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Article in Zoo Biology - April 2023
DOI: 10.1002/zoo.21767

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RESEARCH ARTICLE

Diagnosing gastric ulcers in bottlenose dolphins (Tursiops sp.) using gastroscopy and cytology

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Abstract
Gastric ulcers have been reported in a range of cetacean species. Bottlenose dolphins (Tursiops spp.), the most common cetacean species held in captivity, are known to experience gastric ulcers in both wild and captive environments. Documented causes of gastric ulceration include bacterial infection by Helicobacter sp., parasitic infections, high dietary histamine and foreign body ingestion. Gastric ulceration without any obvious cause might be related to stress. Currently, the most accurate way to determine the presence of gastric ulcers in captive dolphins is through direct examination of the stomach mucosa using endoscopy (gastroscopy); a procedure that requires substantial animal training and specialised medical equipment. In this study, we investigate whether cytology of the gastric fluid, collected through the less intensive method of intubation, can be used as an alternative to gastroscopy to predict the presence and severity of gastric ulcers in eight captive bottlenose dolphins at uShaka Sea World, South Africa. An ulcer grading scale was developed to quantify the severity of the dolphins’ gastric ulcers observed using gastroscopy. Gastric ulcer severity was then compared with the cytological data collected from gastric fluid samples taken during the gastroscopic examinations. The cytological findings were consistent with other studies, but ulcer severity was not found to be linked to the cytological parameters measured. From these results we suggest that routine cytology of the gastric fluid is not a viable alternative to gastroscopy for diagnosing gastric ulcers in bottlenose dolphins.

KEYWORDS
animal welfare, dolphins, endoscopy, stomach ulcers

1 | INTRODUCTION

Gastric ulcers are seen frequently in all cetacean species (Sweeney & Ridgway, 1975) and have been reported from both wild and captive dolphin populations, including common dolphins (Delphinus delphis, Abollo et al., 1998; Davison et al., 2014) and common bottlenose dolphins (Tursiops truncatus, C. G. Harper et al., 2002; Sweeney & Ridgway, 1975). Existing data of cetacean gastric ulceration have been generated through detailed necropsy of wild-stranded specimens (Abollo et al., 1998; Davison et al., 2014; Hrabar et al., 2017), or from live animals in captive settings during health checks (C. G. Harper et al., 2002; Sweeney & Ridgway, 1975; Waples &
The documented causes of gastric ulcers in dolphins include bacterial infection by *Helicobacter* sp., parasitic infections, high dietary histamine and foreign body ingestion (Avalos-Téllez et al., 2010; Dierauf & Gulland, 2001; Goldman et al., 2002; C. M. G. Harper et al., 2000; Sweeney & Ridgway, 1975). There have also been reports of gastric ulceration in wild and captive dolphins without any obvious attributable cause (C. M. G. Harper et al., 2000; Sweeney & Ridgway, 1975). For bottlenose dolphins (*Tursiops* spp.) in captivity, such cases may reflect stress and possibly compromised welfare (Waples & Gales, 2002).

A gastric ulcer is defined as a defect in the wall of the gastric mucosa that extends deep into the gastric submucosa (Yeomans & Naesdal, 2008). The clinical signs of gastric ulcers in humans and other mammals are similar in dolphins and include abdominal tenderness, mental depression, anorexia, inappetence, unresponsive-ness, vomiting and regurgitation (Avalos-Téllez et al., 2010; Fiorucci et al., 2015; C. M. G. Harper et al., 2000). However, diagnosing gastric ulcers based on clinical signs alone can be misleading, since they may also be a sign of other diseases of the stomach, such as gastritis or peptic ulcer disease (Kayacetin & Guresci, 2014; Malfertheiner et al., 2009). Gastritis occurs when there is inflammation of the gastric and duodenal mucosa and can be either acute or chronic (Kayacetin & Guresci, 2014). Peptic ulcer disease is characterised by the erosion of the mucosa of the gastric tract and includes both gastric and duodenal ulcers (Malfertheiner et al., 2009). If the ulcer erodes a major blood vessel, bleeding will occur, which can cause anaemia and severe haemorrhaging that may result in death (C. M. G. Harper et al., 2000; Yeomans & Naesdal, 2008). Understanding the rate and severity of gastric ulceration is an important welfare tool for dolphins in a captive setting, where prolonged or extreme occurrences of ulceration could induce a negative welfare state or mortality (Goldstein et al., 2012; Waples & Gales, 2002).

