

1 **Application of otolith shape analysis in identifying different ecotypes of**  
2 ***Coilia ectenes* in the Yangtze Basin, China**

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23 ABSTRACT

24 The variability in otolith shape of the tapertail anchovy *Coilia ectenes* was investigated as  
25 a tool for identifying its different ecotypes. The outlines of 350 sagittal otoliths of known  
26 ecotypes collected from seven sampling areas, covering most of the distributional range  
27 of the species in the Yangtze Basin, were digitized and analyzed. Canonical Discriminant  
28 Analysis (CDA) of the shape indices and Fourier descriptors pooled together showed  
29 three distinct clusters of individuals representing anadromous, land-locked, and  
30 freshwater-resident ecotypes. A test of the classification success of CDA indicated that  
31 individuals of the freshwater-resident ecotype could be efficiently discriminated from  
32 either the anadromous or the land-locked ecotypes. A high rate of misclassification of the  
33 anadromous ecotype as the land-locked ecotype indicated some weakness when  
34 distinguishing individuals of these two ecotypes from each other. Average otolith shapes  
35 of the anadromous and the land-locked ecotypes shared similarities in the form of the  
36 rostrum and antirostrum, while the freshwater-resident ecotype showed marked  
37 dissimilarity from them. The information generated in this study is useful in  
38 ecotype-adapted resource assessment and management of *C. ectenes* in the Yangtze Basin.

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40 *Keywords: Coilia ectenes; Ecotype discrimination; Otolith shape analysis; Elliptical*  
41 *Fourier analysis; Yangtze Basin.*

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## 44 **1. Introduction**

45

46 Many fish populations consist of multiple stocks. Each stock may have unique  
47 demographic properties and responses to exploitation, and should be managed separately  
48 to ensure sustainable fishery benefits and efficient conservation (Begg and Brown, 2000;  
49 Stransky et al., 2008; Neves et al., 2011). Stock identification of fish is also fundamental  
50 for understanding the population dynamics of a species in an ecological sense (Tracey et  
51 al., 2006). There is a wide variety of methods applied in fish stock identification, e.g.,  
52 morphological analysis, the investigation of environmental signals (markers) and genetics  
53 (Cadrin et al., 2005). Otolith shape may vary according to geographical site, depth and/or  
54 other environmental factors (De Vries et al., 2002; Cardinale et al., 2004), and has been  
55 applied as an effective tool for stock identifications in several species (Pothin et al., 2006;  
56 Tracey et al., 2006; Merigot et al., 2007; Stransky et al., 2008; Neves et al., 2011).

57 The tapertail anchovy *Coilia ectenes* (Jordan and Seale 1905) (Clupeiformes:  
58 Engraulidae) is a small- to moderate-sized fish. It is distributed widely in the river  
59 systems and coastal waters of the western Pacific Ocean, mainly near the Yellow Sea  
60 (Whitehead et al., 1988), and is an important fishery resource in the middle and lower  
61 reaches of the Yangtze River and its affiliated lakes (Li et al., 2007; Ma et al., 2011).  
62 Three ecotypes of this species have been identified for the Yangtze River population: 1)  
63 the anadromous ecotype feeds and over-winters in the Yangtze Estuary and nearby  
64 coastal waters, and the adults migrate up the river for spawning; 2) the land-locked  
65 ecotype originated from the anadromous ecotype and now is confined to some lakes in the

66 lower reaches of Yangtze River, e.g. Taihu Lake and Chaohu Lake; and 3) the  
67 freshwater-resident ecotype inhabits the middle and lower reaches of the Yangtze River  
68 and the affiliated lakes, and does not have a migratory phase between seawater and  
69 freshwater (Zhang, 2001). Resource status of the anadromous ecotype has declined  
70 significantly since the late 1970's due to intensive anthropogenic disturbances such as  
71 overfishing, fry by-catch and habitat degradation (Yuan & Qin, 1984; Liu et al., 2004; Li  
72 et al., 2007; Ma et al., 2011). Annual catches of the anadromous ecotype recorded at the  
73 Yangtze Estuary declined from  $3.9 \times 10^5$  kg in 1973 to  $2.5 \times 10^4$  kg in 2003 (Shi and  
74 Gong, 2003). Historically, upstream migration of the anadromous ecotype initiated in  
75 February, forming a primary catch season in the estuary that lasted through June, but now  
76 the catch season usually begins in late March or early April, and lasts for only several  
77 days (Huang, 2005). Further, the migration historically reached Dongting Lake in the  
78 middle reach of the Yangtze River, but now it remains below the tributary of Poyang Lake  
79 (Huang, 2005). Economic value of the land-locked and the freshwater-resident *C. ectenes*  
80 is much lower than the anadromous fish (Li et al., 2007). While the abundance of the  
81 anadromous ecotype declining, the land-locked and the freshwater-resident ecotypes have  
82 maintained a healthy resource status, and have even shown slight increases (Yuan and Qin,  
83 1984; Liu et al., 2004; Li et al., 2007).

