Geographic variation in pigmentation patterns of Indo-Pacific humpback dolphins (Sousa chinensis) in Chinese waters

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The pigmentation patterns of Indo-Pacific humpback dolphins have not been well studied throughout most of the species’ range. In the present study, both the subjective scoring method and a newly developed quantified scoring method were employed to evaluate the spotting intensity of 137 humpback dolphin individuals from the Xiamen and Beibu Gulf putative populations, including the Dafengjiang-Nanliujiang River Estuary (DRE) and Shatian-Caotan (SC) communities. Both scoring methods indicated that spotting intensity on the dorsal fin was lower than that on the body of humpback dolphins in all 3 groups. The SC and DRE humpback dolphins had significantly greater dorsal fin, body spotting intensity, and greater differences between spotting on the dorsal fin and body than Xiamen dolphins, while no differences were found between SC and DRE. The pigmentation variation is related to age class, young dolphins have more spotting density than adults, and young and adults showed similar geographical variation as above. The present paper, in combination with previous research, clarifies the general pattern of pigmentation for Chinese humpback dolphins. Eastern Taiwan Strait and Pearl River Estuary populations represent 2 extreme patterns of pigmentation, while the Xiamen population, SC community, and DRE community seem to be intermediate. The results suggest that these groups should be viewed as demographically distinct forms.

Key words: Beibu Gulf, coloration, Indo-Pacific humpback dolphin, photo-identification, pigmentation pattern, Xiamen

Compared to most mammals, cetaceans are not a particularly colorful group (Stockin and Visser 2005), but geographical variation of color patterns does occur in cetaceans (Perrin 1972). Despite limited light penetration in the oceans, coloration patterns are important in cetaceans, presumably having ecological function and adaptive value (Perrin 2009; Caro et al. 2011). Most species show rather limited variation within their typical pigmentation patterns (Stockin and Visser 2005); however, a few species or genera show extensive color variation, for example, beluga whales (Delphinapterus leucas—Brodie 1989) and humpback dolphins (Sousa spp.—Jefferson and Karczmarski 2001; Wang et al. 2008; Jefferson and Rosenbaum 2014).

In dolphins of the genus Sousa, young calves and juveniles are typically devoid of spots in all 4 species (Jefferson and Rosenbaum 2014; Brown et al. 2015; Jefferson et al. 2015). Adults of the Indo-Pacific humpback dolphin (Sousa
**Materials and Methods**

The Xiamen, DRE, and SC Indo-Pacific humpback dolphins were photographed in the wild. The research team had a special permit from Animal Research Ethics Committee of Nanjing Normal University. During encounters with dolphins, efforts were made to photograph all members of the group of dolphins seen during a sighting and to avoid disturbance. If dolphins displayed boat avoidance behavior, such as deep dives, long down times, changes in direction, or fleeing and fast swimming, encounters were ended.

The DRE humpback dolphins and SC dolphins possibly belong to the same population inhabiting the northern Beibu Gulf during our research. Because the photographic comparison found no matches of individuals between DRE and SC, they were considered as separate communities in this paper. Their data were therefore analyzed separately. In total, photographs of 137 individual dolphins were selected for comparison: Xiamen \((n = 36)\), SC \((n = 23)\), and DRE \((n = 78)\).

Spotting was scored by 2 methods: 1) a subjective scoring method developed by Wang et al. (2008, 2015), and 2) a quantified scoring method developed by us. For the subjective scoring method, the density of spotting on the dorsal fin and on the body below the dorsal fin was scored on a scale from 1 to 4 (Wang et al. 2008; see examples in Fig. 2, left). The photographs were scored independently by 6 scorers. Four scorers were cetacean researchers (each familiar with this species), and 2 were researchers with no experience with humpback dolphins.
The subjective scoring method resulted in integer score data, which possibly produced experience-related and subjective biases. Therefore, we developed a new quantified scoring method, which is more objective. The threshold method was applied to digitize the photographs of dolphins into white background and black spots (Fig. 2, right). Then, the percentages of black spots on the dorsal fin and on the body were calculated using Image-Pro Plus 6.0 (Ahern 1998). The differences of body-to-dorsal fin spotting (body score minus dorsal fin score) were also calculated.

