



Diet and mitochondrial DNA haplotype of a sperm whale (*Physeter macrocephalus*) found dead off Jurong Island, Singapore

Marcus A.H. Chua^{1,2}, David J.W. Lane¹, Seng Keat Ooi³, Serene H.X. Tay⁴ and Tsunemi Kubodera⁵

¹ Lee Kong Chian Natural History Museum, National University of Singapore, Singapore, Singapore

² Department of Environmental Science and Policy, George Mason University, Fairfax, VA, United States of America

³ Tropical Marine Science Institute, National University of Singapore, Singapore, Singapore

⁴ Civil and Environmental Engineering, National University of Singapore, Singapore, Singapore

⁵ National Museum of Nature and Science, Tokyo, Japan

ABSTRACT

Despite numerous studies across the large geographic range of the sperm whale (*Physeter macrocephalus*), little is known about the diet and mitochondrial DNA haplotypes of this strongly female philopatric species in waters off Southeast Asia. A female sperm whale found dead in Singapore waters provided the opportunity to study her diet and mitochondrial DNA haplotype. Here we report on the identification of stomach contents and mitochondrial DNA haplotype of this individual, and we include coastal hydrodynamic modelling to determine the possible geographic origin of the whale. At least 28 species of prey were eaten by this adult female whale, most of which were cephalopods. The mesopelagic squids *Taonius pavo*, *Histioteuthis pacifica*, *Chiroteuthis imperator*, and *Ancistrocheirus lesueurii* made up over 65% of the whale's stomach contents. Plastic debris was also found in the whale's stomach. Based on the diet, genetics, and coastal hydrodynamic modelling that suggest an easterly drift of the whale carcass over several days, the dead sperm whale in Singapore probably originated from a pod in the Southern Indian Ocean. This study provides an increase in the understanding the diet and natural history of the sperm whale in Southeast Asia. The combined analyses of stomach contents, DNA, and hydrodynamic modeling could provide a context to future studies on the sperm whale strandings, and have broader applicability for other marine mammals in the region.

Submitted 15 December 2018

Accepted 3 March 2019

Published 5 April 2019

Corresponding author

Marcus A.H. Chua,
marcus.chua.ah@gmail.com

Academic editor

James Reimer

Additional Information and
Declarations can be found on
page 12

DOI 10.7717/peerj.6705

© Copyright
2019 Chua et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

Subjects Biodiversity, Ecology, Marine Biology, Zoology

Keywords Stomach contents, Cephalopod, Hydrodynamic modelling, Population genetics, Prey, Plastic, Pyrosoma, Indo-pacific

INTRODUCTION

The sperm whale *Physeter macrocephalus* Linnaeus, 1758 has a large geographic range encompassing all oceans in temperate and tropical waters (Rice, 1989). They are typically found in regions with deep, off-shore waters, and their diet, which consists mainly of cephalopods—with regional differences in species composition—has been studied in the Atlantic, central, eastern and northern Pacific, and Southern Oceans (e.g.,

Clarke, 1956; Okutani *et al.*, 1976; Okutani & Satake, 1978; Kawakami, 1980; Clarke & Young, 1998; Best, 1999; Smith & Whitehead, 2000; Evans & Hindell, 2004; Garibaldi & Podesta, 2014; Harvey, Friend & McHuron, 2014). Pelagic and benthic fishes (actinopterygii and chondrichthyes), crustaceans, and tunicates are also eaten in smaller quantities, indicating different modes of foraging (Kawakami, 1980; Best, 1999; Evans & Hindell, 2004; Santos *et al.*, 2002). Sperm whale population structure and genetic studies of the maternally-inherited mitochondrial DNA have shown differentiation between populations, suggesting female philopatry (Richard *et al.*, 1996; Lyrholm *et al.*, 1999; Engelhaupt *et al.*, 2009; Mesnick *et al.*, 2011; Alexander *et al.*, 2016).

However, even though sperm whales are present in waters off Southeast Asia, which is surrounded by the Indian Ocean and western Pacific Ocean (Indo-West Pacific), the diet and mitochondrial DNA haplotypes of sperm whales there are largely unknown to science. Live sperm whales have been recorded off northwest Malay Peninsula, the Lesser Sunda archipelago, the Sunda Strait of the Indian Ocean, and the South China Sea north of Borneo (Anderson & Kinze, 1999; Barnes, 2005; De Boer, 2000) (Fig. 1). Strandings and carcasses are known from Borneo, Java, the Lesser Sunda archipelago, northwest of the Malay Peninsula, Papua, Raja Ampat Islands, Sulawesi, and Sumatra (Anderson & Kinze, 1999; Anon, 2017; Anon, 2018; Cahya & Simanjuntak, 2017; Mustika *et al.*, 2009; Mueanhawong, 2012; Ponnampalam, 2012). Despite their widespread distribution and numerous physical records in the Indo-West Pacific, little else is known about sperm whales in the region.

On 10 Jul 2015, a 10.6 m long adult female sperm whale with erupted mandibular and maxillary teeth (size and tooth eruption suggesting sexual maturity) was found dead off the coast of Jurong Island (Fig. 1), Singapore, which provided an opportunity to salvage the carcass, and conduct a scientific study on aspects of the natural history of the whale. The objectives of this study were to (1) determine the diet of the sperm whale from stomach contents by morphological identification of remains, (2) describe the relative proportion of each prey species and the size of cephalopods eaten by the whale, (3) determine the mitochondrial DNA control region haplotype of this sperm whale, and (4) investigate the possible location of the whale's origin using a coastal hydrodynamic model. This study provides the first description of the diet and mitochondrial DNA haplotype of a sperm whale from Southeast Asia, could provide a context for future studies of sperm whale strandings, and have broader applicability for other marine mammals in the region.

