



# Article First Suction Cup Tagging on a Small and Coastal Form Bryde's Whale (*Balaenoptera edeni edeni*) in China to Investigate Its Dive Profiles and Foraging Behaviours

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**Abstract:** Small-and-coastal-form Bryde's whales (*Balaenoptera edeni edeni*) are known to inhabit coastal and continental shelf waters. However, little is known on their ethological activities, especially underwater behaviours. Here, we deployed a suction cup tag on a small-and-coastal-form Bryde's whale to study its ethological activities in Dapeng Bay, a coastal water of Shenzhen off the east Pearl River Estuary, China. The whale was tagged for 6 hours in total and displayed a head-lifting feeding behaviour 77 times during two tag deployments. The swim speed, dive duration and depth and bottom duration were collected by the tag to describe detailed information on dive profiles. The rate of feeding behaviours was observed decreasing with the presence of anthropogenic whale-approaching (AWA) boat activities occurring close (within 300 m) to the animal. Our study, for the first time, investigated behaviours of Bryde's whales using suction cup tagging in Chinese waters. The results reveal the dive and feeding patterns of a small-and-coastal-form Bryde's whale with short-term monitoring and provide a fundamental advancement in the knowledge of both the diving behaviour and the behavioural response of this subspecies to human activities. The findings are valuable for possible rescue of the investigated whale and for policy and management regarding conservation of this subspecies as well.

Keywords: biologging; suction cup tag; dive; feeding behaviour; conservation measure

## 1. Introduction

Information on animal behaviour and ecology are essential to improve animal conservation and provide effective management measures. Since many marine species occur primarily or partially outside of the observable realm of researchers, it is often challenging to investigate their movement and ecology in the wild, while in captivity, it is difficult to preserve the health of the study species. As an essential tool in marine mammal research, the development of biotelemetry techniques nowadays allows researchers to track wild marine mammals and fills knowledge gaps in their habitat use and different behaviours [1-4]. The success and progression of telemetry studies on marine mammals have been documented in a number of publications [5-7]. We can infer animal behaviours, including underwater behaviours, from the collection of a wide range of biological and environmental information by the tags integrated with Global Positioning System (GPS), accelerometers, magnetometers, pressure sensors and acoustic recorders. Unlike the anchored and consolidated tags [8-11], tags with suction cups do not penetrate the animals' skin and can be used to investigate movements within a range of meters to kilometers for hours to days [12–18], making them applicable for important insights into habitat preferences [19,20], which is also important for conservation management in the light of increasing anthropogenic impacts on coastal habitats. Suction cup tags are more available for tagging large whales rather than pinnipeds and small odontocetes, not only because large whales cannot be easily captured



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and their sloughing skin makes the use of traditional epoxy or glue methods unfeasible, but also because large whales usually have a long surface time, convenient for deployment of suction cup tags.

The Bryde's whale (*Balaenoptera edeni*) is one of the least known baleen whales [21] and can be sighted throughout the whole year in tropical, subtropical and warm temperate waters, typically between 40° north and south latitude [22]. To date, two provisional subspecies, *B. e. edeni* (small and coastal form) and *B. e. brydei* (large and oceanic form), have been recognized [23,24]. *B. e. edeni*, with body lengths of 10–12 m, inhabits coastal and continental shelf waters primarily, while *B. e. brydei*, with body lengths of 14–15 m, mainly inhabits offshore and pelagic waters. The Bryde's whale was globally classified as "Least Concern" by the Red List of Threatened Species, International Union for Conservation of Nature [24], but the Gulf of Mexico subpopulation is classified as "Critically Endangered" [25]. This difference in assessments indicated considerable knowledge gaps and conservation challenges regarding this species, possibly caused by the deficiency in fundamental information on their occurrence and behaviours, especially in waters of developing countries [24,26].