84 As the three ecotypes of *C. ectenes* are prone to intermixing along Yangtze,  
85 identifying an efficient methodology for their distinction is imperative. In several cases of  
86 recent morphological and genetic studies, controversy arose and ultimately failed to  
87 differentiate the different ecotypes. Significant genetic separation of *Coilia ectenes*

88 samples collected from different regions of Yangtze was identified by Ma et al. (2004) ,  
89 however recent genetic studies showed between-population haplotype frequency  
90 differences as very low, countermanding the idea of genetic heterogeneity (Cheng and Lu,  
91 2005; Cheng et al., 2008). Similarly, morphological studies concluded that the  
92 anadromous and landlocked fish have overlapping character states, and were difficult to  
93 distinguish from one another (Yuan et al., 1976; Liu, 1995; Cheng and Han, 2004).  
94 Otolith shape analysis may provide an alternative way to identify different ecotypes of *C.*  
95 *ectenes*, as environmental and life history variations of ecotypes may induce  
96 corresponding variations in their otolith shape (Pothin et al., 2006; Stransky et al., 2008).  
97 In the present study, we investigated the variability in otolith shape of known-ecotype *C.*  
98 *ectenes* collected from different habitats in the middle and lower reaches of Yangtze  
99 Basin, to determine the efficiency of otolith shape analysis for ecotype identification in  
100 this species. Both otolith shape indices and elliptical Fourier analyses were applied to  
101 characterize otolith shapes. The results of the study may facilitate appropriate  
102 management planning for *C.ectenes* by parsing stock contributions from its multiple  
103 ecotypes.

104

## 105 **2. Material and methods**

106

### 107 *2.1. Sampling*

108

109 *C. ectenes* were sampled from seven areas in the Yangtze Basin during 2006 and 2007:  
110 1) at Chongming in the Yangtze Estuary; 2) in Taihu Lake; 3) at Jingjiang in the lower  
111 Yangtze River; 4) at Wuhu in the lower Yangtze River ; 5) at Anqing in the lower Yangtze  
112 River; 6) at Chibi in the middle Yangtze River; and 7) in the Tiane' zhou Oxbow (Table 1,  
113 Fig. 1). The sampling areas were selected based on current information regarding the  
114 boundaries of current ecotype units. Sampling areas 1, 3, 4 and 5 targeted the anadromous  
115 ecotype; area 2 the land-locked ecotype; and areas 6 and 7 the freshwater-resident ecotype.  
116 Specimens were collected from commercial catches harvested by gillnets (mesh sizes of  
117 30 to 40 mm). Each fish was measured for standard length (SL). Individuals of 175-275  
118 mm SL were selected for otolith analysis to minimize size-induced variations in otolith  
119 shape. Sagittal otoliths were taken as pairs, washed in clean water, air-dried, and stored in  
120 plastic tubes. The left otolith of each fish was used for shape analysis.

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## 122 2.2. Image and shape analyses

123

124 Digital images of each otolith were recorded with a DP72 digital camera fitted to  
125 an SZX16 stereo microscope (Olympus Inc, Tokyo). The microscope magnification  
126 was adjusted to the size of the otoliths to ensure the highest resolution possible,  
127 varying between 3× and 5×. The digitized image was then analysed using Sigma Scan  
128 Pro Version 5.0.0 image analysis software to measure its area ( $A_o$ ), perimeter ( $P_o$ ),  
129 maximum length ( $L_o$ ) and maximum width ( $W_o$ ). Otolith shape indices, including  
130 circularity, ellipticity, rectangularity and form factor, were then calculated (Table 2)