The stomach anatomy is similar for most cetacean species except for beaked whales (Family Ziphiidae) and some river dolphins (Family Platanistidae) (Mead, 2007). Cetaceans have multi-chambered (plurilocular) stomachs. Bottlenose dolphins, for example, have a three-chambered stomach (Figure 1) which comprises the forestomach, the main stomach and the pyloric stomach (Fiorucci et al., 2015; C. M. G. Harper et al., 2000). The forestomach (also known as the oesophageal stomach) is a non-glandular, muscular structure which consists of a thick lining of stratified squamous epithelium that is continuous with the oesophagus. It is a highly distensible structure and is primarily used to hold food. The main stomach (aka the second or duodenal stomach) is largely responsible for digestion. It is glandular and consists of a thin lining of highly convoluted epithelium that is a dark red to purple in colour. It is made up of chief cells, neck cells and parietal cells which actively secrete digestive enzymes, mucous and hydrochloric acid. Gastric fluid from the main stomach is refluxed into the forestomach due to the two chambers having a relatively open connection with one another via an approximately 2.5 cm diameter ostium; therefore, some digestion may also occur within the forestomach. The main stomach is connected to the
"U-shaped" pyloric stomach (aka the third stomach) via a connecting chamber with a small, muscularly controlled opening. The pyloric stomach is also glandular with a thinner lining containing mucous and argentaffin cells. This is where partially digested food is held and neutralised before being moved to the duodenum of the small intestine via the muscular pyloric sphincter (C. M. G. Harper et al., 2000; Mead, 2007; Rommel et al., 2018; Van Bonn & Dover, 2018; Varela et al., 2007). Previous studies have identified gastric ulcers in the forestomach and occasionally in the main stomach of both wild and captive dolphins (C. M. G. Harper et al., 2000). Ulceration in the pyloric stomach cannot be determined in living dolphins by endoscopy, since the channel connecting the main and pyloric stomachs has a small diameter, is intramural and 'J' shaped (Fiorucci et al., 2015; Van Bonn & Dover, 2018). However, ulceration has been reported in the pyloric chambers of Stenella longirostris, Pecococephala electra (Motta et al., 2008) and Stenella frontalis (Suárez et al., 2010) studied through necropsy.

Gastric ulceration has traditionally been diagnosed using one of three methods: (1) radiography, (2) cytology revealing the presence of red blood cells (RBC) and white blood cells (WBC) in the gastric fluid of an individual (Sweeney & Ridgway, 1975) or (3) through endoscopy (termed 'gastroscopy'). Gastroscopy is preferable to the first two methods since it allows for direct visual examination of the oesophagus, forestomach and entrance of the main stomach via live-relayed video imagery (Fiorucci et al., 2015; C. M. G. Harper et al., 2000). A diagnosis of gastric ulcers in the stomach is usually made through gastroscopy when a break in the stomach mucosa >3–5 mm in diameter is observed (Malferttheiner et al., 2009; Yeomans & Naesdal, 2008). Although this definition may be criticised as arbitrary, it is routine in human pathology and clinical trials (Malferttheiner et al., 2009), and is used as the determinant for gastric ulcers in 80% of published studies in humans between 2000 and 2007 (Yeomans & Naesdal, 2008).