Bryde's whales are rarely reported in Chinese waters [27]. Most of the Bryde's whale records in Chinese waters were from animal strandings, including those in the Yellow Sea, East China Sea and South China Sea [27–31]. Some records in South China Sea mistook the Sei whale *B. borealis* and/or Omura's whale *B. omurai* for Bryde's whales [27,32]. However, the populations recorded in the northern Beibu Gulf [33,34] and in the upper Gulf of Thailand [35,36] were believed to be B. e. edeni due to the shallow and inshore area as well as their small body lengths. Scientific studies on the fine-scale occurrence and tread-water feeding observations of the Bryde's whale in a small area around Weizhou Island in the northern Beibu Gulf were conducted until 2018 [33,34,37], which indicated the waters around Weizhou Island might be its feeding ground. The first satellite tracking attempt on an adult Bryde's whale expanded the known movement areas of Bryde's whales in the Beibu Gulf in 2021 [11]. Unlike satellite tagging to investigate potential occurrence areas and migration routes of animals [1,6,38], suction cup tagging could document detailed animal dive profiles and movements in a small area [15,26,39–41]. The data on the dive profiles and feeding behaviours of Bryde's whales are essential to understand their novel behaviours, which will inform conservation management of this species vulnerable to anthropogenic impacts. However, dive profiles of the Bryde's whale have not been investigated in Chinese waters. Dive profiles of Balaenoptera species have been widely investigated by time depth recorders for fin whales in the Mediterranean Sea [42], fin and blue whales in the Gulf of California [40] and blue whales in northern Chilean Patagonia [18]. A few studies have used suction cup tags to analyse the dive profiles and feeding behaviours of Bryde's whale in the waters around Madeira Island [43], Gulf of Mexico [44], the Hauraki Gulf, New Zealand [39] and the upper Gulf of Thailand [36]. In the shallow waters of the Hauraki Gulf with depths around 50 m, Bryde's whales (B. e. edeni/brydei) showed foraging behaviours during the day and rest behaviours during the night time, respectively [39]. B. e. edeni were observed to perform passive tread-water feeding in the upper Gulf of Thailand [36]. To date, knowledge of the dive profiles of B. e. edeni in coastal waters have been limited. While surface feeding behaviour data on *B. e. edeni* in shallow waters were documented [36], no dive data were published.

Dapeng Bay is located between Dapeng Peninsula and Kowloon Peninsula of Hong Kong. There were historic stranding records of Bryde's whales documented in this area [27,31]. The most recent sighting here was in 2005, when one animal was stranded off Sha Tau Kok, Hong Kong, where a putative population of Bryde's whales was reported [30]. However, no evidence has been presented that Bryde's whales currently live in Dapeng Bay. On June 29, 2021, one *B. e. edeni* appeared in Dapeng Bay 200 m offshore, feeding on fish school with seabirds close to the water surface. It was found dead on 30 August 2021, 12 km away from the location where it was first spotted. This is the first time that a Bryde's whale has been sighted living in Dapeng Bay since 2005 [27]. It is unclear why and

how the animal appeared in this water. The occurrence of *B. e. edeni* immediately attracted the attention of marine authorities and marine environmental protection organizations in Shenzhen, who prepared to rescue the whale, whenever needed. The presence of a rare whale also resulted in the gathering and watching of local residents, both on land and in the sea by vessels, which may have brought disturbances and risks to the animal.

This study is an attempt to investigate underwater behaviours of a free-ranging *B. e. edeni* in Chinese waters by suction cup tagging. The aim of this study is not only investigating the dive profiles, foraging behaviours and the potential disturbance of human activities on animal behaviours in Bryde's whales, but also to provide evidence of health conditions of the whale through comparison with previous studies in the same species elsewhere, in order to help rescue the investigated whale if needed. The revealed vertical movements in shallow water area can reflect the environment they live in and highlight the whales' ability to exploit a variety of surface and deeper waters. The information offers an important scientific basis for the rescue of the investigated whale when needed and the conservation of Bryde's whales in other Chinese waters as well.

### 2. Materials and Methods

The investigated Bryde's whale was found close to Xiaomeisha Tourist Resort, Shenzhen (Figure 1A). The depth of the area where the whale occurred was around 8–10 meters. The whale was 7–8 m long with dorsal fin typically falcate and frequently performed tread-water feeding (Figure 1B,C). Based on its morphologic and behavioural characteristics [27,33,34,36], the whale was recognized as *B. e. edeni*. This identification was later confirmed using environmental DNA techniques by a research team from South China Sea Fisheries Research Institute, China. They compared the obtained whale DNA sequences (including 222 bases of mitochondrial genome COI gene, 170 bases of 12 S and 193 bases of Dloop region) with those in International Gene Bank (Detailed information can be found online: https://www.southchinafish.ac.cn/info/1392/22348.htm, accessed on 25 August 2022). According to *B. e. edeni*' s adult body lengths of 10–12 m, the animal individual in this study was empirically considered as sub-adult. The whale presented in a relatively small region within 2 km in range and did not move for a long distance during the investigating period (Figure 1A).

