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Origin of the mass mortality of the flathead grey mullet (*Mugil cephalus*) in the Tanshui River, northern Taiwan, as indicated by otolith elemental signatures

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ABSTRACT

A massive fish kill of approximately 50 tons of flathead grey mullet (*Mugil cephalus*) occurred in August 2006 in the Tanshui River, northern Taiwan. To clarify the origin of these mullets, 42 individuals were randomly collected to determine their ages by reading otolith annuli and to measure the trace elements in the otolith. The total lengths of the fish averaged 32.7 ± 4.4 cm, and most of the individuals were 2 years old, indicating that the mullets in the mass fish kill event were in the immature and growth stages. The Sr/Ca and Ba/Ca ratios of a subsample of 14 otoliths showed that half of the fish killed were estuarine residents, and the rest originated from marine and freshwater habitats. The multiple causes involved in this fish kill implied that additional unknown factors influenced the mullet populations on a large scale and induced them to move quickly and gather at this estuary.

1. Introduction

The flathead grey mullet (*Mugil cephalus* L.) is a cosmopolitan euryhaline fish that is widely distributed in coastal waters, lagoons, and estuaries between the longitudes of 42°N and 42°S. It is an economically important species associated with both capture fisheries and aquaculture in many countries. Fish kill events often occur throughout the world in estuaries as geographically diverse as those in Australia and the US (Walsh et al., 2004; Thronson and Quigg, 2008). Mass mortality events of the mullet population in the Tanshui River in northern Taiwan have been occasionally reported to the public since 2002. The most serious of these mass mortality events occurred on August 10, 2006. Approximately 50 tons of mullet died in this event, which was close to 25% of the annual mullet production of 193 tons in 2006, as reported by the Fisheries Agency of Taiwan. In fact, the actual amount of dead mullet might have been underestimated because the total mass of the dead fish was not completely reported as a result of being washed away and/or sinking to the bottom of the river.

Fish kills clearly lead to fish de-population, economic losses, human health risks, and degradation of water quality. Most investigations concerning the causes of fish kills focus on factors, such as human impacts, pollution, salinity, abrupt temperature changes, oxygen depletion and toxic dinoflagellate blooms (Dawson, 2002; Hoeksema et al., 2006; Becker et al., 2009). However, few studies determine or even question the origin of the fish involved. If the origin and the migratory pattern of the fish involved are not clear, it is difficult to determine the causes of fish kills and to implement effective environmental monitoring, fish conservation and fishery management programs.

Otoliths (ear stones) are located in the inner ear of teleost fish and regulate their balance and hearing (Lowenstein, 1971). Otoliths are deposited in periodic increments in calcium carbonate-rich and protein-rich layers that permit us to determine the age of a fish on daily and annual scales (Pannella, 1971). Generally speaking, the elements deposited in increments in the otolith are positively correlated with the ambient water experienced by the fish (Campana, 1999). Thus, the temporal change of the elemental composition of the otolith allows us to reconstruct the past migratory environmental history of the fish (Campana et al., 2000; Chang et al., 2004a; Wang et al., 2010).

The present study investigated the origin of the dead mullets involved in the mass mortality event in the Tanshui River in 2006 by reconstructing the migratory environmental history of these mullets and by examining the elemental composition of the otoliths of these fish. Understanding the origin of the fish will provide useful information for gaining insight into the cause of this particular event of mass mortality of mullets in the Tanshui River.

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2. Materials and methods

2.1. Study area and sample collection

A total of 42 specimens of *M. cephalus* were randomly collected from the fish kill in the estuary of the Tanshui River at 3 pm on August 11, 2006 (Fig. 1). The in situ environmental factors at the sampling site were as follows: water temperature 29.3 °C, dissolved oxygen 5.24 mg l^{-1}, salinity 4.5 PSU and pH 7.82. After collection, the fish were immediately preserved on ice. The mullets involved in this event were all partially rotted, especially in the abdominal region. Therefore, the body weight of the mullets could have been biased and was therefore not measured. Only the fork length (FL, cm) of the fish was measured.

2.2. Otolith preparation for age determination

The sagittal otoliths were removed, ultrasonically cleaned with de-ionized water, and air-dried. The otoliths were then embedded in epoxy resin (Epofix, Struers). A thin transverse section of the otolith, approximately 300 μm in thickness, was cut with a low-speed diamond saw and then polished using a grinder-polisher (Isomat and Metaserv, Buehler) until the primordium was exposed. The polished otolith section was cleaned with de-ionized water in an extra-sonic water bath. The annulus in the otolith section was then read to determine the age of the mullet, following the methods of previous studies (Hsu and Tzeng, 2009; Wang et al., 2010).