Although direct examination of the stomach mucosa using gastroscopy is considered the most accurate way to determine the presence of gastric ulcers in dolphins (Goldstein et al., 2012), the procedure is minimally invasive (Van Bonn & Dover, 2018) and requires specialised medical equipment. Substantial animal training is needed to desensitise the dolphins to the medical equipment and to voluntarily allow the gastroscopic examination to be conducted. Examinations last approximately 8 min, during which the dolphins must remain still.

Alternatively, examining the cytology (epithelial cells, leukocytes, erythrocytes) within the gastric fluid could potentially be used to indicate gastric abnormalities before the animal displays clinical signs (Fiorucci et al., 2015; Goldstein et al., 2012; Sweeney & Reddy, 2001). The pH of the stomach fluid can also be assessed, and microbial cultures generated, enabling additional diagnostic information with regard to the presence of gastric ulcers (and other gastric abnormalities) in the dolphins (Fiorucci et al., 2015; Sweeney & Reddy, 2001). Although modern endoscopes can collect gastric fluid through built-in collection tubes, gastric fluid can be collected more simply by intubation (Goldman et al., 2002; Sweeney & Reddy, 2001), which is already practiced by many facilities that house cetaceans. Compared to gastroscopy, collecting samples of gastric fluid through intubation is less time-consuming, costly and training intensive for the dolphins and the animal care staff. The ability to diagnose gastric ulcers in dolphins based on the cytology of gastric samples collected through simple intubation would alleviate or reduce the need for endoscopy.

This study investigates the occurrence, frequency and severity of gastric ulcers in a captive group of bottlenose dolphins (Tursiops sp.) and compares the efficacy of cytology to gastroscopy with regard to their diagnosis.

2 MATERIALS AND METHODS

2.1 Study subjects

The study focused on a group of eight bottlenose dolphins (Tursiops sp.) housed under managed care at uShaka Sea World in Durban, South Africa; two T. truncatus (one male, one female), five T. truncatus-aduncus hybrids (two male, three female) and one T. truncatus-aduncus × T. truncatus backcross hybrid (female) (Table 1).

The dolphinarium complex has an approximate total volume of 7500 m³ seawater and is made up of six interconnected pools of varying shape, size and depth. The dolphins were fed daily calorically balanced diets consisting of a combination of fish species with portion sizes dependant on each animal’s body mass.

2.2 Gastroscopy and cytology

Gastroscopy of the forestomach of each dolphin, together with a cytological analysis of gastric fluid samples, was conducted bi-monthly over a period of 6.5 months (October 2017–April 2018). None of the dolphins received specific medication for the treatment of ulcers during the study period. Gastroscopic examinations were conducted using a video-endoscope (11 mm diameter, 1.6 m flexible Olympus CF video-endoscope) to visually assess the oesophagus and forestomach of the dolphins. Insufflation was used to aid visualisation of the surface of the forestomach. The procedures were conducted after a period of overnight fasting, that is, before the dolphin's first

<p>| TABLE 1 Summary information of the dolphins held at uShaka Sea World by sex, age and species. |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Subject code</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>33</td>
<td>Tursiops truncatus</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>24</td>
<td>Hybrid</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>24</td>
<td>Backcross hybrid</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>23</td>
<td>T. truncatus</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>22</td>
<td>Hybrid</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>18</td>
<td>Hybrid</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>13</td>
<td>Hybrid</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>8</td>
<td>Hybrid</td>
</tr>
</tbody>
</table>
feed of the day, to avoid contamination of the dolphin’s gastric fluid with ingested food (Sweeney & Reddy, 2001). A sample of gastric fluid was collected for cytological analysis at the beginning of each gastrosopic procedure, through an opening in the video-endoscope, using a vacuum suction pump. Between 10 and 50 ml of fluid was extracted and decanted into plastic jars for analysis. The sample was taken from the forestomach, which is the same area where an intubation sample would be taken. If the examination was stopped before a gastric sample could be obtained through the video-endoscope, a gastric sample was taken by the method of intubation immediately following termination of the procedure. The video footage obtained from each gastroscope was analysed for presence or absence of ulcers, and their severity, as described in Table 2. Severity was determined on a scale of 1–4, where grade 1 indicated that no lesions were observed, grade 2 denoted up to 10 lesions were observed, grade 3 indicated that more than 10 lesions were observed and with up to 50% of the area displaying lesions and grade 4 indicated that more than 50% of the area was observed with lesions. Additionally, grade 0 was used if no adequate images were obtained for determining ulcer presence or severity, and the associated cytological information was removed from analyses. Lesions present in the forestomach were graded independently from any lesions seen in the oesophagus region.