A CATS (Customized Animal Tracking Solutions, Australia) suction cup base unit specially designed for whales (https://www.cats.is, accessed on 21 January 2022) was used in this study. It is a powerful device that is attached with suction cups on the dorsal side and records behavioural data of the animal (depth orientation, locomotion, etc.). It has been extensively used on a variety of whales [19,45–47]. The model that we choose is the CATS Diary Basic WIFI + Hydrophone add-on (HTI 96 mini). This is a self-contained, battery-powered (rechargeable), internally recording instrument rated for depths of up to 1000 meters. The system is fitted with 4 silicon suction cups, a galvanic release mechanism and a beacon with a VHF transmitter and GPS model for retrieval. The galvanic release was set to release within approximately 3–6 h after tag deployment to ensure timely retrieval. The tag could be recovered after release using specific VHF equipment (ATS receiver R410 and 3-element Yagi antenna). The recording sampling frequency of the tag for accelerometer (tri-axial), compass (tri-axial), gyroscope (tri-axial), temperature and pressure (depth) was set to 50 Hz in the deployments. All the sensors were calibrated on the land near the tagging area. The buoyancy and data acquisition of the suction cup tag was also checked prior to the deployment at sea. As a part of the effort to evaluate the healthy conditions of the rarely present whale in the study area, the tagging deployment was provisionally approved by the local authority for aquatic wildlife protection and under guidelines of the ethical statement involving marine mammal researches with a number of IDSSE-SYLL-MMMBL-01, issued by Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences in May 2016.



**Figure 1.** (**A**) A map of the study area. The red stars illustrate the locations where the tag was deployed or retrieved. '1' and '2' represent the first and second successful tagging on July 2 and 4, 2021, respectively. The grey line shows the accumulated speedboat survey routes from 2–5 July, indicating the occurrence range of the whale during the investigating period. The geographical relationship of *B. e. edeni* between the Gulf of Thailand, Beibu Gulf and this study area is illustrated in bottom-left figure (revised from the previous publication [11]). (**B**) and (**C**) The whale performed tread-water feeding (Photo credit: Dr. Wenzhi Lin). (**D**) The tag was deployed on the whale successfully (Photo credit: Ping Bei). (**E**) and (**F**) The full body of the whale ((**E**) was adopted from https://appdetail.netwin.cn/web/2021/07/696e84b7804533a89fa39bc511ba516f.html, accessed on 6 September 2022, photo credit: Yuxuan Chen and Yaozhong Liu; (**F**) was adopted from https://finance.southcn.com/node\_36f2f52b77/21e9d69d96.shtml, accessed on 6 September 2022, photo credit: Lei Shi).

Efforts were conducted in four days from July 2 to 5, 2021. The encounter locations of the Bryde's whale were concentrated in a small area of ~10 km<sup>2</sup> (Figure 1A). We used a 10 m length speedboat (60 HP two-stroke engine, maximum speed: ~20 knots) to slowly approach the whale in the study area. Three researchers were onboard to observe the whale behaviours with naked eye before tagging. During deployments, we tried to attach the tag on the whale back near the dorsal fin by an 8 m hand-held carbon fibre pole (Figure 1D). In considering the animal welfare, when the whale swam and breathed on the water surface, it was approached slowly at a speed of 2–3 knots, allowing it to acclimate to the investigation

boat. If the deployment failed, we turned off the engine and waited for the next opportunity. After two consecutive failed trials, we took a 10-minute break to eliminate the whale's potential stress. We firstly successfully tagged the whale after five trials at 12:51 p.m. on 2 July 2021. The speedboat moved away slowly (<5 knots) at least 300 m from the whale after the successful deployment and then turned off the boat engine to minimize interference on animal behaviours. During the whole tagging duration, the whale was slowly followed to keep a minimum distance of 300 m if the distance to the whale was over 1000 m. The distances were determined by the vision estimation of experienced sea workers. The second successful tagging deployment occurred after three trials at 6:38 a.m. on 4 July 2021.

During the first successful tagging duration, we also recorded information on whether anthropogenic whale-approaching (AWA) activities were present every half hour (if yes, number of boat trips were recorded). Previous studies on humpback whales considered an interaction threshold distance range of 300–1000 m between whales and boats [48–51]. AWA activities in this study are defined as boat activities actively entering the area within 300 m threshold distance from the whale. The AWA boats are typically the same type of survey speedboat as ours with similar lengths and engines. When the whale appeared on the water surface, the AWA boats were always observed to launch immediately and to approach the animal quickly; when the animal dived down, the AWA boats moved slowly around to try to find out where the animal came out next time and then approached the animal again. For each AWA boat, this process went on and on for at least 15–30 min. Considering that the tagging duration is relatively short, we did not quantitatively examine how the number and types of AWA boats and the interaction duration between the whale and AWA boats affected animal behaviours. Instead, we only focused on the differences in animal behaviours between scenarios with the presence and absence of AWA activities, to illustrate the general influence of AWA activities on animal behaviours. In this case, we did not record detailed specific information of each AWA boat such as engine kind, length or distance to the whale.

