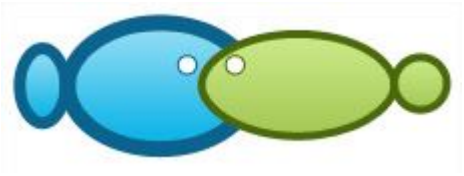

The stomach content analysis of whales in Alor and Lamalera Islands, Savu Sea, Indonesia

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Abstract. There has been little information and no comprehensive study made on whales (Cetacean) in Indonesia, particularly in the region of the Savu sea. Alor Island and Lamalera Island are a part of the Savu sea region. Savu sea is the main migratory route for cetaceans. The research aims to determine the DNA makeup of whales in the Savu sea region, both stranded and caught, and to perform a DNA analysis of their stomach content to determine their natural diet. The research was conducted from May to September 2020. The results of the DNA analysis of stranded whales found the species of *Mesoplodon densirostris*. In the stomach of the stranded whale, a parasitic worm of *Ancylostoma ceylanicum* was found. DNA analysis of the whales caught by fishermen in Lamalera Island was *Physeter catodon*. The squid *Sepioteuthis lessoniana* is determined to be the natural diet of the whales in this region. *Sepioteuthis lessoniana* was determined to be the natural diet of the whales.
Key Words: Alor, DNA amplification, Lamalera, squid, whale.

Introduction. Alor and Lamalera are islands located in the Savu sea region, at the east end of Nusa Tenggara Islands, Indonesia. These islands are bordered by the Sea of Flores and Banda Sea to the north, Ombai strait to the south (separating it from Timor Islands), and Strait of Pantar to the west (separating it from Pantar Island). Pods of whales can be found in the waters of Alor, Lamalera, and their surrounding regions. Every year, cases of stranded whales are reported. Whales are marine mammals that live in seas worldwide and are sought after for their economic value. Two parvorders of whales are currently known: the toothed whale (*Odontoceti*) and Baleen whales (*Mysticeti*). *Odontoceti* is carnivorous, and its diet consists of fish, squids, and cuttlefish. They are closely related to dolphins and snub fin dolphins. Members of *Mysticeti* parvorder are generally ampler in size than those of *Odontoceti*. They possess distinct, brush-like organs called baleens. This unique structure is used to catch their natural diet, planktons (Bergman 2012; Berta et al 2016).

There are 31 species of cetaceans of whales, dolphins, dugong in the Indonesian seawaters (Rosas et al 2012). These animals are spread all over the coast to the deep sea and have both residential and migratory behavior. Several species of cetaceans with migratory behavior occupy the Eastern Indonesia waters as a migratory pathway from the Indian to Pacific ocean through the straits of Komodo island, Solor - Lembata, Alor, Banda Sea, southeast Sulawesi, east Sulawesi, and Sorong – Fakfak of Papua island at the north (Mujiyanto et al 2017). Bottlenose dolphins (*Tursiops truncatus*) were recorded and exhibited bow-riding behavior and feeding; Fraser's dolphin (*Lagenodelphis hosei*) were recorded exhibiting the behavior of traveling and aerials driving arc; Pantropical spinner dolphins (*Stenella longirostris*) were recorded showing traveling bows and aerials; and the Pantropical dolphins (*Stenella attenuata*) were recorded exhibiting aerial avoidance and aerial behavior. Recorded whales were the dwarf killer whale (*Feresa*

attenuate) and dwarf sperm whales (*Kogia breviceps*). The coastal fishing communities of Alor and Lamalera are very familiar with whales, because these animals are one of the marine products that are considered delicacies and precious materials in ethnic crafts. However, the causes of frequent stranding of whales in the region, both individually and *en masse*, have yet to be determined.

More information regarding the Cetacean species based on DNA analysis in Alor and Lamalera is necessary to be obtained because it can contribute to marine wildlife conservation efforts. Effective and appropriate conservation efforts must be based on precise data, as the authors provide in this paper. Therefore, it is necessary to study whales in the Strait of Alor and Lamalera, East Nusa Tenggara, Indonesia. So far, the actual cause of the population decline is unknown. Traditional whaling (through whale capturing) has a minimum destructive impact on fish. The research had two objectives. Firstly, to determine the types of whales stranded in the Alor Strait and those caught on the Lamalera coast as part of the Savu Sea, East Nusa Tenggara, Indonesia. Secondly, to provide DNA analysis of whale guts. The purpose of this research is to determine the DNA analysis of the captured whale, stranded whale, whale guts content to confirm their presence in the Savu sea. The analysis of the whale stomach content reveals the whale natural feed, based on DNA analysis.