MATERIALS & METHODS

Stomach contents collection, morphological identification and analysis

Stomach contents were collected from an adult non-pregnant female sperm whale of 10.6 m body length found dead off the coast of Jurong Island, Singapore (1°16'48.23"N 103°43'57.23"E) on 10 July 2015 (Fig. 1). The subject was identified as an adult based on the body size and presence of erupted mandibular and maxillary teeth. The carcass was towed to a section of a beach not accessible to the public and lifted to land by a lorry crane for defleshing and skeleton preservation. Approval to work on the section of the beach

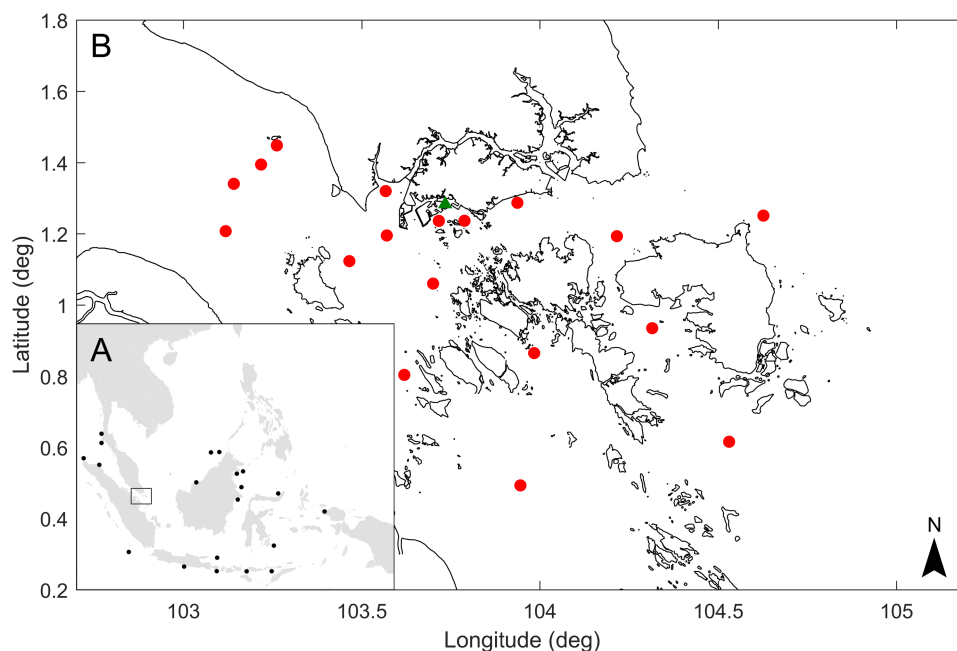


Figure 1 Map of sperm whale records in waters off Southeast Asia, location where the sperm whale was found in Singapore and approximate release points of simulated drogues representing the floating dead whale. (A) Black dots represent sperm whale records in Southeast Asia (Anderson & Kinze, 1999; Barnes, 2005; De Boer, 2000; Anon, 2017; Anon, 2018; Cahya & Simanjuntak, 2017; Mustika et al., 2009; Mueanhawong, 2012; Ponnampalam, 2012). (B) Green triangle shows the location where the sperm whale carcass was found in Singapore, and red dots show the approximate release points in the vicinity of Singapore of simulated drogues.

Full-size  DOI: [10.7717/peerj.6705/fig-1](https://doi.org/10.7717/peerj.6705/fig-1)

was granted verbally by Boon Kiang Chew from the National Environment Agency of Singapore. Examination of the whale revealed a copiously bleeding large gash on the back and associated spinal injury, with a few of the caudal vertebrae smashed, possibly caused by a ship strike. Owing to the rapid deterioration of the carcass in tropical conditions after removal from seawater, a standard necrosis was not possible.

Approximately 80% of the total stomach contents were collected. The stomach contents were washed and preserved in 70% ethanol. Pre-sorting of the stomach contents, in particular, upper and lower cephalopod beaks was first performed, and identification of lower cephalopod beaks was later determined by one of the co-authors (TK), and following Kubodera (2005), and Xavier & Cherel (2009). Other diet items were sorted and identified to the lowest taxonomic level by the authors (MAHC and DJWL), and biologists from the Lee Kong Chian Natural History Museum, National University of Singapore.

The average estimated dorsal mantle length (DML) and mass of individuals of each cephalopod species were calculated from the lower rostral length of beaks where conversion formulas were available (Kubodera, 2005; Xavier & Cherel, 2009; Clarke, 1986; Rodhouse et al., 1990; Lu & Ickeringill, 2002). For species represented by more than 100 beaks, a sample average of 100 beaks was taken. Diet items were counted, and expressed as a percentage total (PT) by number of all prey items present.

