



## Research article

# Improving diagnostic success in stranded dugong necropsy investigations

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## Abstract

Stranding events of dugongs (*Dugong dugon*) provide important opportunities to investigate causes of mortality and population health in the wild. However, successful determination of the cause of death is often constrained by carcass condition and the availability of diagnostic investigations. This study aimed to evaluate factors influencing diagnostic success in stranded dugongs recovered along the Andaman Sea coastline of southern Thailand. A retrospective analysis was conducted using data from a centralized stranding database covering the period 2019 to 2025. A total of 166 stranding records were reviewed, of which 81 carcasses met the criteria for necropsy based diagnostic evaluation after excluding cases lacking examination or affected by advanced decomposition. Necropsy findings, histopathological results, and ancillary diagnostic investigations, including parasitological examination, bacteriological culture, and molecular detection of infectious agents, were compiled for each case. Diagnostic outcomes were categorized into four levels of certainty: undetermined cause of death, presumptive cause of death, tentative diagnosis, and final diagnosis. Overall, 26 cases (32.1%) achieved a final diagnosis, while 11 cases (13.6%) were classified as tentative diagnoses, 21 cases (25.9%) as presumptive causes of death, and 23 cases (28.4%) remained undetermined. Diagnostic resolution improved in later years of the study, with a declining proportion of undetermined cases. Trauma, parasitic disease, infectious disease, and systemic disorders represented the major pathological categories identified. Statistical analysis showed that mild to moderate carcass autolysis and detection of infectious agents tended to increase diagnostic success, whereas female sex and higher body condition score showed the opposite trend, although none of these associations were statistically significant. These findings highlight the importance of carcass condition, systematic necropsy procedures, and ancillary diagnostic investigations in improving cause of death determination in stranded dugongs and supporting effective conservation and health surveillance efforts.

**Keywords:** Cause of death analysis, *Dugong dugon*, Dugong stranding, Marine mammal health, Necropsy investigation, Wildlife pathology

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## INTRODUCTION

The dugong (*Dugong dugon*) is a large herbivorous marine mammal belonging to the order Sirenia and is widely distributed throughout coastal waters of the Indo-West Pacific region (Panyawai and Prathap, 2022). Dugongs play an important ecological role in maintaining seagrass ecosystems through grazing activities that influence seagrass structure, productivity, and nutrient cycling (Marsh, 2018). However, dugong populations have experienced significant declines in many regions, and the species is currently classified as Vulnerable on the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2025). Despite increasing reports of dugong strandings in several regions (Raghunathan et al., 2012; Woolford et al., 2015; Kayanne et al., 2022; Daochai et al., 2024; Sornying et al., 2025), the underlying causes of mortality in many cases remain poorly understood (Khumraksa et al., 2026).

Stranding investigations provide valuable opportunities to assess the health status and mortality causes of marine mammal populations (Chan et al., 2017; Lennon et al., 2025). Necropsy examinations of stranded animals enable detailed evaluation of pathological lesions and other conditions contributing to mortality (Woolford et al., 2015; Eiamcharoen et al., 2025; Díaz-Santana et al., 2026). To improve the accuracy of cause-of-death determination, standardized necropsy and pathology protocols have been developed for dugongs, providing guidance for carcass recovery, examination, and diagnostic interpretation (Eros et al., 2007). These protocols provide a structured framework for carcass recovery, systematic gross examination, tissue sampling, and integration of ancillary diagnostic investigations, while carcass condition is typically classified based on standardized decomposition stages ranging from fresh to advanced decomposition (Eros et al., 2007; Pugliarès-Bonner et al., 2007). However, the practical implementation of these standardized protocols in field-based stranding investigations remains challenging. In real-world conditions, carcasses are often recovered at varying stages of decomposition, may be incomplete, or are subject to logistical constraints that limit comprehensive examination and sample collection (Pietroluongo et al., 2025). In addition, the availability and application of ancillary diagnostic investigations, such as histopathology and laboratory testing, may vary across cases (Khumraksa et al., 2026). These factors can restrict the completeness of necropsy procedures and reduce the reliability of diagnostic interpretation (Khumraksa et al., 2026). Determining the causes of mortality is essential for identifying threats affecting dugong populations and supporting conservation and management strategies (Khumraksa et al., 2026). In Thailand, a national necropsy manual for dugongs has been developed by the Department of Marine and Coastal Resources to guide postmortem investigations (Department of Marine and Coastal Resources, 2013). However, despite the availability of these standardized procedures, limited information is available regarding how these real-world constraints and variations in diagnostic implementation influence the likelihood of achieving a definitive cause-of-death determination in stranded dugongs. This gap is particularly important because information on disease processes and mortality causes in free-ranging individuals remains relatively limited (Khumraksa et al., 2026).

Despite the availability of standardized necropsy protocols, diagnostic success in stranded dugongs remains inconsistent. This discrepancy suggests that failures in cause-of-death determination may not be solely due to biological or environmental factors, but may also reflect limitations in the implementation of necropsy procedures and diagnostic workflows (Khumraksa et al., 2026). In addition, a substantial proportion of stranded carcasses are often not suitable for full necropsy evaluation due to advanced decomposition or logistical constraints (Pietroluongo et al., 2025; Kaewmong et al., 2026). These cases, although frequently excluded from diagnostic analyses, still represent undetermined causes of death at the surveillance level and contribute important epidemiological

information for mortality assessment, as commonly recognized in marine mammal stranding studies (Arbelo et al., 2013; Daochai et al., 2024; Souter et al., 2025). Therefore, this study aimed to evaluate diagnostic success in stranded dugong investigations and to identify factors associated with successful cause-of-death determination, with particular emphasis on the role of necropsy completeness and ancillary diagnostic investigations. Using retrospective data from 166 stranded dugongs recorded between 2019 and 2025, this study examined how variations in carcass condition, diagnostic effort, and workflow completeness influence diagnostic outcomes. This framework provides a basis for improving diagnostic workflows and strengthening mortality surveillance in endangered marine mammals.