The surfacing duration was defined as the time period when the animal was at depths of less than 0.5 m. Considering both fluctuations caused by small waves and body motion and the large body of Bryde's whale, it is appropriate to define surfacing as depth less than 0.5 m since the drift of depth was within 0.5 m after calibrations. This assumption is also consistent with the study on freshwater finless porpoises in shallow water [41]. The dive duration, bottom duration and descent and ascent duration were all defined based on previous studies [41,52]. The descent and ascent angles were calculated by the tri-axial acceleration measured by an accelerometer, relying on the a2pr.m script from http://www.animaltags.org/ (accessed on 15 December 2021) in MATLAB R2019a. We did not use the Jiggle method [53] to estimate the forward speed in this study, because the SpeedFromRMS script relies on having at least one period of steep ascent or descent to calculate orientation-corrected depth rate (OCDR) [54]. However, with the whale being in shallow water for the whole tagging period, it might not be able to get an ideal calibration time window. Hence, the swim speed in this study was calculated from OCDR (swim speed =  $\Delta$ depth  $\times \sin^{-1}$ (pitch)  $\times \Delta$ time<sup>-1</sup>) instead, presuming that the animal's forward motion with respect to the horizontal plane is equivalent to its pitch. In the present study, according to the results of depth distribution of the whale dives (see in Results), the shallow and deep dives were defined as dives with maximum depth shallower than 3.00 m and deeper than 3.00 m, respectively, which differentiates from the general shallow and deep dives of other baleen whale studies in offshore areas [40,43,55,56].

Tread-water feeding was described as head-lifting feeding in Bryde's whales [36], which is distinct from the typical lunge feeding of rorqual whales. The typical lunge feeding forage was oblique, vertical and lateral to prey on small fish species or plankton with a minimum amplitude in ascent of 5–8 m, lasting between 20 s and 2 min [11,25]. During tread-water feeding, the animal typically holds its position for several seconds and waits for the prey to enter the mouth [36]. In this case, the tag was always under water surface when tread-water feeding occurred. According to this characteristic and the visual

observations in the field, the tread-water feeding event was identified when the depth changes became static during the ascent phase at depth of approximately 3 m in a dive based on the body location of tag deployment (Figure 2B). The duration of tread-water feeding events was measured as the period between two consecutive ascent phases for at least 4 s in a tread-water feeding event; thus, the potential feeding events with unacceptable durations were removed for further analyses.



**Figure 2.** (**A**) The whale dive profiles of the total track duration on 2 July 2021. (**B**) An example of bottom duration and tread-water feeding in a dive. The bottom duration was the duration spent at the bottom deeper than 85% of maximum depth in each dive.

Statistics are reported as mean  $\pm$  standard deviation (SD). The dive duration, descending duration, averaged descending rate, bottom duration, maximum depth, ascending duration and averaged ascending rate in each dive were compared between deep dives with and without feeding behaviours. To illustrate the potential effect of human activities on the whale behaviours, the deep dive rate (mean number of deep dives per minute), bottom duration of deep dives, feeding rate (mean number of feeding behaviours per minute) and feeding duration were compared between scenarios with the presence and absence of AWA activities. The normal distribution was tested using the Kolmogorov–Smirnov test for each group of data. When datasets were not normally distributed (p < 0.05), a Mann–Whitney nonparametric test for the independent data was applied to elucidate the differences. We used a linear regression model to test the relationship between dive duration of deep dives with feeding behaviours and the corresponding feeding duration. PASW Statistics 25.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses. The alpha level for all statistical analyses was set at 0.05.

#### 3. Results

The Bryde's whale was successfully tagged on July 2 and 4 for 3 h 41 min and 2 h 54 min, respectively (Table 1). The whale spent 89.42% of the total track duration performing diving and 10.58% on the water surface. In total, 455 dives were recorded with a mean

of 1.1 dives per minute (Table 1). The recorded maximum dive depth in each dive of the Bryde's whale showed a multimodal distribution, having local minimum at around 3.00 m and 5.80 m (Figure 3). Considering the water depth of the study area was 5–10 m, the dives with maximum depth less than 3.00 m were defined as 'shallow dive' and others were defined as 'deep dive' in the present study.