Mitochondrial DNA haplotype identification

Samples of the sperm whale skin and skeletal muscle were collected and frozen at -20°C . Samples of DNA were extracted from each tissue type using QuickExtract (Epicentre, Madison, WI, USA) following the manufacturer's protocols. Two replicate for each tissue type was done to minimize the likelihood of reporting erroneous sequences because of sequencing error. A section of the mitochondrial DNA control region was targeted using primers and PCR protocols from [Southern, Southern & Dizon \(1988\)](#) with a negative control. The resulting PCR product was visualized under UV light after GelRedTM agarose gel electrophoresis. Successful product from PCR was purified using SureClean (Bioline). Cycle sequencing was performed using BigDye Terminator PCR (Applied Biosystems, Foster City, CA, USA) in both directions following the manufacturer's instructions. The resulting single-stranded DNA were purified with CleanSEQ magnetic beads (Agencourt Bioscience Corp), and sequenced on an ABI 3100xl genetic analysis sequencer (Applied Biosystems). The resulting sequences were aligned and edited using the software Sequencher (Gene Codes Corporation, Ann Arbor, WI, USA), and haplotype matching followed [Engelhaupt et al. \(2009\)](#).

Coastal hydrodynamic modelling

Calibrated coastal hydrodynamic models are useful tools for investigating flow circulation in complex coastal environments. The flow circulation of the coastal waters surrounding the Singapore region are relatively complex due to the impact of tidal mixing, seasonal monsoons and larger tropical storm or depression systems ([Sin et al., 2016](#)). To be able to capture this, a relatively large domain model with a fine resolution grid of 2 km in the region of interest was used. This South China Sea Model was built in the Delft3D Modelling Framework and was used as the hydrodynamic model for this study as it provides a good representation of tidal and seasonal forcing in the Singapore Strait and the surrounding region ([Tay et al., 2016](#)). The model is particularly capable of simulating distinct seasonal throughflows in the straits of Singapore and Malacca which was required for the purposes of this study.

To serve as a proxy for a floating dead whale, inert particles called drogues were released during the model simulation. The drogues were released in the model over a seven day period which is assumed to be the maximum flotation time of the dead whale. This was based on the condition of the whale at the time of carcass discovery (Code 2 or early Code 3) ([Geraci & Lounsbury, 1993](#)), which would suggest a floating time of a week or less in tropical conditions. To examine the possible location where the sperm whale became deceased, drogues were released in various locations in the model seven days prior to its discovery off Jurong Island ([Fig. 1](#)). The pathway of drogues that ended up close to Jurong Island on the landfall date were identified as the possible pathways of the floating dead whale.

RESULTS

Diet analysis

Morphological sorting and identification revealed 1,835 upper beaks and 1,657 lower beaks of at least 25 cephalopod species (11 identified to species), forming the bulk of

the stomach contents (Table 1; Table S1). All diet remains were highly digested, with no fresh tissue. Squids (order Teuthida) formed over 97% of the percentage total by number. *Taonius pavo* was the species with the highest percentage total (31.4%), and at an average estimated mass of 1.11 kg per individual, was probably the most important prey item, followed by *Histioteuthis pacifica* (19.1%; estimated weight not available), *Chiroteuthis imperator* (8.34%; 323 g), and *Ancistrocheirus lesueurii* (7.07%; 325 g). Together these species comprised over 65% of the whale's diet remains numerically. The range of average dorsal mantle length (DML) and mass for the species consumed, estimated with beak conversion formulas, was 11.5–63.9 cm, and 60.4–5,360 g.

The stomach contents included *Pyrosomatidae* material (Tunicata: Thaliacea) comprising nine intact, cylindrical specimens of *Pyrosoma atlanticum* Péron, 1804 (Fig. 2) with colony lengths (post-mortem/ethanol preserved) of 29–100 mm (mean = 66.6 mm). Even though the zooids had been digested, and attempts to obtain 18S DNA for GenBank (NCBI, NIH) matching were unsuccessful, there is a high degree of confidence in identification as the opaque colony tunic had resisted digestion, and its characteristics closely matched the description by van Soest (Van Soest, 1981) for *P. atlanticum* (i.e., colony size and shape; zooids densely packed and irregularly arranged; distinct blunt test processes—Fig. 2).

Other than cephalopods and tunicates, an unidentified Thalassinidea (decapod crustacean) cheliped, and unidentified Teleostei (fish) bones were also recorded among the stomach contents.

Non-food items, namely plastic debris were also found in the sperm whale stomach (Fig. 3A). These included plastic drinking cups, food wrappers, and a plastic bag. Two of these items appeared to be of Indonesian origin (Figs. 3B, 3C).

Mitochondrial DNA haplotype identification

Only the skin samples yielded DNA that could be successfully amplified by PCR. The resulting sequences of a pair of replicates were identical, and fully matched Haplotype A (GenBank accession number DQ512921.1) in Engelhaupt et al. (2009).

Coastal hydrodynamic modelling

The results of the drogue tracks from the hydrodynamic model simulations are shown in Fig. 4 and the Supplemental Information 1. Figure 4A shows the tracks or paths of all the drogues released seven days prior to the dead whale being discovered. The results generally agree with the expectation that for the particular time of year the predominant currents are eastward through the Singapore Strait. Figure 4B shows the release points and the tracks of a select set of drogues released 7 days prior to the date of the carcass discovery. Given the discovery of the whale off Jurong Island, the whale was likely to have been free floating in the region bordered by the purple, red and blue drogue west of Singapore.