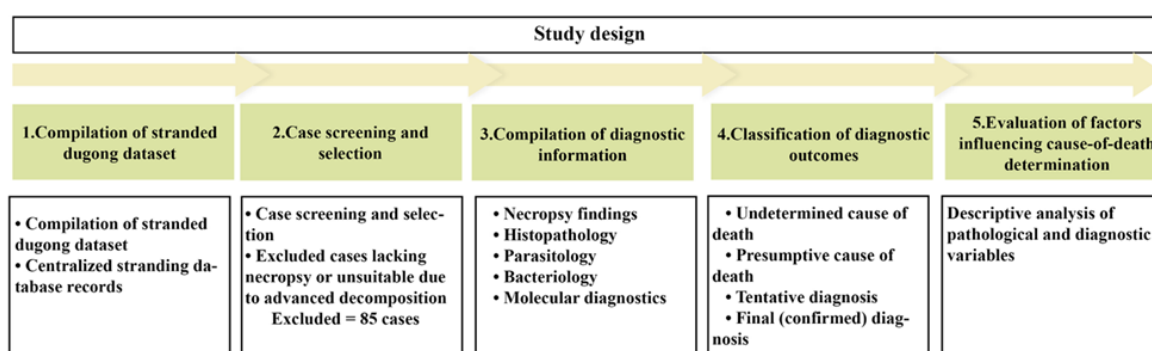
## MATERIALS AND METHODS

### Ethical clearance and sample use authorization

All procedures involving the use of recorded data were conducted in accordance with institutional guidelines and applicable national regulations. No animal tissues were collected or used in this study; the analysis was based solely on previously recorded data. Permission to access and utilize these data was granted by the Department of Fisheries, Thailand (MOAC Document No. 0510.5/12652), in accordance with Section 73(1) of the Wildlife Preservation and Protection Act, B.E. 2562 (2019).

### Study design

This study was designed as a retrospective observational analysis of stranded dugongs recovered along the Andaman Sea coastline of southern Thailand. The objective of the study was to investigate factors influencing the success or failure of disease diagnosis and cause-of-death determination in stranded dugongs based on necropsy investigations and associated laboratory examinations. The overall study design and analytical workflow are illustrated in Figure 1. The analytical framework of the study consisted of several sequential steps.



**Figure 1** Study design and analytical workflow. A total of 166 stranded dugong (*Dugong dugon*) records from 2019–2025 were compiled from the centralized stranding database. After case screening, 85 cases were excluded due to lack of pathological examination or advanced decomposition, leaving 81 carcasses for analysis. Necropsy findings and ancillary laboratory results were compiled for each case and categorized into four diagnostic outcomes: undetermined cause of death, presumptive cause of death, tentative diagnosis, and final (confirmed) diagnosis. Factors influencing successful cause-of-death determination were subsequently evaluated using descriptive analysis.

First, a stranded dugong dataset was compiled from the centralized stranding database, including a total of 166 stranded dugong records collected between 2019 and 2025.

Second, case screening and selection were performed to ensure the reliability of pathological interpretation. Cases that lacked pathological examination or were unsuitable for diagnostic evaluation due to advanced decomposition were excluded. Following this screening process, 85 cases were not included in the necropsy-based diagnostic analysis due to lack of pathological examination or advanced decomposition, which precluded reliable diagnostic evaluation. However, these cases were retained within the overall stranding dataset and considered as undetermined causes of death at the surveillance level. 81 carcasses were retained for further analysis.

Third, diagnostic information was compiled for each individual case. Necropsy findings and ancillary laboratory results were systematically reviewed and organized at the case level. Ancillary examinations included histopathological evaluation, parasitological assessment, bacteriological culture and identification, and molecular diagnostic tests.

Fourth, cases were categorized according to the diagnostic outcome of the investigation. Four levels of diagnostic certainty were defined, including undetermined cause of death, presumptive cause of death, tentative diagnosis, and final (confirmed) diagnosis.

Finally, factors potentially influencing the successful determination of cause of death were evaluated using descriptive analysis based on the available pathological and diagnostic information across cases.

## Stranded dugong dataset

This study was based on recorded data of stranded dugongs recovered along the coastlines of four provinces bordering the Andaman Sea in southern Thailand (Phang Nga, Trang, Satun, and Krabi) between January 2019 and May 2025. All pathological and laboratory findings were compiled as case-based records within a centralized database maintained by the Department of Marine and Coastal Resources (DMCR) and were subsequently used for analysis in this study.

All carcasses were examined through necropsy conducted by trained veterinarians from the Andaman Coastal Research Center (Lower Andaman Sea), DMCR, following standardized marine mammal postmortem protocols (Pugliares-Bonner et al., 2007). The degree of postmortem change was assessed according to established carcass decomposition guidelines (Eros et al., 2007). Carcasses were categorized according to decomposition stage, including live/fresh (minimal postmortem change), fresh (no or minimal autolysis), mildly to moderately decomposed (early to moderate autolysis with partial tissue degradation), and advanced decomposition (severe autolysis, bloating, or tissue disintegration). These categories were used to assess the suitability of carcasses for necropsy and diagnostic evaluation. Carcasses that were severely decomposed or reduced to dry or skeletal remains were excluded from detailed diagnostic evaluation to ensure reliable pathological interpretation. Only carcasses in suitable condition for examination were included for subsequent necropsy-based diagnostic assessment. Body condition was evaluated during necropsy using the Dugong Necropsy Manual of Thailand (Department of Marine and Coastal Resources, 2013). Body condition score (BCS) data were subsequently harmonized with the updated field-based BCS assessment criteria described by Khumraksa et al. (2026) to ensure consistency in body condition classification across cases. Necropsy findings were systematically recorded for each individual carcass. In addition, an initial diagnosis of the cause of death, based on gross pathological findings observed during necropsy, was recorded by the attending veterinarian. Corresponding diagnostic data, including histopathological findings and ancillary laboratory investigations, were also documented. Ancillary examinations included parasitological evaluation (parasite identification and estimation of parasite

burden), bacterial culture and identification, molecular detection of viral pathogens using PCR, protozoal screening by PCR, and fungal examination using PCR.