**Table 1.** Summary of tagging information on the Bryde's whale in Dapeng Bay, China, on 2 and 4 July 2021.

Date	Tag Deployment Time (GMT + 8)	Tag Retrieve Time (GMT + 8)	Deployment Location	Retrieve Location	Track Duration (min)	Total Numbers of Dives	Total Numbers of Feeding
2 July 2021	12:51:16	16:32:14	22°35.681′ N 114°25.279′ E	22°35.686′ N 114°25.551′ E	220.97	278	45
4 July 2021	6:38:37	9:32:10	22°35.740′ N 114°25.473′ E	22°35.478′ N 114°25.830′ E	173.55	177	32
Total					394.52	455	77



**Figure 3.** The distribution of maximum depth in each dive (n = 455). The shallow and deep dives are distinguished with depth boundary at 3.00 m.

The dive statistics are shown in Table 1. The maximum depth of each dive was measured up to 15.68 m, with an average of  $4.03 \pm 3.17$  m in total and a mean of  $6.49 \pm 2.48$  m for deep dives and  $1.33 \pm 0.61$  m for shallow dives, respectively. The dive duration of deep dives was measured as 72.20 s on average with a range of 15.34–200.92 s and the bottom duration of deep dives was 38.11 s on average with a range of 0.84-179.28 s. The comparison between these two dive types showed that deep dives were longer, deeper and with a longer bottom duration than shallow dives (Table 2). During the tagging period, the whale's swimming speed was estimated at  $0.61 \pm 0.18$  m/s in total,  $0.67 \pm 0.15$  m/s for deep dives and  $0.53 \pm 0.17$  m/s for shallow dives, respectively. The max speed of each dive was up to 2.40 m/s on average. The degree of descent and ascent angles was measured as  $29.35 \pm 12.61$  and  $18.77 \pm 14.33$ , respectively. The descending and ascending rate were calculated as  $0.37 \pm 0.19$  m/s and  $0.49 \pm 0.37$  m/s, respectively (Table 2).

	All Dives $(n = 455)$	Deep Dives $(n = 238)$	Shallow Dives $(n = 217)$
Max. depth (m)	$4.03\pm3.17$	$6.49 \pm 2.48$	$1.33\pm0.61$
Dive duration (s)	$46.52\pm39.89$	$72.20\pm38.71$	$18.36\pm13.30$
Bottom duration (s)	$24.57\pm30.67$	$38.11 \pm 34.45$	$9.73 \pm 15.90$
Max speed $(m/s)$	$2.40 \pm 1.30$	$2.84 \pm 1.52$	$1.91\pm0.74$
Mean speed (m/s)	$0.61\pm0.18$	$0.67\pm0.15$	$0.53\pm0.17$
Degree of descent angle	$29.35\pm12.61$	$34.42 \pm 13.60$	$23.77\pm8.46$
Degree of ascent angle	$18.77\pm14.33$	$25.50\pm15.73$	$11.36\pm6.97$
Descending rate $(m/s)$	$0.37\pm0.19$	$0.44\pm0.19$	$0.29\pm0.15$
Ascending rate $(m/s)$	$0.49\pm0.37$	$0.45\pm0.20$	$0.53\pm0.49$
Number of Tread-water feeding events	77	77	0

**Table 2.** Summary of descriptive statistics (mean  $\pm$  standard deviation) for dive parameters of the Bryde's whale on 2 and 4 July 2021.

The tread-water feeding behaviours were visually observed on every survey day (Figure 1B,C). During the tagging period, 77 feeding behaviours in total were detected and the cumulative feeding durations were 686.22 s, accounting for 2.79% of the whole tagging time. All tread-water feeding behaviours were recorded during the ascent phase of deep dives, with 32.63% of all the examined deep dives being presented with the feeding behaviour. The mean and maximum feeding durations were 8.91  $\pm$  2.47 and 15.44 s, respectively. A comparison between deep dives with and without feeding behaviours is shown in Table 3. Dive duration, descending rate, maximum depth, ascending duration and ascending rate exhibit significant differences between deep dives with and without feeding behaviours was 66.59  $\pm$  39.51 s on average and showed a significant weak positive relationship with the feeding duration (Figure 4, R<sup>2</sup> = 0.12, *p* = 0.002).

**Table 3.** Dives statistics (mean  $\pm$  standard deviation and Mann–Whitney nonparametric test *p* values) for the two deep dive types. \* *p* < 0.05.