DISCUSSION

Results from the study support the understanding that the sperm whale is a predator mainly of small to medium-sized squids, with a smaller proportion of other marine invertebrates

Table 1 Number, percentage total by number (PT), average estimated dorsal mantle length (DML) and estimated mass of prey items found in the sperm whale stomach.

Species	Number	PT (%)	Ave. est. DML (cm)	Ave. est. mass (g)	Reference
Mollusca					
Ancistrocheiridae					
<i>Ancistrocheirus lesueurii</i>	118	7.08	17.2	325	Clarke (1986)
Chiroteuthidae					
<i>Asperoteuthis acanthoderma</i>	25	1.50	N.A.	N.A.	
<i>Chiroteuthis imperator</i>	139	8.34	23.8	323	Clarke (1986)
<i>Chiroteuthis</i> sp. A	115	6.90	13.4	60.4	Clarke (1986)
Cranchidae					
<i>Megalocranchia maxima</i>	1	0.06	N.A.	N.A.	
<i>Taonius</i> cf. <i>belone</i>	15	0.900	42.5	142	Rodhouse et al. (1990)
<i>Taonius pavo</i>	523	31.4	54.9	1,110	Rodhouse et al. (1990)
<i>Taonius</i> sp. A	3	0.18	53.4	238	Rodhouse et al. (1990)
Histioteuthidae					
<i>Histioteuthis inermis</i>	115	6.90	12.3	N.A.	Lu & Ickeringill (2002)
<i>Histioteuthis pacifica</i>	318	19.1	11.5	N.A.	Lu & Ickeringill (2002)
<i>Histioteuthis</i> sp. A	45	2.70	17.9	N.A.	Lu & Ickeringill (2002)
Octopeuthidae					
<i>Taningia danae</i>	6	0.36	63.9	5,360	Clarke (1986)
Unidentified Octopeuthidae	115	6.90	N.A.	N.A.	
Onychoteuthidae					
<i>Onykia loenberggii</i>	31	1.86	N.A.	N.A.	
Pholidoteuthidae					
<i>Pholidoteuthis massyae</i>	17	1.02	26.9	479	Clarke (1986)
Unidentified Teuthida					
Unidentified A	3	0.180	N.A.	N.A.	
Unidentified B	2	0.120	N.A.	N.A.	
Unidentified C	6	0.360	N.A.	N.A.	
Unidentified D	3	0.180	N.A.	N.A.	
Unidentified E	2	0.120	N.A.	N.A.	
Unidentified F	2	0.120	N.A.	N.A.	
Unidentified G	1	0.0600	N.A.	N.A.	
Unidentified H	3	0.180	N.A.	N.A.	
Unidentified I	9	0.540	N.A.	N.A.	
Alloposidae					
<i>Haliphron atlanticus</i>	40	2.40	N.A.	425	Xavier & Cherel (2009)
Arthropoda					
Decapoda					
Unidentified Thalassinidea	1	0.0600	N.A.	N.A.	
Chordata					
Pyrosomatidae					
<i>Pyrosoma atlanticum</i>	9	0.540	N.A.	N.A.	

(continued on next page)

Table 1 (continued)

Species	Number	PT (%)	Ave. est. DML (cm)	Ave. est. mass (g)	Reference
Actinopterygii					
Unidentified Teleostei	N.A.	N.A.	N.A.	N.A.	

Notes.

N.A., not available.



Figure 2 *Pyrosoma atlanticum* from stomach of deceased sperm whale. Colony 85 mm × 25 mm in size. The open end of the somewhat flattened, opaque colony is to the left. Some of the protruding zooid test processes (arrows) are clearly visible.

Full-size DOI: 10.7717/peerj.6705/fig-2

and fish. The number of species of prey, and relative importance of cephalopods found in the stomach of the Singapore whale is similar to that of sperm whales in the northeastern and southeastern Atlantic, and southwestern Pacific (Best, 1999; Smith & Whitehead, 2000; Clarke & Young, 1998). However, it differed from male sperm whales off Iceland with a high representation of fish eaten (Martin & Clarke, 1986), and the whales from seas partially enclosed by landforms (e.g., Mediterranean Sea), which typically have less than 10 prey species recovered (Garibaldi & Podesta, 2014; Santos et al., 2002; Roberts, 2003). This could reflect the availability of prey in the waters where the different sexes of sperm whales forage.

The majority of cephalopod prey species eaten by the sperm whale prior to death are distributed mesopelagically (200–1,000 m) in the Indo-West Pacific (e.g., *Asperoteuthis acanthoderma*, *Chiroteuthis imperator*, *Histoteuthis pacifica*), with some having a wider or global distribution (e.g., *Ancistrocheirus lesueurii*, *Haliphron atlanticus*, *Taningia danae*) (Chun, 1908; Voss, Nesis & Rodhouse, 1998; Norman, Nabhitabhata & Lu, 2016). This, together with the relatively high diversity of prey indicate that the whale was foraging outside the relatively shallow waters within the Singapore Strait (mostly <100 m deep) or surrounding enclosed seas (e.g., Java or South China Sea) (Voris, 2000; Tan et al., 2016).

Most of the cephalopod prey species consumed were small to medium-sized (1–6% of whale's length) squids with bioluminescent organs, consistent with findings of other studies



Figure 3 (A) Plastic debris found in the sperm whale stomach. Scale: each square measures 1 × 1 cm. (B) Drinking cup, and (C) food wrapper with origins from Indonesia.

