black carp and it highlights the importance of the feeding regimen if black carp are produced for snail control. The feeding of juvenile black carp should include snails from as early as possible in the production cycle. This study also supports the conclusion that food source affects the morphology and feeding performance of fishes (Slootweg et al., 1993; Huysseune, 1995; Mittelbach et al., 1999; Bouton et al., 2002; Grubich, 2003; Hulsey et al., 2005, 2008; Binning et al., 2010).

In conclusion, the pharyngeal bones and teeth of black carp *M. piceus*, show significant adaptive modification which was associated with the food sources.

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