Material and Method

Sample collection. The meat samples of stranded whales were collected from coastal areas of Alor Strait, and the samples of caught whales were collected from fishermen operating in the vicinity of Lamalera. The samples collected were slices of the whale meat and the stomach content of whales. After collection, the samples were stored in a cooler box and transported to Genetica Science Indonesia laboratory, Jakarta, for analysis. To analyze the identification of whales with molecular methods, it is enough to collect any fish body parts weighing 100 g.

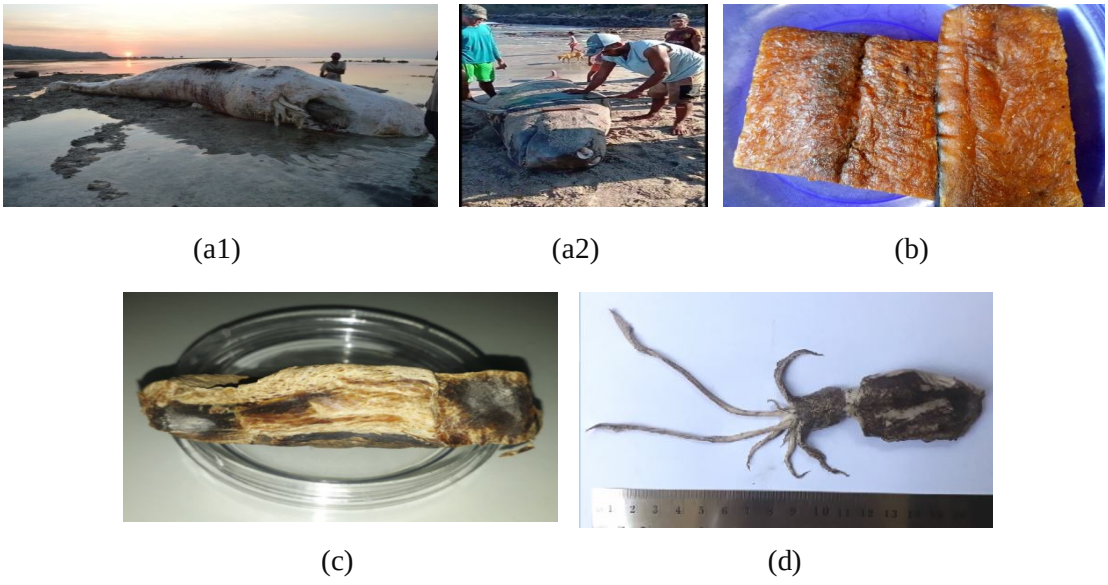
DNA extraction, ***DNA amplification***, ***and sequencing***. DNA extraction of all samples was performed using two commercial kits, ZR Tissue and Insect DNA MiniPrep genomic DNA. The sample was directly placed into a ZR BashingBead ™ Lysis Tube and was processed. The DNA from the samples was isolated and purified with Fast-Spin technology.

Genomic DNA extraction was carried out using ZR Tissue and Insect DNA MiniPrep. The amplification PCR utilized MyTaq Red Mix (Bioline) BIO-25047. PCR Master Mix consisted of several components (25µl), namley: dd H2O reagent, MyTaq Red Mix (Bioline) BIO-25047, VF2_t1: TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC, FishF2_t1: TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC, FishR2_t1: CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA, FR1d_t1: CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA dan template DNA. The sequencing process was carried out using ABI PRISM 3730xl Genetic Analyzer developed by Applied Biosystems, USA. The kit used for the sequencing purpose is BigDye® Terminator v3.1 Cycle Sequencing.

Homology Using BLAST. Analysis of the sample DNA sequence was compared with the sequence in the DNA database. The search was carried out using the *Basic Local Alignment Search Tool* (BLAST) database in *National Center for Biotechnology Information*, *National Institute for Health*, USA (Pearson 2013) to determine the percentage match of species based on the level of homology of the nucleotide base sequence. The sequence of nucleotide bases is then compared with a database available on GenBank. Visual qualitative data, DNA isolation and amplification profiles, and electropherograms are presented descriptively.