Gastric fluid samples were analysed immediately after collection. The gastric fluid samples were analysed according to the standard procedure described by Sweeney and Reddy (2001). This involved determining the sample pH, generating a description of the sample (colour) and undertaking microscopy to determine cell counts. The pH of the gastric fluid was determined to the nearest whole value, using the Macherey–Nagel pH-Fix test. Gastric fluid samples were categorised into six colour groups, namely white, cream, green, tan, brown and maroon, based on personal observation and the experience of the uShaka Sea World veterinarians (F. Lampen and C. Knox [personal communication, May 23, 2017]).

The occurrence and abundance of WBC, red blood cells (RBC), epithelial cells and basal cells were determined for each sample by adding 10 μl of gastric fluid to microscope slides prepared with Rapid-Diff II stain. Slides were viewed at ×40 magnification to screen for parasites, then at ×400 magnification to conduct cell counts. The number of WBC, RBC, epithelial and basal cells were counted from five random fields of view (FOV) according to the method described in Sweeney and Reddy (2001). This process was repeated three times. For each cell type, the total number of cells observed were then divided by 15, to calculate the average number of cells seen in one FOV at high magnification, that is, the number of cells per high-power field (hpf) (Varela et al., 2007).

### TABLE 2

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No adequate images</td>
</tr>
<tr>
<td>1</td>
<td>No lesions</td>
</tr>
<tr>
<td>2</td>
<td>Up to 10 lesions</td>
</tr>
<tr>
<td>3</td>
<td>More than 10 lesions, up to 50% area displaying lesions</td>
</tr>
<tr>
<td>4</td>
<td>More than 50% of area displaying lesions</td>
</tr>
</tbody>
</table>

### 2.3 Statistical analyses

All statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 27.0. A comparison of the cytological parameters measured, where ulcers were present or absent, was conducted using a Mann-Whitney U test. To compare the cytology and forestomach ulcer grades, a one-way analysis of variance (ANOVA) was used for epithelial and WBC only, because the assumptions of normality of residuals were tested using a one-sample Kolmogorov–Smirnov test and were not met for basal cells and pH. A Kruskal–Wallis test was used for basal cells and pH instead. RBC were observed only on one occasion; therefore, their relationship with forestomach ulcer grades was not tested.

In addition, the relationships between pH and the various cell types were tested with a Spearman rank correlation, as the cytological data were not normally distributed (one-sample Kolmogorov–Smirnov test, \( p = .05 \)). Last, the cytology and colour of the gastric samples were compared using a Pearson’s chi-square analysis.

### 3 RESULTS

During the 6-month study period, 12 gastrosopic procedures were performed on each of the dolphins except for one female (subject 8), where only four procedures were possible. In total, 85 examinations were successfully carried out during the study. A total of 94 gastric samples were collected during the investigation; 85 samples collected via the video-endoscope, referred to as ‘true gastric samples’ henceforth, and nine via the gastric intubation method. Cytology results were calculated only from 84 true gastric samples because it was certain that these samples were taken from the forestomach (one true gastric sample was collected without the forestomach being clearly visualised; therefore, no ulcer grade could be determined, and the sample was excluded from the analyses).