The differences of spotting scores of humpback dolphins from different groups were tested. One-way analyses of variance (ANOVAs) and multiple pairwise t-tests were used to test between populations (Xiamen, DRE, and SC), based on the scores of dorsal fin, body, and the difference of body-to-dorsal fin. A 1-sample t-test was conducted comparing the pigmentation scores of Xiamen, DRE, or SC with the mean scores of ETS or PRE from data in Wang et al. (2008) (Table 1). All the tests were performed with a 0.05 significance level, using IBM SPSS Statistics version 19 (Gray and Kinnear 2012). For the subjective score scatterplot, the data (subjective body and dorsal fin score) for ETS and PRE were preliminarily estimated from figure 2 of Wang et al. (2015).

To explore the influence of age class and consider sample size, we re-classified all dolphins into 2 main age classes (young: mottled and speckled, adult: spotted and unspotted). Then, we compared differences in their pigmentation pattern.

### Results

Based on the subjective scoring method, the mean intensity of spotting on dorsal fins of all 3 groups was less than that on the bodies (Table 1), i.e., Xiamen (2.03 versus 2.74), SC (2.97 versus 3.34), and DRE (2.76 versus 3.16). The mean difference of body-to-dorsal fin of Indo-Pacific humpback dolphins of the 3 populations varied from 0.37 to 1.40, but was mostly < 1.0 (Fig. 3). Xiamen had significantly less spotting intensity on the dorsal fin and body than SC, and had significantly less spot intensity on the dorsal fin and greater score difference of body-to-dorsal fin (Tables 1 and 2). The frequency distribution of different spotting intensity of the dorsal fin of Xiamen humpback dolphins was significantly different from that of SC or DRE groups, whereas the frequency distributions for the body and dorsal fin were not significantly different.

#### Table 1

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Body score (mean ± SE)</th>
<th>Dorsal fin score (mean ± SE)</th>
<th>Difference of body-to-dorsal fin scores (mean ± SE)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETS</td>
<td>31</td>
<td>2.92 ± 0.13</td>
<td>3.3 ± 0.13</td>
<td>-0.38 ± 0.08</td>
<td>Wang et al. (2008)</td>
</tr>
<tr>
<td>Xiamen</td>
<td>10</td>
<td>2.6 ± 0.37</td>
<td>1.53 ± 0.14</td>
<td>1.07 ± 0.24</td>
<td>Wang et al. (2008)</td>
</tr>
<tr>
<td>Xiamen</td>
<td>36</td>
<td>2.74 ± 0.15</td>
<td>2.03 ± 0.14</td>
<td>0.72 ± 0.09</td>
<td>This study</td>
</tr>
<tr>
<td>PRE</td>
<td>186</td>
<td>2.66 ± 0.07</td>
<td>1.43 ± 0.04</td>
<td>1.23 ± 0.05</td>
<td>Wang et al. (2008)</td>
</tr>
<tr>
<td>SC</td>
<td>23</td>
<td>3.34 ± 0.14</td>
<td>2.97 ± 0.19</td>
<td>0.37 ± 0.11</td>
<td>This study</td>
</tr>
<tr>
<td>DRE</td>
<td>78</td>
<td>3.16 ± 0.10</td>
<td>2.76 ± 0.11</td>
<td>0.40 ± 0.06</td>
<td>This study</td>
</tr>
</tbody>
</table>

*1 adult (a black patch in the left front body) was the same individual used in Wang et al. (2008).
body-to-dorsal fin difference (Fig. 3, left) among the 3 groups were similar.