Results and Discussion

Stranded whales in Alor coast and caught whales in Lamalera coast. There are reports of whales stranded in Alor waters almost every year. They mention up to 7 whales between 5 and 7 meters long. However, the authors got information from the research site that the longest stranded whale recorded in the area exceeded 10 meters. April to November is when it is common to find beaching whales on the coast of Alor Island. Stranded whales are commonly dark blue/gray on top, and lighter gray on the underside, and their heads are usually in the shades of brown. Almost all of them have injuries on their bodies. These whales are generally found in physical decomposition (Figure 1a). They are then buried on the beach using heavy equipment. Whales caught in the coastal area of Lamalera and its surroundings are usually cut and distributed to coastal village communities to be consumed. Each family processes them into various products. One processed product made from whale meat is the sun-dried marinated fish jerky (Figure 1b). The stomach content of the stranded or captured whales and the digestive material found in their stomachs are presented in Figures 1c and 1d. Squid (*Sepioteuthis lessoniana*) is the whale's natural feed and a species abundant in Alor and Lamalera seawater.



(a1) (a2) (b)

(c) (d)

Figure 1. (a1) stranded whale in Alor beach; (a2) caught whale in Lamalera beach, (b) dried whale meat for consumption, (c) stomach content of a stranded whale, (d) squid (*Sepioteuthis lessoniana*) found in the stomach of caught whale (Source: authors' personal archive).

Identification of whale, their stomach content and diet, and DNA amplification. The stomach content of a stranded whale was extricated from its intestines. Cuts of the stomach content were collected and were analyzed using the molecular DNA method. Amplified COI gene was measured at 700 bp of length using the 10,000 bp DNA ladder for comparison. Amplified COI gene was measured at 700 bp of length using the 10,000 bp DNA ladder for comparison. The results of DNA amplification of stranded whale isolates, caught whales, and their stomach content are presented in Figure 2. It can be seen that the isolates produce a single band with a size of 700 bp (base pair) according to the comparison using DNA markers. The measured isolates had base lengths of, in increasing order, 100, 200, 300, 400, 500 600, 700, 800, 900, 900, 1000, 1500, and 3000 base pairs, respectively.

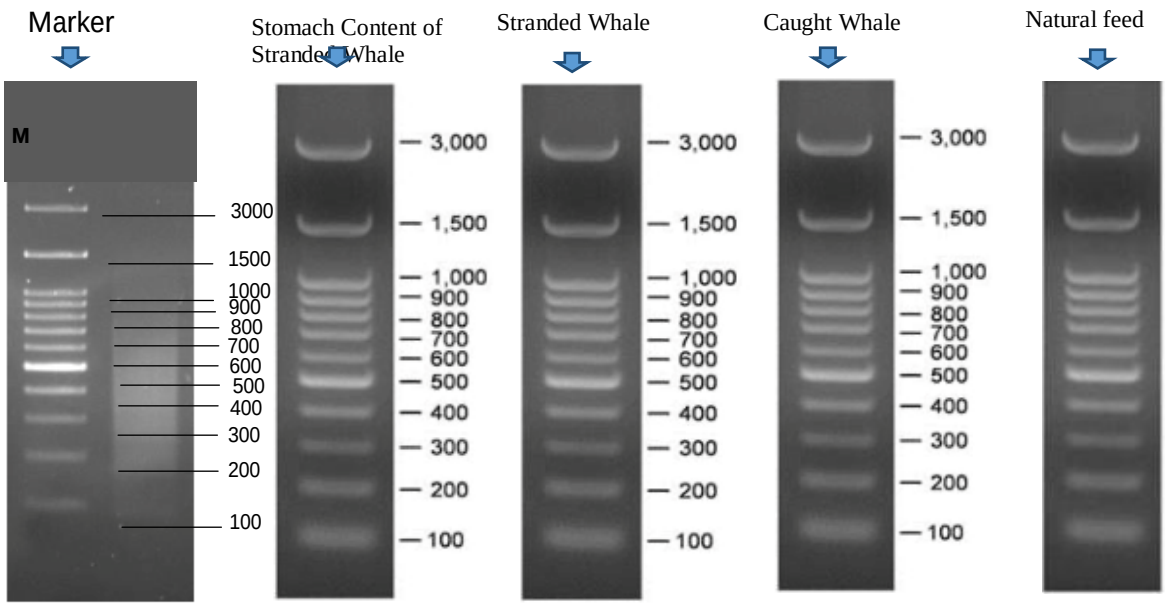


Figure 2. DNA amplification of the stomach content of stranded and caught whales (source: photo taken by authors).

Material identification using BLAST analysis indicates that the stranded whale stomach content isolate (spesific amino acid compound that is separated chemically indicating the DNA) with access number KY640299.1 has the value of 98% homology with the parasite *Ancylostoma ceylanicum*. It also indicated that the stranded whale isolate with access number KF032875.2 has 97% homology with the whale species *Mesoplodon densirostris.* Meanwhile, caught whale gut isolate with access number KJ168062.1 has the value of 98% homology with squid from the species of *Sepioteuthis lessonianai.* Whale isolate with access number KU891394.1 has the value of 100% homology with *Physeter catodon* whale species (Table 1 and Figure 2).