None of the cytological parameters measured from the dolphins’ gastric fluid samples differed significantly in the presence or absence of gastric ulcers (pH: \( U = 600, p = .489 \); epithelial cells: \( U = 606, p = .721 \); WBC: \( U = 579, p = .521 \); basal cells: \( U = 634.5, p = .927 \)). RBC were identified in only one true gastric sample (only six cells identified) and were therefore not included in the analysis. Similarly, we found no significant relationship between ulcer grades and any of the measured cytological parameters (pH: \( \chi^2 \) (Chi-square test value) = 3.599, \( p = .308 \); epithelial cells: \( F \) (F-ratio) = 0.603, \( p = .615 \); WBC: \( F = 1.360, p = .261 \); basal cells: \( \chi^2 = 3.492, p = .322 \); Table 3).
**TABLE 3** Gastric sample cytology (number of cells/high-power field) and pH across ulcer grades for males and females combined.

<table>
<thead>
<tr>
<th>Grade</th>
<th>$\bar{x}$ pH ± $\sigma$</th>
<th>$\bar{x}$ epithelial cells ± $\sigma$</th>
<th>$\bar{x}$ white blood cells ± $\sigma$</th>
<th>$\bar{x}$ basal cells ± $\sigma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ($n=20$)</td>
<td>1.20 ± 0.41</td>
<td>1.15 ± 0.76</td>
<td>0.79 ± 0.65</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td>2 ($n=41$)</td>
<td>1.15 ± 0.57</td>
<td>1.30 ± 0.79</td>
<td>0.58 ± 0.41</td>
<td>0.02 ± 0.04</td>
</tr>
<tr>
<td>3 ($n=17$)</td>
<td>1.65 ± 1.66</td>
<td>1.03 ± 0.73</td>
<td>0.72 ± 0.43</td>
<td>0.02 ± 0.04</td>
</tr>
<tr>
<td>4 ($n=6$)</td>
<td>1.33 ± 0.52</td>
<td>1.41 ± 1.33</td>
<td>0.93 ± 0.71</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: $n =$ number in subsample; $\bar{x} =$ sample mean; $\sigma =$ standard deviation.

**TABLE 4** Gastric sample cytology (number of cells/high-power field) and pH across gastric sample colour of all samples for males and females combined.

<table>
<thead>
<tr>
<th>Colour</th>
<th>$\bar{x}$ pH ± $\sigma$</th>
<th>$\bar{x}$ epithelial cells ± $\sigma$</th>
<th>$\bar{x}$ white blood cells ± $\sigma$</th>
<th>$\bar{x}$ basal cells ± $\sigma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown ($n=5$)</td>
<td>2.00 ± 1.73</td>
<td>1.15 ± 0.77</td>
<td>0.57 ± 0.59</td>
<td>0.07 ± 0.07</td>
</tr>
<tr>
<td>Cream ($n=10$)</td>
<td>1.60 ± 1.58</td>
<td>1.25 ± 0.63</td>
<td>0.56 ± 0.17</td>
<td>0.01 ± 0.02</td>
</tr>
<tr>
<td>Green ($n=4$)</td>
<td>1.00 ± 0.00</td>
<td>1.70 ± 0.53</td>
<td>1.08 ± 0.41</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Maroon ($n=12$)</td>
<td>1.83 ± 1.11</td>
<td>1.74 ± 1.16</td>
<td>0.97 ± 0.64</td>
<td>0.04 ± 0.07</td>
</tr>
<tr>
<td>Tan ($n=21$)</td>
<td>1.38 ± 1.16</td>
<td>1.29 ± 0.74</td>
<td>0.87 ± 0.51</td>
<td>0.00 ± 0.01</td>
</tr>
<tr>
<td>White ($n=42$)</td>
<td>1.33 ± 1.07</td>
<td>1.17 ± 0.86</td>
<td>0.66 ± 0.74</td>
<td>0.02 ± 0.04</td>
</tr>
</tbody>
</table>

Note: $n =$ number in subsample; $\bar{x} =$ sample mean; $\sigma =$ standard deviation.