Full-size  DOI: [10.7717/peerj.6705/fig-3](https://doi.org/10.7717/peerj.6705/fig-3)

across oceans (*Kawakami, 1980; Clarke & Young, 1998; Evans & Hindell, 2004; Harvey, Friend & McHuron, 2014; Clarke, Martin & Pascoe, 1993*). In this study, bioluminescent photophores are present in a majority of the prey species, i.e., *Ancistrocheirus* species, *Asperoteuthis acanthoderma*, *Chiroteuthis* species, *Histioteuthis* species, *Megalocranchia maxima*, *Taningia danae*, *Taonius pavo* (*Lu, 1977; Herring, 1987; Herring, Dilly & Cope, 2002; Kubodera, Koyama & Mori, 2007*), but absent in *Onykia loennbergii* (*Kubodera, Piatkowski & Okutani, 1998*). Sperm whales are known to forage at depth in the aphotic mesopelagic zone using echolocation (*Watwood et al., 2006*), but with up to 77.5% of cephalopod prey species reported to possess luminous organs, *Clarke, Martin & Pascoe (1993)* suggested it is probable that sperm whales detect and capture most of their food using a combination of echolocation and vision while approaching and swimming through shoals of bioluminescent slow-swimming squids.

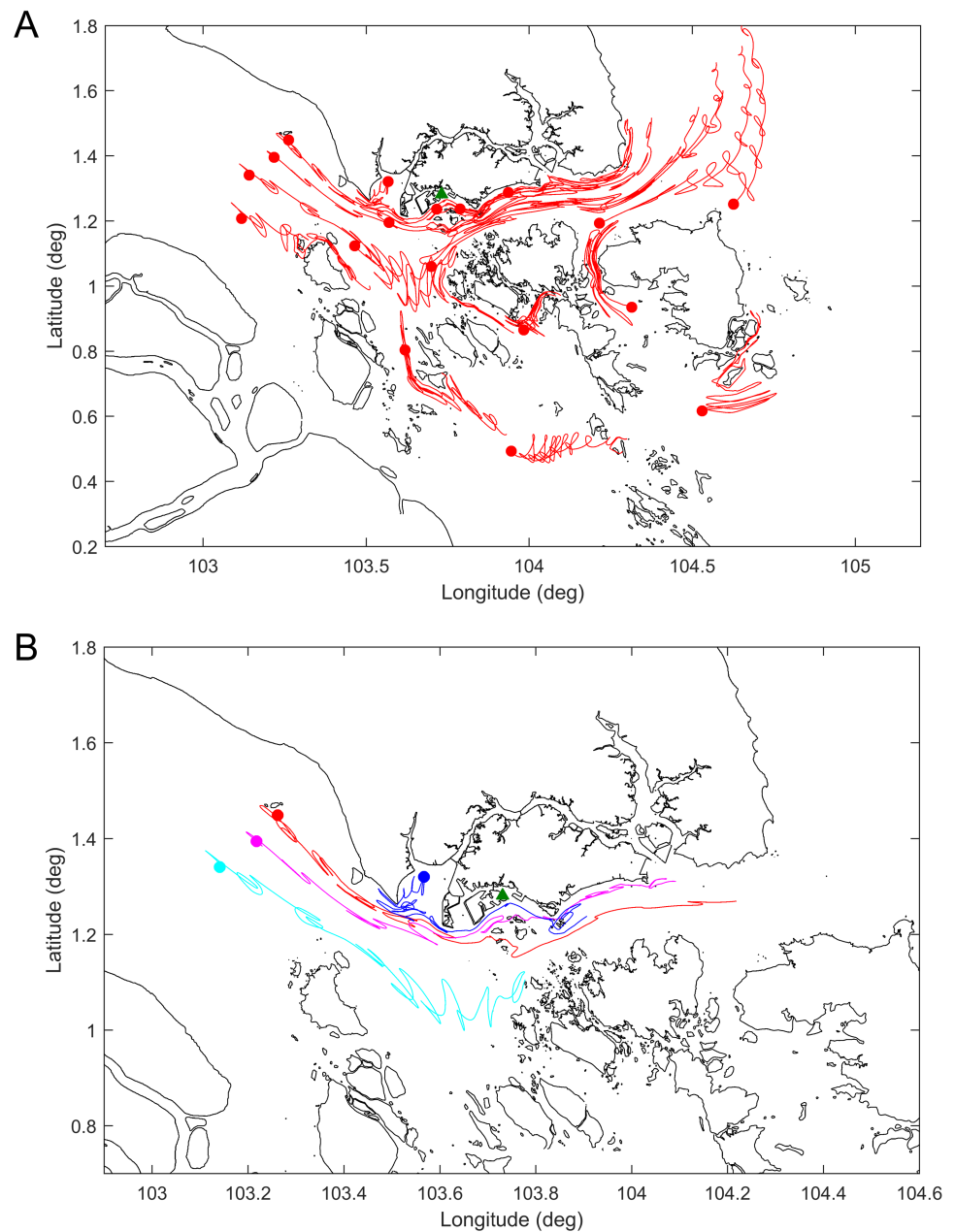


Figure 4 Tracks of the simulated drogues over seven days. (A) All the released drogues; (B) the likeliest drogue tracks to have ended up on the southwestern coast of Singapore on 10 July 2015. The green triangle represents the location where the whale carcass was found. Dots represent the location of the drogues at the start of the simulation.