## Classification of diagnostic outcomes

The classification of diagnostic outcomes was conducted based on the necropsy procedures and diagnostic evaluation principles described in Procedures for the Salvage and Necropsy of the Dugong, Second Edition (Eros et al., 2007), particularly the sections on specimen collection and preservation and determination of causes of death. These guidelines provide a standardized framework for postmortem investigation and interpretation of pathological findings in stranded dugongs. Based on these principles, the available necropsy findings, specimen collection records, and ancillary diagnostic results were reviewed for each case. The strength of diagnostic evidence was subsequently evaluated according to general pathological and necropsy diagnostic principles (King et al., 2007; Zachary, 2022). Cases were then categorized into levels of diagnostic certainty, referred to as diagnostic outcome categories. Four levels of diagnostic certainty were defined: 1) undetermined cause of death, 2) presumptive diagnosis (presumptive cause of death), 3) tentative diagnosis, and 4) final (confirmed) diagnosis.

Cases were classified as undetermined cause of death when no primary cause of death or systemic pathological lesion could be identified and supporting diagnostic information was insufficient. Cases were categorized as presumptive cause of death when gross pathological lesions suggested a plausible cause of death but confirmatory diagnostic evidence was unavailable. Cases were classified as tentative diagnosis when histopathological findings supported a specific disease process, but definitive etiological confirmation was lacking. Finally, cases were classified as final (confirmed) diagnosis when the cause of death was supported by consistent pathological findings together with confirmatory diagnostic evidence.

This classification framework allowed cases to be organized according to the degree of diagnostic certainty and was used to evaluate factors influencing successful cause-of-death determination in stranded dugongs. The criteria used for assigning cases to each diagnostic outcome category are summarized in Table 1.

## Statistical analysis

Statistical analyses were performed to evaluate factors potentially associated with diagnostic success in necropsied dugongs. For analytical purposes, cases categorized as tentative diagnosis and final (confirmed) diagnosis were grouped together and defined as *diagnostic success*, whereas cases classified as presumptive cause of death and undetermined cause of death were grouped as *diagnostic failure*.

Associations between diagnostic success and selected explanatory variables were evaluated using Fisher's exact test, due to the relatively small sample size and the presence of low expected frequencies in several contingency table cells. The explanatory variables included stage of carcass, gender, body condition score (BCS), parasitic detection, bacterial detection, viral detection, and the presence of infectious agents identified through ancillary diagnostic investigations.

For each variable, odds ratios (ORs) were calculated to estimate the direction and magnitude of the association with diagnostic success. Statistical significance was defined as  $p < 0.05$ . All statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA).

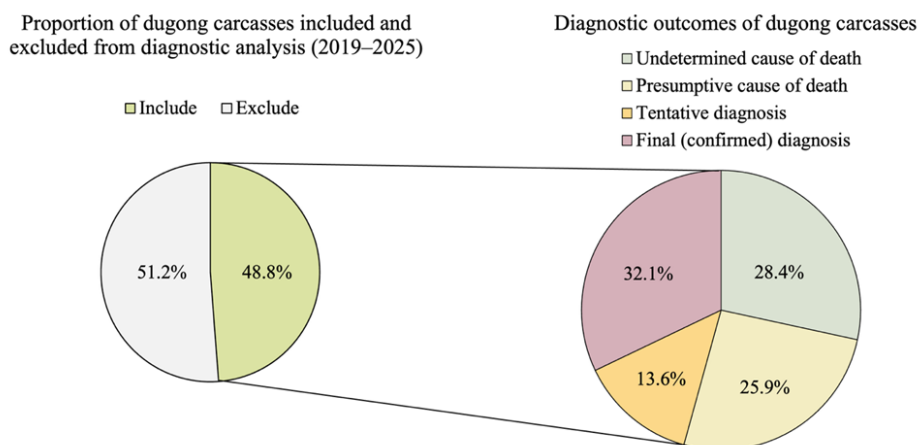
**Table 1** Criteria used for assigning cases to each diagnostic outcome category in stranded dugongs.

| Diagnostic outcome category                               | Definition                                                                                                | Pathological evidence                                                                                                                     | Ancillary diagnostic evidence                                                                                                              | Interpretation                                                                          |
|-----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| <b>Undetermined cause of death</b>                        | No identifiable cause of death based on available information.                                            | No significant gross or microscopic lesions associated with mortality, or carcass condition precluded meaningful pathological evaluation. | Ancillary tests absent, inconclusive, or not performed.                                                                                    | Cause of death cannot be determined due to insufficient diagnostic evidence.            |
| <b>Presumptive diagnosis (presumptive cause of death)</b> | A probable cause of death is suggested based on gross necropsy findings.                                  | Gross lesions suggest a plausible disease process or traumatic event; histopathology may be unavailable or inconclusive.                  | Ancillary testing not performed or insufficient to confirm the etiology.                                                                   | Cause of death inferred primarily from macroscopic pathological findings.               |
| <b>Tentative diagnosis</b>                                | A specific disease process is supported by pathological findings but etiological confirmation is lacking. | Gross and histopathological lesions are consistent with a particular disease process.                                                     | Ancillary tests unavailable, negative, or insufficient for definitive etiological confirmation.                                            | Diagnosis supported by pathology but remains provisional without confirmatory evidence. |
| <b>Final (confirmed) diagnosis</b>                        | Cause of death confirmed by consistent pathological findings together with etiological evidence.          | Gross and histopathological lesions clearly support the disease process responsible for death.                                            | Confirmatory diagnostic evidence present (e.g., microbiology, molecular diagnostics, toxicology, parasitology, or other laboratory tests). | Cause of death determined with high diagnostic certainty.                               |

## RESULTS

### Overall carcass inclusion and diagnostic classification (2019–2025)

Between 2019 and 2025, a total of 166 dugong carcasses were reported through the stranding surveillance system. Of these, 81 carcasses (48.8%) underwent necropsy and were considered suitable for diagnostic evaluation, while 85 carcasses (51.2%) were not examined or were affected by advanced decomposition. These cases were considered as undetermined causes of death at the surveillance level, reflecting inherent limitations in field-based mortality investigations. Only cases subjected to necropsy were included in subsequent analyses (Figure 2).