WBC counts varied across the six gastric sample colour categories (all gastric samples included; $\chi^2 = 11.58$, $p < .05$) and were most abundant in green (1.08 ± 0.41 cells/hpf) and maroon samples (0.97 ± 0.64 cells/hpf), and least abundant in cream-coloured samples (0.56 ± 0.17 cells/hpf). Basal cells occurred across all colour categories apart from green samples. Although only observed in two samples (one true gastric and one intubation sample), RBC occurred exclusively in maroon samples, but not all maroon samples contained RBC (2/12 samples, 16.7%) (Table 4).

Additionally, the only cytological parameters that showed a significant correlation were epithelial and WBC counts (rho [Spearman's rank correlation coefficient] = 0.365, $p < .05$).

4 | DISCUSSION

This study tested the efficacy of using the cytology of gastric fluid routinely collected from the dolphins for determining the presence and severity of gastric ulcers, as an alternative method to gastroscopy. No relationship was observed between epithelial, basal and WBC counts and ulceration (Table 3). Similarly, there was no relationship found between pH and ulcer severity (Table 3). There also appears to be no clear trend in changes in pH and cell counts from one ulcer grade to the next. It was therefore not possible to confidently use cytological findings to predict the presence or severity of ulceration in the uShaka Sea World dolphin population.

Leukocytes (WBC) present in gastric fluid are usually an indication of inflammation and infection. It is generally accepted that a normal ratio of leukocytes to epithelial cells is approximately 1:1 (Sweeney & Reddy, 2001), and a finding of more than 20 leukocytes/hpf may be an indication of gastritis (Fiorucci et al., 2015). No dolphin in this population showed evidence of high numbers of WBC/hpf, even though ulceration was present; nevertheless, the greatest number of WBC (0.93 ± 0.71 cells/hpf) was observed at grade 4 ulceration (Table 3).

Whilst the presence of basal cells and erythrocytes (RBC) may be a sign of ulceration (Mitchell et al., 2008), basal cells were present in the gastric fluid where ulceration was absent on two occasions in the present study and were not seen in the gastric fluid where grade 4 ulceration was observed. RBC were found on only two occasions in this study, once in a sample taken by gastroscopy from a dolphin with grade 4 ulceration; and the other at an unknown ulcer severity where the gastric sample was collected through intubation. However, there were other ($n=5$) occasions where grade 4 ulceration was observed without the presence of RBC in the gastric sample. Furthermore, RBC will rapidly lyse in the highly acidic gastric fluid (Sweeney & Reddy, 2001) and can only be expected to be found in the presence of profusely bleeding ulcers. In such an instance, the gastric fluid pH would be out of the normal range for dolphins, actively bleeding ulcers would be visible upon gastroscopy and the animal may present other clinical signs of illness. None of these symptoms were present in this study, indicating that the presence of basal cells and RBC in the gastric fluid cannot be used as an indicator of ulcers in the dolphins.

The mean pH of the gastric samples across all ulcer grades in this study was within the normal range for fasted, clinically healthy dolphins (1.0–3.0, Fiorucci et al., 2015). The results of our study further support Fiorucci et al.’s (2015) observation that there is no correlation between the pH of gastric fluid and the number of epithelial cells or WBC in the
dolphins studied. However, they also found no correlation between the number of epithelial cells and WBC ($r^2 = .08$), in direct contrast to our observations, which showed that there was a significant connection between the number of epithelial cells and WBC.