Full-size  DOI: [10.7717/peerj.6705/fig-4](https://doi.org/10.7717/peerj.6705/fig-4)

The hypothesized foraging strategy of sperm whales for bioluminescent squids may also explain the presence of the planktonic, bioluminescent, colonial tunicate, *Pyrosoma atlanticum*, in the diet of this whale. *Pyrosoma atlanticum* has a distribution (50°N–50°S in all oceans) similar to that for female and juvenile sperm whales, as well as mature males

for at least part of the male's life cycle (Van Soest, 1981; Bowlby, Widder & Case, 1990). *Pyrosoma atlanticum* also occurs over a depth range (0–965 m) comparable to that of squid prey (Anderson & Sardou, 1994). These pyrosomes grow to a size of 60 cm by 6 cm (Van Soest, 1981) which is within the size range of squid eaten by sperm whales in this and other studies (Clarke & Young, 1998; Evans & Hindell, 2004; Harvey, Friend & McHuron, 2014; Clarke, Martin & Pascoe, 1993) and, furthermore, their bioluminescence has been noted to be intense and sustained (Bowlby, Widder & Case, 1990; Wrobel & Mills, 1998). Thus, strongly bioluminescent *P. atlanticum* colonies occupy the same mesopelagic niche (Van Soest, 1981; Anderson & Sardou, 1994) as squid prey and it is possible that these tunicates are tracked visually in the same way.

Elsewhere, pyrosomatid colonies have been recorded as prey items in the stomachs of sperm whales captured during whaling operations off the Azores (Clarke, 1956; Clarke, Martin & Pascoe, 1993) and South Africa (Best, 1999). Off South Africa, 73 of 1,268 whales captured (5.76%) contained *Pyrosoma* colonies (Best, 1999). Usually the numbers of colonies per whale stomach are small, as found in the present necropsy. Interestingly, diet data from whaling studies indicate that it is exclusively (Clarke, Martin & Pascoe, 1993) or predominantly (92%: Best, 1999) males that consume these planktonic tunicate colonies, whereas pyrosomatids in this study were consumed by a mature female whale. Sperm whale captures in the South African fishery were typically biased towards males but annual capture inventories were large (>1,000) and of 291 females only two were reported to have consumed *Pyrosoma* (Best, 1999). Best (1999) considered the consumption of *Pyrosoma* by sperm whales to be opportunistic feeding on a secondary prey item. It is not known whether *P. atlanticum* would be taken in large numbers when these colonial tunicates occur in superabundant swarms (Wrobel & Mills, 1998; Lebrato & Jones, 2009; Milstein, 2017; Welch, 2017) but this is a possibility. The importance of pyrosomes, and other pelagic tunicates (Henschke et al., 2016) in the diet of toothed whales, as well as other marine predators, and in pelagic food webs generally, may be underestimated.

The presence of plastic debris in the stomach of this whale, although not large or copious enough to have resulted in death, adds a further report of such debris in the stomachs of sperm whales across oceans since the 1970s (Clarke & Young, 1998; Evans & Hindell, 2004; Clarke, Martin & Pascoe, 1993; Martin & Clarke, 1986; Roberts, 2003; De Stephanis et al., 2013) and highlights the current prevalence of marine trash in the oceans. The ingestion of plastic debris has been known to result in the death of sperm whales due to gastric blockage or rupture (De Stephanis et al., 2013; Jacobsen, Massey & Gull, 2010). Further, plastic debris can also result in problems such as injury or entanglement of whales and other marine mammals (Pace, Miragliuolo & Mussi, 2008; Gregory, 2009). With the amount of marine litter (including plastic debris) generated by Southeast Asian nations equaling or exceeding global averages (Todd, Ong & Chou, 2010), this may be of conservation concern to threatened marine species, such as the sperm whale, in the region.

The Singapore sperm whale had a control region haplotype that is present in the northern Atlantic, northern and southwestern Pacific, central, western and southern Indian, and Southern Oceans (Haplotype 1: (Lyrholm & Gyllensten, 1998); Haplotype A: (Engelhaupt et al., 2009; Alexander et al., 2016)). It is among the most common haplotypes worldwide

(*Alexander et al., 2016*), and this study extends its known distribution to the Southeast Asian Indo-West Pacific. Although widespread, this haplotype appears in the highest frequency in the northern Pacific, specifically off Japanese coastal areas (*Lyrholm & Gyllensten, 1998*), and off Cocos (Keeling) Islands in the southern Indian Ocean (*Alexander et al., 2016*). In contrast, it was found to be absent off Sri Lanka (*Alexander et al., 2016*).

The coastal hydrodynamic model results suggest that the Singapore sperm whale was likely to have been to the west of Singapore prior to her being found dead off Jurong Island. Furthermore a selected group of drogue tracks as used in the model point to a likely location for the start of free drifting of the whale carcass close to the shipping routes rather than further out in the Malacca Straits.

In summary, current circumstantial evidence from the diet, origin of ingested plastic debris, mitochondrial DNA haplotype, and hydrodynamic modeling suggests that the sperm whale could have originated from a population in the Indian Ocean, close to Cocos (Keeling) Islands or Indonesia. However, until more detailed genetic sampling and kinship analyses of sperm whales off Southeast Asian waters can be done, it would not be possible to confidently determine the origin of the Singapore specimen. Although no parasites or barnacles were found on this whale, future studies of parasite presence or identity, and ageing of whales based on teeth may also help narrow down the origins of stranded animals.