**Figure 2** Distribution of diagnostic outcomes among necropsied dugong carcasses (n = 81) recorded between 2019 and 2025. Diagnostic outcomes were classified as undetermined cause of death, presumptive cause of death, tentative diagnosis, or final (confirmed) diagnosis.

Among the 81 necropsied dugongs, carcass preservation status at the time of examination varied. Fresh carcasses constituted the majority of cases (48/81, 59.3%), followed by mild to moderately decomposed carcasses (29/81, 35.8%). A small proportion were categorized as live/fresh (3/81, 3.7%), and one carcass (1.2%) was classified as actively decomposed. Sex was determined in nearly all examined cases, comprising 44 males (54.3%), 36 females (44.4%), and one individual of undetermined sex (1.2%). Body condition score (BCS), recorded on a scale of 1 to 5 when available, ranged from poor to good condition, with most individuals presenting with moderate body condition (BCS 2–3).

Diagnostic outcomes were categorized into four hierarchical levels of certainty: undetermined cause of death, presumptive cause of death, tentative diagnosis, and final (confirmed) diagnosis. Across the entire study period, a confirmed cause of death was established in 26 of 81 cases (32.1%). Presumptive causes of death were assigned in 21 cases (25.9%), while 11 cases (13.6%) were classified as tentative diagnoses. The remaining 23 cases (28.4%) were categorized as undetermined cause of death, indicating that despite necropsy examination, a definitive primary lethal mechanism could not be identified (Figure 2). Representative gross and histopathological findings from selected cases with final diagnosis are shown in Figure 3.



**Figure 3** Representative gross and histopathological findings from stranded dugongs with confirmed causes of death. (a–c) Severe blunt trauma affecting the trunk, with extensive hemorrhage in deep musculature and associated soft tissues, consistent with vessel strike. (d) Lung showing severe diffuse suppurative pneumonia. (e) Cross section of lung illustrating purulent exudate within the pulmonary parenchyma. (f) Intestinal perforation associated with heavy infection by *Paradujardinia halicoris*. (g) Diffuse airway infestation by *Pulmonicola cochleotrema*, associated with moderate diffuse eosinophilic pneumonitis. (h–i) Representative histopathological features of eosinophilic pneumonitis, characterized by inflammatory infiltration of alveolar walls and peribronchiolar regions. (j–k) Representative histopathological features of severe transverse parasitic enteritis, with mucosal necrosis associated with *P. halicoris*. Note: Panels (h–k) stained with hematoxylin and eosin (H&E). Original magnifications: (j)  $\times 4$  (scale bar = 500  $\mu\text{m}$ ); (h, i, k)  $\times 40$  (scale bar = 50  $\mu\text{m}$ ).

Overall, approximately two-thirds of necropsied carcasses (58/81, 71.6%) yielded at least a presumptive or higher level of diagnostic interpretation, whereas just under one-third remained undetermined. These findings provide a baseline characterization of diagnostic resolution within the dugong mortality investigation system over the seven-year study period.

### Annual distribution of diagnostic outcomes

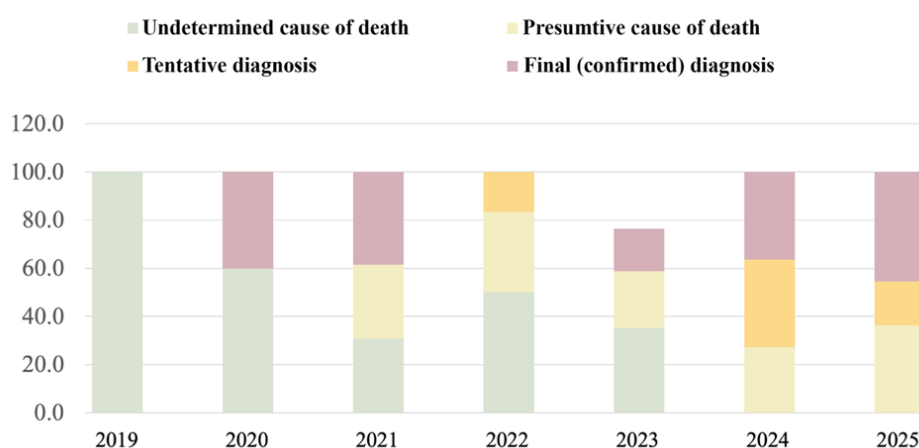
The distribution of diagnostic outcomes varied across years during the study period (Table 2; Figure 4). In 2019, all necropsied carcasses (7/7, 100.0%) were classified as undetermined cause of death. In 2020, diagnostic resolution slightly improved, with 2 of 5 cases (40.0%) reaching a final diagnosis, while the remaining cases were categorized as undetermined (60.0%).

**Table 2** Annual distribution of diagnostic outcomes among necropsied dugong carcasses from 2019 to 2025.

| Year         | n         | Undetermined cause of death<br>n (%) | Presumptive cause of death<br>n (%) | Tentative diagnosis<br>n (%) | Final diagnosis<br>n (%) |
|--------------|-----------|--------------------------------------|-------------------------------------|------------------------------|--------------------------|
| 2019         | 7         | 7 (100.0)                            | 0 (0.0)                             | 0 (0.0)                      | 0 (0.0)                  |
| 2020         | 5         | 3 (60.0)                             | 0 (0.0)                             | 0 (0.0)                      | 2 (40.0)                 |
| 2021         | 13        | 4 (30.8)                             | 4 (30.8)                            | 0 (0.0)                      | 5 (38.5)                 |
| 2022         | 6         | 3 (50.0)                             | 2 (33.3)                            | 1 (16.7)                     | 0 (0.0)                  |
| 2023         | 17        | 6 (35.3)                             | 4 (23.5)                            | 0 (0.0)                      | 3 (17.6)                 |
| 2024         | 22        | 0 (0.0)                              | 6 (27.3)                            | 8 (36.4)                     | 8 (36.4)                 |
| 2025         | 11        | 0 (0.0)                              | 4 (36.4)                            | 2 (18.2)                     | 5 (45.5)                 |
| <b>Total</b> | <b>81</b> | <b>23 (28.4)</b>                     | <b>20 (24.7)</b>                    | <b>11 (13.6)</b>             | <b>23 (28.4)</b>         |

N = Number of the dugong carcasses

**Annual variation in diagnostic outcomes among necropsied carcasses**



**Figure 4** Annual distribution of diagnostic outcomes among necropsied dugong carcasses (n = 81) recorded between 2019 and 2025. Diagnostic outcomes were categorized as undetermined cause of death, presumptive cause of death, tentative diagnosis, or final (confirmed) diagnosis.