2.3. Measurement of the otolith elemental composition by LA-ICP-MS

Following age determination, a subsample of 14 otoliths representative of the length and age distributions of all 42 specimens (Fig. 2) was selected to measure their elemental composition. The otolith was immersed in 5% ultrapure hydrogen peroxide (H_2O_2) for 80 s to oxidize the remaining organic material from the section's surface, then rinsed using de-ionized water, air-dried in a class 100 clean room and subsequently adhered to a petrographic slide (Buehler). The petro slide, containing three otolith sections, was placed in a sealed chamber. Measurement of the elemental composition in the otolith was programmed to transect along the dorsal–ventral axis of the otolith using an inductively coupled plasma mass spectrometer (ICP-MS, Finnigan MAT ELEMENT II, Thermo Electron Corp.) equipped with a laser microprobe (Merchantek LUV 266 nm Nd: YAG UV, New Wave Research Inc.). The laser was set to a repetition rate of 20 Hz, scan speed of 15 μm per second, ablation diameter of 150 μm, and to collect one data point approximately every 2.46 s. The measured elements included ^{23}Na, ^{24}Mg, ^{44}Ca, ^{55}Mn, ^{88}Sr, and ^{138}Ba, which consistently remained at least 10 times higher than the background levels.

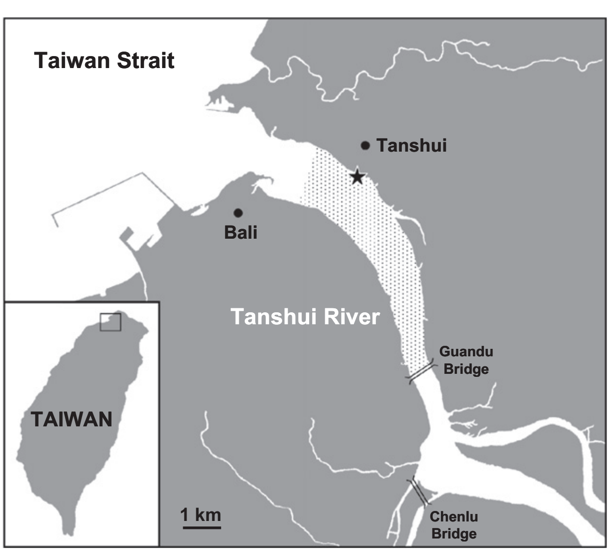

Fig. 1. Distribution of *Mugil cephalus* mass mortality in the Tanshui River (shaded area) and the location where the fish and water samples were collected on August 11, 2006 (star).

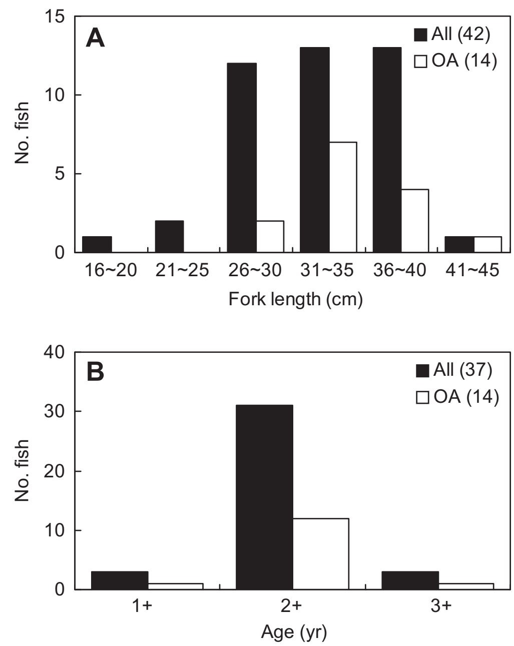

Fig. 2. Length (A) and age (B) frequency distributions of *Mugil cephalus* collected in the fish kill event of August 2006 referring to all specimens (All, solid histogram) and the subsample used for otolith elemental composition analysis (OA, open histogram). Numeral in parenthesis: sample size.

At the beginning of each otolith analysis, background counts were collected for 30 s, and the average was subtracted from the sample counts to correct for the background levels. Then, standards (National Institute of Standards and Technology 612 glass standard, NIST 612) were collected before each analysis series. The ablation chamber was purged for 60 s after sampling each otolith and standard. All counting data were expressed as ratios of the element to Ca concentrations (ppm ppm^{-1}) by estimating the relative response factor of the instrument to the known concentration in the standard.

2.4. Data analysis

The Sr/Ca and Ba/Ca concentration ratios in the otoliths vary according to fish migrating between seawater and freshwater (Chang et al., 2004b; Elsdon and Gillanders, 2005). For *M. cephalus*, Sr/Ca ratios in the otolith between 3×10^{-3} and 6×10^{-3} indicate an estuarine resident, and those with a ratio smaller or larger than this range are defined as freshwater and seawater residents, respectively (Chang et al., 2004a,b; Wang et al., 2010). Moreover, if the otolith Ba/Ca ratios are higher than 124×10^{-6}, this implies that the fish remain in freshwater (Wang et al., 2010). Therefore, temporal changes in the Sr/Ca and Ba/Ca ratios in the otolith are reliable for reconstructing the migratory environmental history and habitat use of *M. cephalus*. Discriminant function analysis was used to test for differences in the elemental composition of the otolith among different life stages using STATISTICA© Version 6.1. The life stages were classified by seasons (summer and winter)