131 (Russ, 1990; Tuset et al., 2003). The digitized image was then subjected to elliptical  
132 Fourier analysis in the programme SHAPE (Iwata and Ukai, 2002). The Fourier  
133 power (FP) spectrum was calculated in order to determine the sufficient and necessary  
134 number of harmonics for the best reconstruction of the otolith outline (Pothin et al.,  
135 2006). We randomly chose a subsample of 35 otoliths (5 samples  $\times$  7 areas) for this  
136 purpose. As the first 13 harmonics reached 99% of the mean cumulated power (Fig. 2),  
137 the Fourier analysis indicated that the otolith shape of *C. ectenes* could be  
138 summarized by these thirteen harmonics, i.e. 52 ( $13 \times 4 = 52$ ) Fourier descriptors  
139 (FDs). However, the first three FDs derived from the 1st harmonic were not taken into  
140 account, because the outline reconstructed with these coefficients is a simple ellipse  
141 resulting in a maximum Fourier power (Merigot et al., 2007; Neves et al., 2011). So,  
142 only 49 FDs (1 FD from harmonic 1 and 4 FDs from harmonics 2–13) were used for  
143 the subsequent analyses. All FD amplitudes appeared to be normally distributed  
144 (Kolmogorov–Smirnov test of normality;  $P > 0.05$ ).

145

### 146 *2.3. Size correction and multivariate analyses*

147

148 To remove size-induced bias we used the residuals of the common within-group slope  
149 (Reist, 1985; Stransky, 2005), derived from the relationship between SL and shape  
150 indices, for subsequent analyses. Differences in shape indices between sampling areas  
151 were tested by one-way analysis of variance (ANOVA) followed by ad-hoc comparisons  
152 (Duncan's multiple range test). A Canonical Discriminant Analysis (CDA) was

153 performed with shape indices and FDs pooled together, to compare otolith shape  
154 variations among the sampling areas. The classification success of the discriminant  
155 analysis was checked using jackknifed cross-validation (SPSS Inc., 1999). To visualize  
156 the shape differences between the groups, average otolith shapes were plotted for each  
157 group by means of the reproduced outlines of the averaged normalised FDs within a  
158 group.

159

### 160 **3. Results**

#### 161 *3.1. Shape analysis*

162

163 Otolith shape indices varied significantly among areas ( $p < 0.05$ ) (Table 3). Areas 1, 3,  
164 4, and 5 consistently were not significantly different from each other for all of the  
165 different shape indices, while area 7 consistently varied from areas 1, 2, 3, 4 and 5 in all  
166 shape categories except ellipticity. The pattern of grouping of areas was not found  
167 consistent between the different shape indices (Table 3).

168 The first two discriminant functions of the CDA performed with shape indices and  
169 FDs pooled together accounted for 88.5% of the variance (Table 4). Individuals could be  
170 significantly discriminated into three distinct groups based on the first two functions  
171 (Wilk's  $\lambda = 0.08$ ,  $p < 0.001$ ) (Fig. 3). The first group was composed mainly of individuals  
172 from areas 1, 3, 4, and 5, representing the anadromous ecotype; the second group was  
173 composed mainly of individuals from area 2, representing the land-locked ecotype; and  
174 the third group was composed mainly of individuals from areas 6 and 7, representing the

175 freshwater-resident ecotype. The jackknifed classification results validate most of the  
176 patterns observed in the CDA analysis (Table 5). Within-ecotype misclassifications for the  
177 anadromous ecotype areas and the freshwater-resident ecotype areas were high (up to  
178 34.5% and 41.7% respectively, Table 5), but samples from either of these ecotype areas  
179 were rarely (up to 4.9%) misclassified as being from areas in the other ecotype.  
180 Misclassifications of fish from area 2 (representing the land-locked ecotype) into the  
181 anadromous ecotype areas (up to 11.4%) and vice versa (up to 18.1%) were higher than  
182 that with the freshwater-resident ecotype areas (2.3% and 0%, respectively; Table 5).  
183 Overall area-classification success was 53.2%, ranging from 24.5% for area 1 to 86.9%  
184 for area 7 (Table 5). Jackknifed cross-validation, testing the classification success of the  
185 three ecotypes, showed that the classification success for the freshwater-resident ecotype  
186 was 100%, and for the anadromous and the land-locked ecotypes were 82.7% and 90.9%,  
187 respectively (Table 6). Misclassifications of the anadromous ecotype into the land-locked  
188 ecotype, and vice versa, were 14.9% and 9.1% respectively (Table 6).