Si	tatistics	With Feeding Behaviours $(n = 77)$	Without Feeding Behaviours (n = 161)	p Value
Dive duration (s)		$66.59\pm39.51$	$76.19\pm37.78$	0.015 *
Descent	Duration (s) Averaged rate (m/s)	$\begin{array}{c} 15.67 \pm 7.88 \\ 0.50 \pm 0.21 \end{array}$	$\begin{array}{c} 18.16 \pm 13.24 \\ 0.42 \pm 0.17 \end{array}$	0.100 0.005 *
Bottom	Duration (s) Maximum depth (m)	$\begin{array}{c} 40.47 \pm 38.84 \\ 6.71 \pm 2.19 \end{array}$	$\begin{array}{c} 36.98 \pm 32.20 \\ 6.39 \pm 2.61 \end{array}$	0.810 0.041 *
Ascent	Duration (s) Averaged rate (m/s)	$\begin{array}{c} 10.45 \pm 3.37 \\ 0.55 \pm 0.15 \end{array}$	$\begin{array}{c} 21.05 \pm 16.98 \\ 0.40 \pm 0.21 \end{array}$	0.000 * 0.000 *

There were no AWA activities being observed before 14:00 during the first tagging period, while afterwards, AWA activities were observed around the whale and the number of AWA boat trips per hour ranged from three to five. During the absence of AWA activities, totally, 42 deep dives and 23 feeding behaviour events were detected with a mean of 0.61 and 0.33 per minute, respectively. When AWA activities were present around the animal, totally, 77 deep dives and 22 feeding behaviour events were detected with a mean of 0.51 and 0.14 per minute, respectively (Figure 5A,C). The deep dive rate showed no significant differences (Mann–Whitney U Test, p = 0.142, Figure 5A) between the absence and presence of AWA activities, while feeding rate during the absence of AWA activities was significantly higher than that during the presence of AWA activities (Mann–Whitney U Test, p = 0.019, Figure 5B). There was no significant difference

on feeding duration between the absence and presence of AWA activities (9.31  $\pm$  2.25 s and 9.57  $\pm$  2.50, respectively) (Mann–Whitney U Test, *p* = 0.496, Figure 5D).



**Figure 4.** Linear regression model results for the correlation between the feeding duration and dive duration.



**Figure 5.** Histograms (mean and standard deviation) showing (**A**) deep dive rate, (**B**) bottom duration of deep dives, (**C**) feeding rate and (**D**) feeding duration, for the absence and presence of the anthropogenic whale-approaching (AWA) activities, respectively. The histograms with symbol '\*' refer to significant results at p < 0.05.

## 4. Discussion

Considering that the last sighting of Bryde's whales in the study area was 16 years before [27,30], the tagged individual appearing in Dapeng Bay, Shenzhen, was likely misguided and not native to the investigated waters. While the behaviour of this whale in a possibly unfamiliar environment may not represent the natural biological characteristics of this species in free-ranging areas [57–59] and only one individual was tagged with 77 tread-water feeding events being recorded in a relatively short-term period, the present study represents the first suction cup tagging attempt on baleen whales in Chinese waters. To our knowledge, this is also the first report of quantitative dive profile for *B. e. edeni* along

the Chinese coast. Therefore, our data on the vertical movement behaviours of *B. e. edeni* by the biotelemetric technique addressed a knowledge gap.

The depth of 3.00 m was considered as the boundary of shallow and deep dives in this study, which was comparable to the study where shallow-deep-dive boundary was defined as 2.70 m for Yangtze finless porpoises in a similar shallow water environment [41]. The maximum dive depth in each dive was up to 15.68 m with a mean of 4.03 m, which is consistent with the same species in the upper Gulf of Thailand [36] but shallower than that for the same species (*B. e. edeni/brydei*) in the shallow waters of the Hauraki Gulf (maximum dive depth was over 50 m) [39] and waters around Madeira Island (maximum recorded dive depth was 267 m) [43]. This could be due to the limited water depth or differences in behaviours. A dive depth less than 10 m was also reported in *B. e. edeni* for the animals when performing tread-water feeding in the upper Gulf of Thailand [36].