Regarding the colour of the gastric samples as a diagnostic tool, white-coloured samples indicate the presence of cells, mucus and fat, whilst brown to red samples indicate digested fish and, potentially, the presence of erythrocytes and free haemoglobin (Sweeney & Reddy, 2001), which may indicate a break in the stomach mucosa. Our study showed that gastric lesions were observed on many occasions, but only two gastric samples, both of which were maroon in colour, contained RBC. This finding, along with the tendency of RBC to lyse in highly acidic gastric fluid (Sweeney & Reddy, 2001), suggests that the dark colour of a gastric sample cannot be used as an accurate indicator of gastric ulcers in dolphins.

It is important to note that the oropharynx in dolphins shares a connection with the lumen of the larynx. This connectedness between the respiratory and gastric tracts may result in cells from the respiratory tract being translocated to, and observed in, the gastric fluid (Goldstein et al., 2012; Varela et al., 2007), which are unrelated to potential gastric abnormalities. Additionally, whilst it is possible to examine the main stomach of dolphins using endoscopy, it was not yet practiced at uShaka Sea World at the time of the study. Ulcers have occurred in the main stomachs of bottlenose dolphins (C. M. G. Harper et al., 2000), and since gastric fluid typically moves between the forestomach and main stomach, ulcers present in the main stomach could also potentially impact the cytology of the gastric fluid found in the forestomach.

5 | CONCLUSION

Dolphins in the wild and captivity are susceptible to gastric ulcers, and frequent or prolonged bouts of ulceration in captive dolphins could compromise their welfare. While gastroscopy is the preferred method used to diagnose gastric ulcers, substantial animal training and specialised medical equipment are needed to carry out the examinations. Intubation, which is practised in many facilities that house dolphins, is often used to collect gastric fluid for cytological analysis; however, our results suggest that the cytology of gastric fluid cannot be used as a viable alternative to diagnose the presence and/or severity of gastric ulcers in bottlenose dolphins.

ACKNOWLEDGEMENTS

We would like to thank the Mammals and Birds and the Animal Health Departments at uShaka Sea World for conducting the endoscopes and collecting the samples. We are especially thankful to Ms. Kelly De Klerk and the animal care specialists who have trained the dolphins to participate in the endoscopic examinations voluntarily, and to Dr. Caryl Knox, Dr. Francois Lampen and Dr. Paolo Martelli for their support with the veterinary aspects of this study. We also thank the Sea World Foundation for Research, Education and Development for their financial support of this research.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Ethical clearance to conduct the study was obtained from SAAMBR's Animal Ethics Committee, following the procedure detailed in SAAMBR's Animal Ethics Policy in agreement with the University of KwaZulu-Natal's ethics policy. No ethical issues were raised as all interactions formed part of the dolphins' medical routine carried out by their trainers and uShaka Sea World's veterinarians.

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REFERENCES


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Corrine A. Buhrmann, is PhD candidate with a particular interest in the behaviour and welfare of animals in managed care. His current research aims to generate a greater understanding of dolphin emotion using both quantitative and qualitative behaviour assessment (QBA) methods. Though his research primarily focuses on bottlenose dolphins, also contributed his knowledge of ethology and welfare to studies involving crabeater seals, Cape fur seals and the endangered African penguin.

Tess Gridley, his body of work bridges the gap between pure ethology and applied ecology. By generating fundamental knowledge of animal acoustic behaviour, and the evolutionary and cultural factors shaping it, can inform programmes of passive acoustic monitoring and understand individual movement, distribution, abundance, density and responses to noise. His research contributes directly and indirectly to conservation management of threatened marine species.

Lawrence K. Oellermann, is an NGO Chief Executive Officer with experience in the nature conservation, zoos and aquaria, research, education and capacity building realms; and a Research Director with an interest in animal welfare and husbandry, aquaculture, aquariology & system design, fish taxonomy, aquatic biodiversity, and natural resource management.

**How to cite this article:** Buhrmann, C. A., Gridley, T., & Oellermann, L. K. (2023). Diagnosing gastric ulcers in bottlenose dolphins (Tursiops sp.) using gastroscopy and cytology. *Zoo Biology*, 1–7. https://doi.org/10.1002/zoo.21767