This study of the diet and haplotype of a sperm whale found dead in Singapore waters represents the first opportunity to understand these aspects of sperm whale biology in the Southeast Asian Indo-West Pacific region. Although most of the dietary components were identified and described, it was not possible to determine the precise biomass contribution of each prey species. This is because (a) soft tissues of squid prey items were completely digested, (b) and mass conversion formulas are not available for all recorded species, and (c) several morphological types remain unidentified as identification guides for squid beaks from the region are not available. Also, owing to the whale's back and spinal injury, with a few of the caudal vertebrae smashed, possibly caused by a ship strike, it is assumed that the whale did not forage normally or feed for some days before her death. Hence, the true relative importance of each species in the diet of this individual may not be accurately reflected. However, the data for the majority of the species eaten, their numerical importance, and the whale's mitochondrial DNA haplotype nonetheless provides the first steps to understanding the diet of this sperm whale. Further, the combined analyses of stomach contents, DNA, and hydrodynamic modeling could provide a context to future studies on the sperm whale, and have broader applicability on other marine mammals in the region.

CONCLUSIONS

In this study we provided an increase in the understanding the diet and natural history of the sperm whale in Southeast Asia. A dead adult female sperm whale found in Singapore fed mainly on small to medium-sized mesopelagic Indo-West Pacific squids, with a smaller proportion of other marine invertebrates and fish. The sperm whale had the most widespread and common mitochondrial DNA control region haplotype that is present in

the northern Atlantic, northern and southwestern Pacific, central, western and southern Indian, and Southern Oceans. Current circumstantial evidence from the diet, origin of ingested plastic debris, mitochondrial DNA haplotype, and hydrodynamic modeling suggests that the sperm whale could have originated from a population in the Indian Ocean. The combined analyses of stomach contents, DNA, and hydrodynamic modeling could provide a context to future studies on the sperm whale, and have broader applicability on other marine mammals in the region.

ACKNOWLEDGEMENTS

The Singapore sperm whale salvage operation that enabled this study to be conducted was made possible by the support rendered by the dedicated staff, volunteers and contractors of the Lee Kong Chian Natural History Museum, the Maritime and Port Authority of Singapore, the National Environment Agency, and public assistance. Chen Mingshi, Foo Maosheng, Iffah Iesa, and Kate Pocklington were instrumental in the dissection of the whale. Marina Chaw, Chen Mingshi, Iffah Iesa, and Tan Siong Kiat assisted with the initial sorting of the stomach contents. Peter K.L. Ng, J.C. Mendoza, and Tohru Naruse helped with the crustacean identification. Rudolf Meier and Amrita Srivathsan provided advice on the genetic analysis. Sarah Mesnick and Thomas Lyrhohn kindly provided information and references on sperm whale genetics. We thank the peer reviewers and editors for their helpful comments.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This study was funded by the Jubilee Whale Fund. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Jubilee Whale Fund.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Marcus A.H. Chua and Seng Keat Ooi conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- David J.W. Lane performed the experiments, analyzed the data, authored or reviewed drafts of the paper, prepared figures and/or tables, approved the final draft.
- Serene H.X. Tay and Tsunemi Kubodera conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Permission to work on the beach was granted verbally by Boon Kiang Chew from the National Environment Agency Singapore.

Data Availability

The following information was supplied regarding data availability:

The raw data from the sperm whale stomach contents are available in [Table S1](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.6705#supplemental-information>.

REFERENCES

- Alexander A, Steel D, Hoekzema K, Mesnick SL, Engelhaupt D, Kerr I, Payne R, Baker CS. 2016. What influences the worldwide genetic structure of sperm whales (*Physeter macrocephalus*)? *Molecular Ecology* 25:2754–2772 DOI 10.1111/mec.13638.
- Anderson M, Kinze CC. 1999. Annotated checklist and identification key to the whales, dolphins, and porpoises (Order Cetacea) of Thailand and adjacent waters. *Natural History Bulletin of the Siam Society* 47:27–62.
- Anderson V, Sardou J. 1994. *Pyrosoma atlanticum* (Tunicata, Thaliacea): diel migration and vertical distribution as a function of colony size. *Journal of Plankton Research* 16(4):337–349 DOI 10.1093/plankt/16.4.337.
- Anon. 2017. Ikan raksasa terdampar di pesisir pantai Minsel. Redaksi Manado. Available at <http://www.redaksimanado.com/2017/09/ikan-raksasa-terdampar-di-pesisir.html?m=1>.
- Anon. 2018. Sperm whale found stranded in East Aceh. The Jakarta Post 19 2018. Available at <http://www.thejakartapost.com/news/2018/06/18/sperm-whale-found-stranded-in-east-aceh.html>.
- Barnes RH. 2005. Indigenous use and management of whales and other marine resources in East Flores and Lembata, Indonesia. *Senri Ethnological Studies* 67:77–85.
- Best PB. 1999. Food and feeding of sperm whales *Physeter macrocephalus* off the west coast of South Africa. *South African Journal of Marine Science* 21(1):393–413 DOI 10.2989/025776199784126033.
- Bowlby MR, Widder EA, Case JF. 1990. Patterns of stimulated bioluminescence in two pyrosomes (Tunicata: Pyrosomatidae). *Biological Bulletin* 179(3):340–350 DOI 10.2307/1542326.
- Cahya GH, Simanjuntak H. 2017. Four of 10 beached whales in Aceh die. The Jakarta Post 14 2017. Available at <http://www.thejakartapost.com/news/2017/11/14/four-of-10-beached-whales-in-aceh-die.html>.
- Chun C. 1908. Ueber cephalopoden der deutschen tiefsee-expedition. *Zoologischer Anzeiger* 33(2):86–89.