Based on the quantified scoring method, the mean intensity of spotting on dorsal fins of all 3 groups was less than that on the bodies (Table 1), which is the same pattern detected by the subjective scoring results. The mean spotting percentage on the dorsal fins and on the bodies of humpback dolphins was greatest in DRE and was lowest in Xiamen (Table 3). The difference of body-to-dorsal fin was the lowest in Xiamen and the highest in SC. The frequency distributions of spotting intensity of the dorsal fin and difference of body-to-dorsal fin were similar among the 3 groups, whereas it was different in spotting of the body (Fig. 3, right). When comparing the pigmentation percentage of the 3 dolphin groups, the SC and DRE humpback dolphins had

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**Fig. 3.**—The frequency distributions of subjective scores (left) and quantified scores (right) of spotting intensity of the dorsal fin (top), body (middle), and body-to-dorsal fin comparison (bottom) of Indo-Pacific humpback dolphins (*Sousa chinensis*). SC = Shatian-Caotan population; DRE = Dafengjiang-Nanluijiang River Estuary population.
significantly greater dorsal fin and body spotting intensity than Xiamen dolphins, while no distinctive difference in pigmentation was found between SC and DRE groups (Table 4).

The scatterplot of the quantified scores indicated that SC and DRE humpback dolphins were separated from those in Xiamen (Fig. 4, top left). In the trend line analysis, the following functions were calculated: Xiamen: $Y = 0.3651X + 0.0152$; SC: $Y = 0.8006X - 0.0783$; and DRE: $Y = 0.6726X - 0.0494$. The slope for Xiamen was significantly shallower than that for SC and DRE, and the intercept for Xiamen was positive, whereas the intercepts for SC and DRE were negative. These points indicated that the spotting intensity of Xiamen humpback
dolphins was different from that of SC and DRE communities. The young dolphins in SC showed differences from Xiamen and DRE (middle left), but the adults (bottom left) were similar to the general trend (top left).

When comparing all 6 geographic areas from our data plus those of Wang et al. (2008, 2015), including ETS and PRE groups, the scatterplot of subjective score and trend lines showed differences in pigmentation among 5 groups (Fig. 4, top right). The trend line equations produced were: ETS: $Y = 0.8315X + 0.9393$; Xiamen: $Y = 0.7659X + 0.073$; PRE: $Y = 0.4577X + 0.2335$; SC: $Y = 1.0525X - 0.5493$; and DRE: $Y = 0.8481X + 0.078$. The slopes varied from 0.4577 in PRE to
1.0525 in SC. The intercept of ETS (0.9393) was significantly larger than that in all other populations, and the intercept of SC (−0.5493) was the smallest. The young (middle right) and adult (bottom right) dolphins showed patterns similar with the general trend (top right).

For mottled–speckled (young) and unspotted–spotted adults, significant geographical pigmentation differences were found (Table 4). For the quantified score of adults, the density of body spotting ($F_{2,80} = 4.928$, $P = 0.01$; multiple comparisons: Xiamen–SC, $P = 0.026$; Xiamen–DRE, $P = 0.011$) and differences of body-to-dorsal fin ($F_{2,80} = 4.786$, $P = 0.011$; Xiamen–SC, $P = 0.014$; Xiamen–DRE, $P = 0.019$) were geographically significant. The young dolphins showed geographical differences in pigmentation on dorsal fins ($F_{2,54} = 13.516$, $P < 0.001$; Xiamen–DRE, $P < 0.001$; Xiamen–SC, $P < 0.001$), body ($F_{2,54} = 12.12$, $P < 0.001$; Xiamen–DRE, $P = 0.001$; Xiamen–SC, $P = 0.007$), and difference of body-to-dorsal fin ($F_{2,54} = 3.875$, $P = 0.027$; DRE–SC, $P = 0.017$; SC–Xiamen, $P = 0.013$).