A deep-dive duration of 72.20 s in this study was similar to the result for B. e. edeni/brydei (dive duration 1~2 min) in the shallow waters of the Hauraki Gulf, New Zealand [39], but much shorter than that in *B. e. brydei* ( $5.00 \pm 1.20 \text{ min}$ , n = 50) in the waters around Madeira Island, calculated from dives with depths of 40–267 m [43]. The bottom duration (0.83  $\pm$  0.75 min) documented for *B. e. brydei* [43] was also longer than that observed in deep dives for B. e. edeni in this study. The averaged descent and ascent rates of *B. e. edeni* in the present study were 0.37 and 0.49 m/s, respectively, which are also consistent with the results for Bryde's whales (B. e. edeni/brydei, around 0.40 m/s) in the Hauraki Gulf [39], but lower than those documented for *B. e. brydei* (0.80–1.30 m/s) [43]. In a shallow inshore system, B. e. edeni seemed to dive with a shorter dive duration and bottom duration with slower descent and ascent rates compared to the same species inhabiting deeper, offshore and pelagic waters. These differences are probably associated with the water depth of habitat environment. The investigated whale's moving speed was calculated to be 0.60 m/s on average, which was lower than the mean speed of 1.50 m/s deduced for this species during migration by using a satellite tag [11] and those values documented for other baleen whale species [60,61]. Such a difference in moving speed may be due to the frequent tread-water feeding behaviours and small range of movements during the tracking period in this study.

The tread-water feedings of *B. e. edeni* in the northern Beibu Gulf, China, have been coming to the attention of local fishermen since 2016 [33,34], as well as during our observations in the present study area. Its characteristic mouth opening and head lifting could be easily distinguished from other feeding techniques of other rorquals and is considered to take advantage of the behaviour of the prey, whereby the hypoxic environment limits fishes to the water surface where oxygenated water concentrates [26,36]. Tread-water feeding was speculated as a cultural behaviour, since it had never been observed in Bryde's whales in any other area except for the upper Gulf of Thailand before 2016 [36]. However, the recent finding that the tread-water feeding behaviours have been observed in both the northern Beibu Gulf [33,34] and the present study area seems not to support the above speculation. The development of this behaviour seems to be owing to the oceanographic characteristics and the behaviour of the prey species [36]. Interestingly, unlike B. e. edeni appearing in above waters, Bryde's whales (B. e. edeni/brydei) in the Hauraki Gulf, New Zealand, did not display tread-water feeding behaviours, although they all lived in shallow waters at depth of less than 50 m. This difference in foraging strategies may be due to different distribution and behaviour of the prey species in different locations.

The feeding behaviours during the absence of AWA activities were detected with a mean feeding rate of 0.33 per minute in this study, consistent with those of *B. e. edeni* in the northern Beibu Gulf, China (0.2–0.5 per minute, personal observation). The mean and maximum feeding durations observed in the upper Gulf of Thailand (14.5 s and 32 s, respectively) [36] are longer than those in this study. The difference may result from prey intake per feeding, prey abundance and interferences from the surroundings, such as vessel approaching. Deep dives with feeding behaviours performed in a shorter duration, deeper dive depth, higher descending and ascending rates than deep dives without feeding

behaviours were observed in this study (Table 3). This was not consistent with the findings for *B. e. brydei* in Madeira Island [43], where feeding dives were longer and with a longer bottom duration than dives without feeding activities. It should be noted that *B. e. brydei* in waters around Madeira Island feed at depth and its prey consumption occurs mainly during the bottom phase of the deep dive, while *B. e. edeni* in this study preys on the water surface. The different feeding behaviour types could lead to different results of feeding dives. The significant weak positive relationship between feeding dive durations and feeding durations (Figure 4) suggested that longer dive duration in deep dives may be conducive to assembling prey on the water surface.

In the past 20 years, almost all records of *Balaenoptera sp.* in Shenzhen waters were stranded or dead animals [11,27,34]. In this case, when one B. e. edeni accidentally entered the region of Dapeng Bay, the health situation of this animal attracted the attention of both the local authority and the public. Sickness behaviours in response to non-lethal infections had been documented in wild animals [62–66]. Changes in behavioural parameters may indicate that an animal is in pain [67]. Therefore, behavioural comparison can provide indirect evidence for health status of mammals. The similar descending and ascending rates were observed in this study to those in the Hauraki Gulf with Bryde's whales [39]. The feeding rates of 0.33 per min in this study were also similar to those of *B. e. edeni* observed in the northern Beibu Gulf (personal observation). Together with the normal foraging process and mass prey intake, which were obviously observed through surface behaviours, it was proposed that the investigated whale in Dapeng Bay was of good health. The accidental occurrence event of this animal was probably due to prey pursuit. The current fisheries resources in Dapeng Bay predominate on short-lived, small pelagic noneconomic fishes, such as small sardines and anchovies, etc. [68,69], all of which are suggested to be preferred prey for Bryde's whales [34,36]. Foraging Bryde's whales are less likely to reside in a fixed place for a long period if their prey is on the move or insufficient to supply for the whole whale population [70–72]. We recommended that the continental shelf waters nearby the Shenzhen area should be further investigated to explore the potential occurrence of Bryde's whales. In Chinese coastal waters, at present, there is one confirmed hotspot area of Bryde's whales in the Beibu Gulf [11,33,34]. The Dapeng Bay and Beibu Gulf have similar environments with vast shallow waters with abundant fisheries resources [68,69,73]. The coastal distance between these two areas is less than 700 km. The relationship and possible connectivity of *B. e. edeni* appearing in Dapeng Bay and Beibu Gulf should also be explored in future research by genetic and ecological (i.e., satellite-tagging) methods [60,61,74,75].