In 2021, a broader distribution of diagnostic categories was observed among the 13 necropsied carcasses, including undetermined (4/13, 30.8%), presumptive (4/13, 30.8%), and final diagnoses (5/13, 38.5%). In contrast, no confirmed diagnoses were recorded in 2022; among the six examined carcasses, three cases (50.0%) were classified as undetermined, two (33.3%) as presumptive, and one (16.7%) as tentative diagnosis.

In 2023, diagnostic outcomes remained heterogeneous among 17 necropsied carcasses, comprising undetermined (6/17, 35.3%), presumptive (4/17, 23.5%), and final diagnoses (3/17, 17.6%). A marked shift in diagnostic distribution was observed in later years. In 2024, none of the examined carcasses were categorized as undetermined; instead, cases were distributed among presumptive (6/22, 27.3%), tentative (8/22, 36.4%), and final diagnoses (8/22, 36.4%).

Similarly, in 2025, no cases were classified as undetermined among the 11 necropsied carcasses. Presumptive diagnoses accounted for 4 cases (36.4%), tentative diagnoses for 2 cases (18.2%), and final diagnoses for 5 cases (45.5%), representing the highest proportion of confirmed diagnoses observed during the study period.

Overall, the proportion of undetermined cases decreased in later years, whereas tentative and final diagnoses became more frequent. This temporal

pattern suggests that the observed variation across years reflects changes in diagnostic resolution among necropsied carcasses rather than fluctuations in the number of strandings.

## Distribution of cause-of-death categories

Across the four diagnostic outcome categories, different patterns of pathological findings were observed (Table 3). Cases classified as undetermined cause of death generally lacked sufficient evidence to identify a primary lethal lesion. In these cases, necropsy findings were either nonspecific or limited to secondary observations such as mild gastrointestinal infection, parasitism without quantification of parasite burden, emaciation, or postmortem changes including autolysis and decomposition. In several cases, no clear primary cause of death could be identified during gross examination, and histopathological examination was not available.

**Table 3** Distribution of major pathological conditions within each diagnostic outcome category.

| Cause category                                             | Undetermined cause of death (n=23) | Presumptive cause of death (n=21) | Tentative diagnosis (n=11) | Final diagnosis (n=26) |
|------------------------------------------------------------|------------------------------------|-----------------------------------|----------------------------|------------------------|
| <b>Trauma</b>                                              | –                                  | 4                                 | –                          | 10                     |
| <b>Parasitic disease*</b>                                  | 3                                  | 6                                 | 5                          | 6                      |
| <b>Infectious disease (bacterial / viral / protozoa)**</b> | 1                                  | 2                                 | 1                          | 3                      |
| <b>Systemic / metabolic disorders***</b>                   | 4                                  | 5                                 | 3                          | 2                      |
| <b>Asphyxiation / drowning****</b>                         | –                                  | 2                                 | –                          | 2                      |
| <b>Unidentified / non-specific lesions</b>                 | 15                                 | 2                                 | 2                          | –                      |

\* Parasitic disease: The diagnostic level depended on parasite burden and the presence of associated severe lesions considered sufficient to cause death. Detection of parasites alone without assessment of parasite intensity or associated pathological damage was not considered sufficient for a definitive diagnosis.

\*\* Infectious disease: Final diagnosis required confirmation of the etiologic agent through diagnostic investigation (e.g., histopathology, microbiology, or molecular detection). Cases lacking etiologic confirmation were classified as presumptive or tentative diagnoses.

\*\*\* Systemic or metabolic disorders: Some systemic conditions were interpreted as contributory disease processes rather than the immediate cause of death. The diagnostic level therefore depended on the severity of pathological lesions and evidence linking the condition to the fatal outcome.

\*\*\*\* Asphyxiation or drowning: Diagnosis required clear pathological evidence consistent with drowning or asphyxiation, including characteristic lesions in respiratory or associated organs, as described in marine mammal pathology literature.

Cases categorized as presumptive cause of death presented with gross pathological findings suggesting a possible cause of death but lacked sufficient diagnostic evidence to confirm the primary lethal mechanism. These cases frequently included suspected parasitic or inflammatory conditions such as gastrointestinal abscesses, enteritis, bronchopneumonia, or systemic inflammatory lesions. Some trauma-related cases, including entanglement or suspected drowning events, were also classified as presumptive when gross lesions were described but the severity of injury or direct lethal mechanism could not be clearly established.

Cases assigned as tentative diagnosis showed more consistent pathological findings supporting a specific disease entity but lacked definitive etiologic confirmation. These cases included suspected respiratory diseases such as eosinophilic bronchopneumonia, systemic eosinophilic syndromes, chronic parasitic infections affecting the gastrointestinal tract or pancreas, and metabolic disorders such as chronic hepatic disease.

In contrast, final diagnoses were assigned when the primary cause of death could be clearly identified based on necropsy findings. These cases most frequently involved traumatic injuries such as boat strikes, severe blunt trauma, or confirmed drowning or asphyxiation events, as well as infectious diseases including

bacterial septicemia, viral infection, and severe parasitic infections causing acute respiratory failure or systemic disease.

## Factors influencing diagnostic success

Diagnostic success in necropsied dugongs was influenced by several sequential factors, including carcass condition, completeness of necropsy procedures, and the extent of ancillary diagnostic investigations. Improvements in carcass recovery and management during the later years of the study reduced the number of cases that were not examined or considered non-diagnostic due to advanced decomposition. Consequently, a greater proportion of carcasses were available for systematic necropsy and diagnostic evaluation.