Table 1

The identity of stranded whales, caught whales and the contents of their stomachs by BLAST analysis

No	*Isolate*	*Relative match*	*Homology (%)*	*Access No.*
1	Stranded whale	*M. densirostris* isolate SWFSC ID z0079838 mitochondrion, partial genome	97%	KF032875.2
2	Stomach content of stranded whale	*A. ceylanicum*	98%	KY640299.1
3	Caught whale	*P. catodon* isolate mtGen81 mitochondrion, complete genome	100%	KU891394.1
4	Natural feed, squid	*S. lessoniana*	98%	KJ168062.1

The results of phylogenetic tree processing indicate the clustering of isolates with other organisms in the closest to the furthest levels. The intestinal contents of the stranded whales were related to the parasitic roundworm *A. ceylanicum*, with *U. sanguinis* haplotype, *Rhabditida sp*, and *Nematode sp* as the outer group. The phylogenetic tree of stranded whales indicates that the isolates are related to whale species of *M. densirostris*. The phylogenetic tree of stomach content isolates of caught whales shows clustering with the genus *Teuthida* and the species *S. lessoniana*. The phylogenetic tree of caught whales clustering with whales of the species *Physeter catodon*. Morphologically, *P. catodon* whale

has a distinguishably large and blunt cranium. The size of its head can take up to one-third of its total body length. The tip of its lower mandible is located precisely at the rear-end of its frontal cranium.

There are also reports that smaller whales, such as members of the genus *Berardius*, washed ashore of Lamalera island. Whales of the species *B. minimus,* a newly named species, can also be found in the local waters of Hokkaido, Japan (Yamada et al 2019). Information on the distribution and ecology of whales is still limited, especially for regions in Indonesia, because the distribution of species and their behavior is unknown. This research focuses on two types of whales, stranded whales and caught whales. DNA analysis indicated that the strand whale sample type was *M. densirostris*, while the DNA sample of the caught whale was *P. catodon*. According to information from people around the coast of Alor and Lamalera, *Mesplodon* (beaked whale) is often stranded around the coast of Alor, and *P. catodon* is the primary source of livelihood for whale hunters in Lamalera.

P. catodon with box-like head morphology is one the most common in temperate and tropical latitudes and most likely to be found in waters inhabited by squid because it is a carnivore feeding on squid or molluscivore. It has a swimming depth from 1,000 m to the sea's surface and is adapted to cold-water upwellings. Because they are so well-adapted for deep-water swimming, they are in real danger of stranding when they move inshore. This study found a parasitic worm of the species *A. ceylanicum* in the stomach content of the stranded whales. The presence of these pathogenic organisms may explain why the whales can become unhealthy, which may eventually lead to stranding. As happened to other types of mammals (seals) these hookworms are also the cause of death (Seguel et al 2018). *A.ceylanicum* is a hookworm commonly found in domestic dogs and cats throughout Asia and Australia. Studies in Australia inform intervention and prevention strategies to control hookworm parasites such as *A. ceylanicum* in dogs and humans. A "One Health" approach is essential for preventing these diseases (Beknazarova et al 2020).

A. ceylanicum is more common in tropical soils and areas, and its presence can pose a health and environmental threat (Smout et al 2017). The results of this study are also supported by Papaiakovou (Papaiakovou et al 2017), which explains that *A. ceylanicum* is a zoonotic hookworm, which is most likely the second most common hookworm species infecting humans in Asia. WHO also reports that *A. ceylanicum* is the cause of 'neglected diseases' which are zoonotic, and this parasite can harm general public health (Thompson 2015).

The discovery of this parasite in the whale's stomach can be an indicator of the poor health of the whales so that they cannot navigate properly and eventually die from being stranded on the beach. This finding is related to the research findings reported by (García-Grajales et al 2017). A survey was conducted along the central coast of Oaxaca, Mexico. The results of the study reported the condition of several *M. peruvianus* whale carcasses that were washed ashore. The main pathological research findings refer to the respiratory and digestive systems of whales. They describe air that appeared from the chest cavity, indicating pneumothorax, collapsed lungs, and showed consistency of atelectasis if touched. A collapsed lung of a stranded individual may be the result of apnea, which is associated with dysbarism (García-Grajales et al 2017).