High anthropogenic activities are the common traits of environments in coastal areas of Shenzhen. Therefore, in Dapeng Bay, the investigated whale was potentially exposed to and suffered from risk of vessel strike and anthropogenic noise from shipping. Anthropogenic activities not only disturb behaviours of marine mammals, but also lead to auditory masking, potential temporary or permanent shifts in hearing thresholds and even damage to the vestibular, reproductive and nervous systems [76–78]. When several vessels approached the investigated whale within 300 m for animal watching, the deep dives and feeding behaviours of the whale decreased by 17% and 58%, respectively. The bottom duration became significantly longer as the frequency of deep dives decreased (Figure 5). Our results showed direct evidence for the animal avoidance of anthropogenic activities and the effect on tread-water feeding behaviours was particularly obvious. It is unknown if the investigated whale foraged with oblique, vertical and lateral lunging feeding as B. e. edeni in the northern Beibu Gulf [33] during the tagging period, since the lunge is difficult to detect in shallow water with depth of only 8-10 m according to the definition of a lunge [40,43]. Our observations are consistent with the findings on minke whales that whale-watching boat interactions reduced both underwater and surface foraging activities of animals in Faxaflói Bay, Iceland [79]. Biological managers and ecologists have all concerned about understanding the population-level consequences of changes in the behaviour or physiology of individual animals that are caused by external stressors, but assessment of nonlethal effects of disturbance on wildlife populations is challenging [80]. In this study, the decrease in feeding activities of *B. e. edeni* due to AWA activities may suggest a decrease in the energy availability for both feeding and breeding and, therefore, an alteration in the survival and calving success of this species. However, we should note the potential temporal feeding variation in *B. e. edeni* individuals could lead to bias in our comparison results between scenarios of the absence and presence of AWA activities. Future studies should, hence, aim at investigating more individuals in their confirmed natural habitat by long-term biotelemetry monitoring, to verify our present observations and to further assess and determine the impact of anthropogenic activities on whales, which help to formulate more effective conservation measures for this species.

The investigated whale could be frequently observed in Dapeng Bay and neighbouring areas by the local authority and public after our field work in both July and August, 2021. On 30 August 2021, a dead male Bryde's whale, 7.2 m long and weighing 2.7 tons, was found 12 km away from our study area. Based on the body length, species information and the fact that our investigated whale had not been definitely seen in the area since a dead whale was found, there is a high probability that the dead individual is our investigated whale. This whale was last surely sighted on 26 August 2021 (https://xw.qq.com/amphtml/20210830A0F73D00). It is about 10 days after an annual fishing ban ended on August 16 in Dapeng Bay water. The certain cause of the death is unknown, since no post-mortem has been conducted so far. However, it is highly possible that the death of the whale was related to fishing activities after the closed fishing season. Both our observations of the behavioural alteration with AWA activities and the death of the whale at the end highlight the importance of reducing interference from human activities, including AWA and fishing, for whale protection. Unfortunately, in recent years, there have been intensive whale watching activities targeting *B. e. edeni* in north Beibu Gulf during the spring season [33,34,37]. The obvious disturbance on feeding behaviours of the whale caused by the presence of AWA activities in this study should be taken in consideration for regulation and management of whale watching activities in north Beibu Gulf. The regulation of moving speed of whale watching vessels [81] and the distance from whales could decrease injury or mortality by collision from approaching vessels, as well as adverse effects from the underwater noise produced by vessel activities. In addition, given the sad ending of the investigated whale, we also recommend that specialized marine mammal health personnel should be established in Chinese waters in order to reduce animal survival risks during emergency events, such as stranding of whales and dolphins.

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**Institutional Review Board Statement:** Suction cup tagging does not penetrate the animals' skin and was conducted in accordance with the guidelines of the Law of the People's Republic of China on the Protection of Wildlife, the Regulations for the Implementation of Wild Aquatic Animal Protection (promulgated in 1993) and the Ethical Statement involving marine mammal research of the Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences with the Ethical Statement Number of IDSSE-SYLL-MMMBL-01 (Issued in May 2016).

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