Carcass condition was a critical determinant of diagnostic interpretation. Carcasses classified as live/fresh, fresh, or mild to moderately decomposed allowed thorough gross examination and tissue sampling, whereas severely decomposed or skeletal remains frequently precluded meaningful pathological assessment. All included cases underwent necropsy examination, either complete or partial, enabling evaluation of major organs and identification of gross lesions potentially associated with mortality.

The level of diagnostic certainty was closely associated with the extent of diagnostic procedures performed (Table 4). Cases categorized as undetermined causes of death generally lacked sufficient pathological evidence to identify a specific cause, often due to non-specific lesions or limited ancillary investigations. Presumptive diagnoses were typically based on compatible gross pathological findings suggesting a particular disease process but without definitive etiologic confirmation. Tentative diagnoses were supported by histopathological evidence of disease, providing stronger pathological correlation but still lacking confirmed etiologic identification. In contrast, final diagnoses were achieved in cases where pathological lesions were consistent with a disease process and were supported by etiologic confirmation through ancillary diagnostic investigations, such as microbiological testing, molecular detection of infectious agents, or parasitological identification.

**Table 4** Diagnostic procedures performed across levels of diagnostic certainty in necropsied dugongs (n = 81). The table summarizes the extent of diagnostic investigations conducted at different levels of diagnostic certainty. Undetermined cases were generally limited to gross necropsy findings without sufficient pathological or etiologic evidence. Presumptive diagnoses were primarily based on compatible gross lesions. Tentative diagnoses were supported by histopathological examination, whereas final diagnoses required both pathological evidence and confirmation of the etiologic agent through ancillary diagnostic investigations.

| Diagnostic procedure                    | Undetermined cause of death (n=23) | Presumptive cause of death (n=21) | Tentative diagnosis (n=11) | Final diagnosis (n=26) |
|-----------------------------------------|------------------------------------|-----------------------------------|----------------------------|------------------------|
| <b>Complete necropsy</b>                | ✓ / partial                        | ✓                                 | ✓                          | ✓                      |
| <b>Gross lesion evaluation</b>          | Limited / non-specific             | ✓                                 | ✓                          | ✓                      |
| <b>Histopathology</b>                   | – / limited                        | –                                 | ✓                          | ✓                      |
| <b>Parasitological examination</b>      | ±                                  | ✓                                 | ✓                          | ✓                      |
| <b>Bacteriology / microbial culture</b> | – / limited                        | ±                                 | ✓                          | ✓                      |
| <b>Molecular detection (PCR)</b>        | –                                  | ±                                 | ✓                          | ✓                      |
| <b>Etiologic confirmation</b>           | –                                  | –                                 | –                          | ✓                      |

Note: ✓ performed routinely, ± performed in selected cases, – not performed

Overall, diagnostic success increased when carcasses were in suitable condition for examination, necropsy procedures were conducted comprehensively, and additional diagnostic investigations were performed to support pathological interpretation (Table 4).

## Statistical analysis of diagnostic success

For analytical purposes, cases categorized as tentative diagnosis and final (confirmed) diagnosis were grouped together and defined as diagnostic success, whereas cases classified as presumptive cause of death and undetermined cause of death were grouped as diagnostic failure. This approach was adopted because both tentative and final diagnoses provide meaningful pathological interpretation and allow identification of the underlying disease process, even when definitive etiological confirmation is not available. In contrast, presumptive and undetermined categories reflect limited diagnostic certainty due to insufficient pathological evidence or lack of supporting diagnostic information.

Associations between selected predictors and diagnostic success were evaluated using Fisher's exact test (Table 5). Although no statistically significant associations were identified ( $p > 0.05$  for all variables), several variables showed observable trends. Cases with mild to moderate carcass autolysis, parasitic detection, bacterial detection, and the presence of infectious agents tended to show a higher proportion of diagnostic success compared with their respective reference categories. In particular, the presence of infectious agents showed the strongest tendency toward increased diagnostic success (OR = 2.19), followed by parasitic detection (OR = 1.94), bacterial detection (OR = 1.61), and mild to moderate carcass autolysis (OR = 1.47).

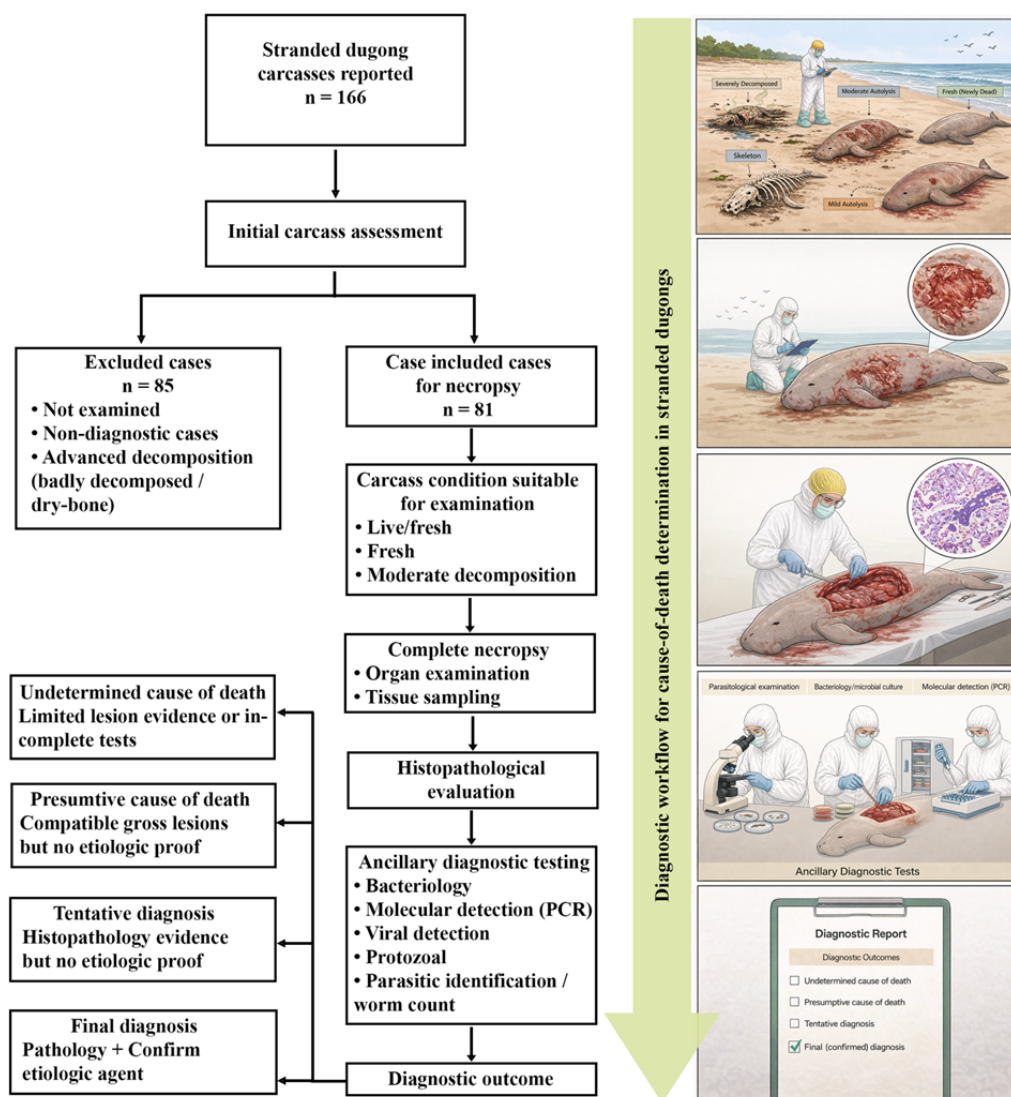
**Table 5** Factors associated with diagnostic success in stranded dugongs based on Fisher's exact test