189

### 190 *3.2. Shape visualization*

191

192 The average shape of the otoliths showed distinct differences among the three  
193 ecotypes (Fig. 4). There was a well-developed antirostrum for both the anadromous and  
194 the land-locked ecotypes, but not for the freshwater-resident ecotype. On the other hand,  
195 the rostrum in the freshwater-resident ecotype was more pronounced and pointed than the  
196 other two ecotypes. The ventral side was deeper for the land-locked ecotype otoliths than

197 the other two, while the dorsal was more raised and the posterior end more pointed for the  
198 freshwater-resident ecotype. Generally, the otolith shapes were more similar between the  
199 anadromous and the land-locked ecotypes than either of those with the  
200 freshwater-resident ecotype.

201

#### 202 **4. Discussion**

203

204 Our results demonstrate the efficiency of otolith shape analysis as a tool to  
205 discriminate individuals of different ecotypes of *C. ectenes* in the middle and lower  
206 reaches of the Yangtze Basin. CDA of otolith shape data revealed three distinct clusters of  
207 individuals representing anadromous, land-locked and freshwater-resident ecotypes  
208 respectively. The jackknifed classification tests validate the observed patterns in the CDA  
209 analysis. The test of the classification success verified the high efficiency of CDA to  
210 distinguish the freshwater-resident ecotype from both the anadromous and the  
211 land-locked ecotypes, while it was weak in separation success between the anadromous  
212 and the land-locked ecotypes. The results of shape visualization demonstrated that otolith  
213 of the anadromous and land-locked ecotypes share many similarities while the  
214 freshwater-resident ecotype showed marked differences, especially in the nature of the  
215 antirostrum and rostrum. For *C. ectenes* in the middle and lower Yangtze Basin, the  
216 anadromous fish mix with the freshwater-resident fish only in some parts of the  
217 mainstream of the Yangtze River or in the affiliated lakes (Cheng and Han, 2004); the  
218 land-locked fish are commonly mixed with the freshwater-resident fish in some lakes

219 isolated from the Yangtze River; and the anadromous fish usually do not mix with the  
220 land-locked fish. Thus, identifying individuals of the anadromous ecotype from the  
221 land-locked ecotype, and vice versa, is actually unnecessary. Otolith shape therefore can  
222 be used as an effective tool for discriminating the different ecotypes of *C. ectenes* in the  
223 Yangtze Basin. These results mirror some other studies, where otolith shape variation has  
224 also proven effective for identifying different stocks of a variety of marine fish species,  
225 e.g. goat fish *Mulloidichthys flavolineatus* (Pothin et al., 2006), striped trumpeter *Latris*  
226 *lineata* (Tracey et al., 2006), common sole *Solea solea* (Merigot et al., 2007), horse  
227 mackerel *Trachurus trachurus* (Stransky et al., 2008) and bluemouth, *Helicolenus*  
228 *dactylopterus* (Neves et al., 2011).

229 Both environmental and genetic factors can influence the otolith shape (Cardinale et  
230 al., 2004). Historically, Taihu Lake was connected to the Yangtze River at its downstream  
231 end, and *C. ectenes* used to move freely between the river and the lake for feeding and  
232 spawning (Cheng and Han, 2004). The lake later was disconnected from the river, and the  
233 anadromous *C. ectenes* that had entered the lake were thus blocked, forming a  
234 land-locked population (Cheng et al., 2005). Recent morphological and genetic studies  
235 also provide evidence for the higher similarity between the anadromous and land-locked  
236 ecotypes of *C. ectenes* in Yangtze Basin compared to the similarity between each of them  
237 to the freshwater-resident ecotype (Cheng and Han, 2004; Cheng et al., 2006; Cheng et al.,  
238 2008). Thus, we suggest that the high similarity of otolith shapes between the anadromous  
239 and the landlocked ecotype, and the apparent deviation of them to the freshwater-resident  
240 ecotype, may mainly reflect the genetic relationships and distinctions between these

241 ecotypes.

242 Our results provide an efficient approach for distinguishing individuals of the  
243 different ecotypes of *C. ectenes* through otolith shape analysis. These results could then  
244 be applied to clarify the contribution of different ecotypes to specific intermixed  
245 populations. Combined with the demographic analysis and stock assessment of the  
246 different ecotypes, a multiple-ecotype adaptive management strategy could be developed,  
247 which may improve sustainable management and conservation of this important fisheries  
248 resource in the Yangtze Basin. Our results are also an addition to verifying the efficiency  
249 of otolith shape analysis for stock identification, particularly for species with multiple  
250 ecotypes/stocks.

251

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