Tracing the natural diet of whales in the Strait of Alor and Lamalera using DNA analysis of stomach content indicated that their food in the area predominantly consists of squid from the species *S. lessoniana*. A study by Clarke & Pascoe (1997) on the analysis of stomach content of stranded, male *P. catodori* whales in Penzance, Cornwall reported a diet consisting of four families of cephalopods, namely *Histioteuthis* spp., *Architeuthis* spp., *T. sagittatus,* and *Galiteuthis* sp. This diet is enriched with the intake of Oceanic Octopod, *Haliphron atlanticus*, and only one neritic species: *Loligoforbesi*. The results of this study indicated that most of the squid consumed were oceanic species, namely *Histioteuthis* spp., *Architeuthis* spp., *T. sagittatus*, and *Galiteuthis* sp. This finding is similar to what was reported in a study by Santos et al (2001), who discovered that the whale's diet consisted of oceanic squid, oceanic fish remains, and crustaceans. The squid found in the stomach of *P. catodori* from the coast of Alor, Indonesia, is *S.*

lessoniana, a neritic species. This finding could indicate that the coasts of Alor and Lamalera are the feeding grounds for these two whale species because of the concentration of the number of the squid species and the suitability of the currents in the two regions. Whale migration and residence patterns are closely related to currents, which in turn affects the potential abundance of food sources in the waters (Aulich et al 2019). This condition makes the straits of Alor and Lamalera as ideal stopping points in the migration routes of these two whale species. *S. lessoniana* is a squid species with a very short life cycle, only four months, with more than 1000 spawns being hatched per cycle. After they lay eggs, they still have a chance to spawn again. This fertility is the source of abundance and the speed of population recovery for this species (Pringgenies et al 2000; Arkhipkin et al 2015; Pringgenies & Ariyanto 2021). In comparison, the squid of the *L. duvaucelii* species has a life cycle of 12 months. Mature individuals of this species can spawn eggs only once because afterward, the parent will die (Lipiński et al 2016). Visual and field observations at the coastal fish markets of Alor and Lamalera indicate abundant populations of squid and fish species from deep waters in these two areas. Jefferson et al (1993) estimated that *M. densirostris* had various species of deep-sea fish and squids as its natural diet. Furthermore, the paper also reported that Blainville's beaked whale was found in the temperate and tropical waters. The species is commonly found off the shores of Hawaii, where they have been observed both in the water and stranded on the coast. They are found in deep water, either individually or in small pods, where the depth of the oceans reaches 1000 fathoms (about 660 m). Due to their deep-water habitat, Blainville's beaked whale has rarely been seen swimming freely over the years, and it was identified as a species only by its skeletal remains.

The molecular DNA analysis of this study revealed that the caught whale in Lamalera was *Physeter catodon* and the species of the stranded whale was *Mesoplodon densirostris.* The Baleen whale, its common name, was seen in the waters around Denmark with a length of almost 9 meters, possessing a very large head covering nearly the entirety of its body (Friel & Sleeman 2003), similar to those seen in the waters of Alor and Lamalera, Indonesia. An increase in *P. catodon* population was reported among waters in England and Ireland (Cabot 1967). The information is exciting because this could mean that the whales of the species *P. catodon* may thrive due to their low mortality rate. Another interesting finding regarding this species is the commensal bacteria that exist in its body. Commensal bacteria live in symbiosis in the body of whales, are not harmful, and can even be beneficial in some cases. Like bacteria from the stomach contents of sea cucumbers, they have the potential to act as an anti-bacterial (Pringgenies et al 2019). Bacterial symbionts in gastropods can be anti-bacterial multi-drug resistant (Bahry et al 2017). However, in certain conditions, for example, in immuno-suppressants, these bacteria can be a health risk to the host. Commensal microbes of mammals play an essential role in the health of their host. The research results on tissue samples of whale blood, feces, and muscle microbiome showed differences in symbiotic microorganisms among the three tissues. Pathogenic bacteria detected in blood, muscle, and fecal samples can cause several health problems in whales (Li et al 2019). The findings of this research on whales in the waters of Alor and Lamalera contribute to the new knowledge regarding the health problems as a cause of whale mortality. They can be used as a benchmark for monitoring the health of marine mammals. This very first study of whale (Cetacean) species identification using molecular method of DNA analysis will give the high accuracy and universal result, but may have some difficulties in whale meat sample collection in remote areas. This is because the Alor Island and Lamalera areas are beautiful small islands but with limited access possibilities.

Conclusions. DNA analysis found that the species of the stranded whale was *Mesoplodon densirostris*, whereas the caught whale species was *P. catodon*. The results of the DNA analysis showed that the parasitic worm *A. ceylanicum* was present in the stomach content of the stranded whale. They also revealed that the squid *S. lessoniana* was the natural diet of the whales in this region.

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Conflict of interest. The authors declare that there is no conflict of interest.

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