| Predictor                                     | Diagnostic success n (%) | Diagnostic failure n (%) | Odds ratio (OR) | p-value |
|-----------------------------------------------|--------------------------|--------------------------|-----------------|---------|
| Stage of carcass (mild to moderate autolysis) | 14 (46.7)                | 16 (53.3)                | 1.47            | 0.485   |
| Gender (female)                               | 13 (37.1)                | 22 (62.9)                | 0.77            | 0.651   |
| BCS (>3)                                      | 15 (32.6)                | 31 (67.4)                | 0.46            | 0.112   |
| Parasitic detection                           | 17 (50.0)                | 17 (50.0)                | 1.94            | 0.174   |
| Bacteria detection                            | 15 (48.4)                | 16 (51.6)                | 1.61            | 0.355   |
| Virus detection                               | 1 (100)                  | 0 (0)                    | $\infty$        | 0.407   |
| Presence of infectious agents                 | 18 (51.4)                | 17 (48.6)                | 2.19            | 0.112   |

In contrast, female sex (OR = 0.77) and higher body condition score (BCS > 3) (OR = 0.46) tended to be associated with a lower probability of diagnostic success. However, none of these associations reached statistical significance (Table 5). The absence of statistically significant associations may partly reflect the relatively limited sample size available for statistical analysis.

## Diagnostic workflow for cause-of-death determination

The diagnostic workflow used to determine the cause of death in stranded dugongs is summarized in Figure 5. Among the 166 reported carcasses, 85 cases were excluded due to lack of examination or advanced decomposition and were therefore considered non-diagnostic.



**Figure 5** Diagnostic workflow for determining the cause of death in stranded dugongs. Of the 166 reported carcasses, 85 were excluded due to lack of examination or advanced decomposition. The remaining 81 carcasses underwent necropsy, histopathology, and ancillary diagnostic investigations. Based on the level of pathological and etiologic evidence, cases were classified into four levels of diagnostic certainty: undetermined cause of death, presumptive cause of death, tentative diagnosis, and final diagnosis.

The remaining 81 carcasses were suitable for necropsy and pathological evaluation. Based on the integration of necropsy findings, histopathology, and ancillary investigations, cases were categorized into four levels of diagnostic certainty.

Cases with insufficient lesion evidence or incomplete investigations were classified as undetermined cause of death. When compatible gross lesions were observed but etiologic confirmation was lacking, cases were categorized as presumptive cause of death. Cases with histopathological findings suggestive of a disease process but without etiologic confirmation were recorded as tentative diagnoses. Finally, cases in which pathological findings were supported by confirmatory identification of an etiologic agent were classified as final diagnoses.

This workflow illustrates how increasing levels of diagnostic investigation contributed to higher diagnostic certainty in determining the cause of death. In addition, the framework enabled retrospective evaluation of factors associated with diagnostic success or failure, allowing identification of conditions under which determination of the cause of death was achievable or remained inconclusive.

## DISCUSSION

Dugongs are key marine megafauna in tropical seagrass ecosystems (Marsh, 2011), yet increasing stranding and mortality reports raise conservation concerns in Thailand (Khumraksa et al., 2026). Stranding investigations provide critical opportunities to understand mortality patterns and health threats in wild populations (Chan et al., 2017; Lennon et al., 2025). In the present study, 81 carcasses (48.8%) underwent necropsy and met inclusion criteria, whereas 85 carcasses (51.2%) were excluded due to non-examination or advanced decomposition. Carcass decomposition is a well-recognized challenge in marine mammal stranding programs worldwide and can obscure lesions, compromise tissue quality, and reduce diagnostic confidence in cause-of-death determination (Cunha Ramos et al., 2024; Esposito et al., 2025). Among included cases, fresh carcasses predominated (48/81, 59.3%), followed by mild to moderately decomposed carcasses (29/81, 35.8%), with only a few live/fresh (3/81, 3.7%) and actively decomposed (1/81, 1.2%), broadly comparable to other stranding datasets where diagnostic interpretation relies largely on fresh to moderately decomposed carcasses (Woolford et al., 2015; Díaz-Delgado et al., 2018). The sex distribution (44 males, 54.3%; 36 females, 44.4%) and the predominance of moderate body condition (BCS 2–3) are also consistent with general patterns described in dugong and other marine mammal stranding investigations (Lanyon et al., 2015). Collectively, these baseline carcass characteristics provide essential context for interpreting both mortality patterns and diagnostic outcomes in necropsy-based investigations.