For subjective scores, the adult dolphins showed geographical variation in pigmentation on dorsal fins ($F_{2,80} = 6.224$, $P = 0.003$; Xiamen–DRE, $P = 0.006$; Xiamen–SC, $P = 0.008$) and body ($F_{2,80} = 4.521$, $P = 0.014$; Xiamen–SC, $P = 0.006$). Young dolphins also showed differences in pigmentation on dorsal fins geographically ($F_{2,54} = 17.346$, $P < 0.001$; Xiamen–DRE, $P = 0.001$; Xiamen–SC, $P < 0.001$; SC–DRE, $P = 0.038$), body ($F_{2,54} = 8.051$, $P = 0.001$; Xiamen–DRE, $P = 0.029$), and difference of body-to-dorsal fin ($F_{2,54} = 8.405$, $P < 0.001$; Xiamen–SC, $P = 0.001$; SC–DRE, $P = 0.001$).

**DISCUSSION**

In this study, we used only high-quality photos for analyses. Only photos with sharp focus were analyzed, and any blurred photos or photos with poor lighting were excluded. Although pigmentation changes over time, the change is limited throughout a 6- to 8-year period. Therefore, for each dolphin, only a single clear photograph was selected, and we believe this is adequate.

Both subjective and quantified scoring methods produced similar results, and we believe they are both appropriate methods. For the Xiamen population, the scores in the present paper were different from those of Wang et al. (2008), although the general pattern was similar. This might have resulted from different target dolphins, sample sizes, or age structures. Individual variation in scoring methods may have also played a part. The photos of the Xiamen animals used in the Wang et al. (2008, 2015) study were taken in the late 1990s ($n = 10$), whereas ours were taken in 2007–2010 ($n = 36$). Fortunately, we photographed the same individual dolphin (Btop in figure 2 of Wang et al. 2008). The pigmentation of this dolphin changed very little over at least 9 years.

Ontogenetic changes in coloration are widespread in cetaceans, such as those found in killer whales (*Orcinus Orca*—Evans et al. 1982), humpback whales (*Megaptera Novae-angliae*—Rosenbaum et al. 1995), pantropical spotted dolphins (*Stenella Attenuata*—Perrin and Hohn 1994), and Indo-Pacific bottlenose dolphins (*Tursiops aduncus*—Bichell et al. 2018), and geographic differences aid in taxonomic studies and the identification of various geographic forms, subspecies, and species (see Perrin 2009).

Of the 6 studied populations, the ETS and PRE groups seemed to represent 2 extreme pigmentation patterns, while Xiamen, SC, and DRE were likely to show more intermediate patterns (Tables 1 and 2; Fig. 4). Significant differences were found among these populations, and this suggests that there is distinctive geographical variation. Further, the differentiation of pigmentation patterns in the ETS population was used as one of the critical features for describing that form as a new subspecies, *S. c. taiwanensis* (Wang et al. 2015). The variation in pigmentation of these populations is irregular along their geographic locations (east to west, or south to north). Analyses of DNA supported the irregular geographic variation (Chen et al. 2010). The unique sample from the Beibu Gulf shared the same haplotype (CH02) with dolphins in Xiamen (Chen et al. 2010). Further research is needed to understand how DNA evolution is related to the change of pigmentation.

Pigmentation and spotting intensities in *S. chinensis* are known to be related to age and sex (Jefferson et al. 2012; Jefferson and Rosenbaum 2014), like the situation in some other cetaceans, e.g., *S. Attenuata* and *Tursiops* sp. (Krzeszczyn and Mann 2012). Despite the age-related variation in pigmentation, young dolphins (mottled and speckled) and adults (spotted and unspotted) both showed similar patterns of geographic variation of pigmentation. However, effects of sex remain unclear, due to the challenge of sex identification in the wild.

Combined, the present paper and previous work by Wang et al. (2008, 2015) have analyzed 4 of 8 putative Indo-Pacific humpback dolphin “populations” in China. There is general agreement among these studies, and they each reflect the general framework of variation in pigmentation of Indo-Pacific humpback dolphins in Chinese waters. For the other 4 populations, similar research to that presented here should be done. However, Ningde and Shantou appear to have small populations, and few identified individuals are currently available (e.g., there are only 4 dolphins from Ningde in our database). This would make such research challenging. However, due to its importance, we suggest that this be pursued in the future, despite the difficulties.

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LITERATURE CITED


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