Across necropsied dugongs, major pathological categories included trauma, parasitic disease, infectious disease, systemic/metabolic disorders, and asphyxiation or drowning. Comparable mortality categories have been reported widely in dugongs, reflecting both anthropogenic pressures (e.g., vessel strikes, fishing gear interactions) and natural disease processes (Daochai et al., 2024). Trauma remains a prominent cause of mortality in coastal sirenians (Bonde et al., 2012), while parasitic and infectious diseases are increasingly recognized as important contributors to morbidity and mortality in stranded individuals (Eiamcharoen et al., 2025; Kaewnoi et al., 2025; Sornying et al., 2025; Sukkarun et al., 2025; Keawchana et al., 2026). Despite necropsy, some cases in the present study remained presumptive or undetermined, underscoring common diagnostic constraints in wildlife postmortem investigations, especially when carcasses are incomplete, decomposed, or lack ancillary testing (Khumraksa et al., 2026). Nevertheless, necropsy-based stranding surveillance remains a cornerstone of wildlife health monitoring because it can reveal mortality drivers and provide population-level signals of endemic or emerging disease syndromes relevant to conservation and management (Geraci et al., 2005). In addition, the annual variation in diagnostic outcomes observed in this study should not be interpreted as changes in the number of stranded dugongs, but rather as differences in diagnostic resolution among necropsied carcasses across years. The reduction in undetermined cases (from 100% in 2019 to none in 2024–2025) likely reflects improved diagnostic capability and data quality over time, consistent with previous studies demonstrating that reclassification and data integration substantially enhance cause-of-death determination (Lopes et al., 2018). This pattern is likely associated with enhanced necropsy practices and improved diagnostic accuracy, as postmortem examinations are well recognized to resolve diagnostic discrepancies and establish definitive causes of death (Silas et al., 2009). This trend

may also reflect broader enhancements in postmortem investigation, including diagnostic approaches, carcass recovery, and the use of ancillary diagnostic techniques that enhance diagnostic accuracy (Orakpoghenor and Terfa, 2024). Environmental and stranding-related factors, including carcass drift dynamics, site accessibility, and time to recovery, may influence carcass condition and consequently affect diagnostic outcomes. These processes are known to shape stranding patterns through interactions among drift, carcass arrival, and detection probability, as well as post-mortem decomposition prior to examination (Santos et al., 2018; Peltier et al., 2020); however, these factors were beyond the scope of the present study.

The present study also explored factors potentially associated with diagnostic success in necropsied dugongs. Although no statistically significant associations were detected, several variables showed observable trends. Carcasses classified as mild to moderately decomposed tended to show higher diagnostic success compared with other carcass conditions. This finding may reflect the fact that carcasses recovered before advanced decomposition still allow adequate gross examination and tissue sampling for histopathology and laboratory testing (Brooks, 2016). In addition, the detection of infectious agents, including parasites and bacteria, was associated with a higher probability of achieving diagnostic success. This trend likely reflects the role of ancillary diagnostic investigations in strengthening etiological confirmation and supporting pathological interpretation (Makki, 2016). Previous studies in marine mammal pathology have similarly emphasized that integrating necropsy findings with histopathology and ancillary diagnostic investigations, including microbiological testing, molecular diagnostics, and/or assessment of parasite burden or infection intensity, can substantially improve the accuracy of cause-of-death determination (Van Bressem et al., 2014; Eiamcharoen et al., 2025; Sornying et al., 2025; Díaz-Santana et al., 2026; Keawchana et al., 2026). Conversely, female sex and higher body condition score tended to show lower diagnostic success in the present dataset, although the biological significance of these trends remains uncertain and may reflect sampling variability rather than true biological effects. In addition, other contextual factors not systematically analyzed in the present study, such as stranding location and the presence of gross traumatic indicators, may also influence diagnostic outcomes. These variables may affect carcass recovery conditions, accessibility, and the ability to detect key pathological features, particularly in advanced stages of decomposition. Although these factors were not consistently available across all cases and were therefore not included in the current analysis, they represent important considerations in the interpretation of diagnostic success in field-based investigations. The relatively small sample size available for statistical analysis may also have limited the ability to detect significant associations (Faber and Fonseca, 2014).

Overall, this study demonstrates that successful disease diagnosis in stranded dugongs requires systematic necropsy procedures following standardized protocols. Histopathological examination together with ancillary diagnostic investigations are key components for confirming etiological agents and improving cause-of-death determination. Strengthening these diagnostic approaches can help reduce undetermined mortality cases in dugong stranding investigations.

## CONCLUSIONS

This study evaluated factors influencing diagnostic success in necropsy investigations of stranded dugongs along the Andaman Sea coast of Thailand. The results demonstrate that successful determination of cause of death depends largely on systematic necropsy procedures, appropriate carcass condition, and the integration of pathological and laboratory investigations. Histopathological

examination represents a critical component for accurate pathological interpretation, while ancillary diagnostic investigations, including microbiological, molecular, and parasitological analyses, are essential for confirming etiological agents. Strengthening these diagnostic approaches will improve cause-of-death determination and help reduce undetermined mortality cases in dugong stranding investigations. Such improvements are important for supporting long-term conservation efforts and enhancing marine mammal health surveillance in the region.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

**Najyamee Inthacho:** Conceptualization (equal); data curation (equal); formal analysis (lead); methodology (lead); writing – original draft (supporting).

**Peerapon Sornying:** Methodology (supporting).

**Narissara Keawchana:** Methodology (supporting).

**Piyarat Khumraksa:** Data curation (supporting); formal analysis (lead); methodology (equal); resources (equal).

**Santi Ninwat:** Resources (lead).

**Watcharapol Suyapoh:** Conceptualization (lead); data curation (lead); formal analysis (supporting); funding acquisition (lead); investigation (lead); methodology (supporting); project administration (lead); software (lead); supervision (lead); validation (lead); visualization (lead); writing – original draft preparation (lead); writing – review & editing (lead).

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