- Clarke MR. 1986.** *A handbook for the identification of cephalopod beaks.* Oxford: Clarendon Press, 273.
- Clarke MR, Martin HR, Pascoe P. 1993.** The diet of sperm whales (*Physeter macrocephalus*) off the Azores. *Philosophical Transactions of the Royal Society B: Biological Sciences* **339**(1287):67–82 DOI [10.1098/rstb.1993.0005](https://doi.org/10.1098/rstb.1993.0005).
- Clarke M, Young R. 1998.** Description and analysis of cephalopod beaks from stomachs of six species of odontocete cetaceans stranded on Hawaiian shore. *Journal of the Marine Biological Association of the United Kingdom* **78**:623–641 DOI [10.1017/S0025315400041667](https://doi.org/10.1017/S0025315400041667).
- Clarke R. 1956.** Sperm whales of the Azores. *Discovery Reports* **28**:237–298.
- De Boer MN. 2000.** A note on cetacean observations in the Indian Ocean Sanctuary and the South China Sea, Mauritius to the Philippines, 1999. *Journal of Cetacean Research and Management* **2**(3):197–200.
- De Stephanis R, Giménez J, Carpinelli E, Gutierrez-Exposito C, Cañadas A. 2013.** As a main meal for sperm whales: plastic debris. *Marine Pollution Bulletin* **69**:206–214 DOI [10.1016/j.marpolbul.2013.01.033](https://doi.org/10.1016/j.marpolbul.2013.01.033).
- Engelhaupt D, Hoelzel AR, Nicholson C, Frantzis A, Mesnick S, Gero S, Whitehead H, Rendell L, Miller P, De Stefanis R, Cañadas A, Airoidi S, Mignucci-Giannoni AA. 2009.** Female philopatry in coastal basins and male dispersion across the North Atlantic in a high mobile marine species, the sperm whale (*Physeter microcephalus*). *Molecular Ecology* **18**:4193–4205 DOI [10.1111/j.1365-294X.2009.04355.x](https://doi.org/10.1111/j.1365-294X.2009.04355.x).
- Evans K, Hindell MA. 2004.** The diet of sperm whale (*Physeter macrocephalus*) in southern Australian waters. *ICES Journal of Marine Science* **61**:1313–1329 DOI [10.1016/j.icesjms.2004.07.02](https://doi.org/10.1016/j.icesjms.2004.07.02).
- Garibaldi F, Podesta M. 2014.** Stomach contents of a sperm whale (*Physeter macrocephalus*) stranded in Italy (Ligurian Sea, north-western Mediterranean). *Journal of the Marine Biological Association of the United Kingdom* **94**(6):1087–1091 DOI [10.1017/S0025315413000428](https://doi.org/10.1017/S0025315413000428).
- Geraci J, Lounsbury VJ. 1993.** *Marine mammals ashore: a field guide for strandings.* Galveston, Texas: Texas A & M University Sea Grant College Programme, 309.
- Gregory M. 2009.** Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**:2013–2025 DOI [10.1098/rstb.2008.0265](https://doi.org/10.1098/rstb.2008.0265).
- Harvey JT, Friend T, McHuron EA. 2014.** Cephalopod remains from stomachs of sperm whales (*Physeter macrocephalus*) that mass-stranded along the Oregon coast. *Marine Mammal Science* **30**(2):609–625 DOI [10.1111/mms.12063](https://doi.org/10.1111/mms.12063).
- Henschke N, Everett JD, Richardson AJ, Suthers IM. 2016.** Rethinking the role of salps in the ocean. *Trends in Ecology & Evolution* **13**(9):720–733.
- Herring PJ. 1987.** Systematic distribution of bioluminescence in living organisms. *Journal of Bioluminescence and Chemiluminescence* **1**:147–163 DOI [10.1002/bio.1170010303](https://doi.org/10.1002/bio.1170010303).
- Herring PJ, Dilly PN, Cope C. 2002.** The photophores of the squid family Cranchiidae (Cephalopoda: Oegopsida). *Journal of Zoology* **258**:73–90.

- Jacobsen JK, Massey L, Gull F. 2010.** Fatal ingestion of floating net debris by two sperm whale (*Physeter macrocephalus*). *Marine Pollution Bulletin* **60**:765–767 DOI [10.1016/j.marpolbul.2010.03.008](https://doi.org/10.1016/j.marpolbul.2010.03.008).
- Kawakami T. 1980.** A review of sperm whale food. *Scientific Reports of the Whales Research Institute* **32**:199–218.
- Kubodera T. 2005.** Manual for the identification of cephalopod beaks in the northwest Pacific. Available at <http://www.kahaku.go.jp/research/db/zoology/Beak-E/index.htm>.
- Kubodera T, Koyama Y, Mori K. 2007.** Observations of wild hunting behavior and bioluminescence of a large deep-sea, eight-armed squid, *Taningia danae*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **274**:1029–1034 DOI [10.1098/rspb.2006.0236](https://doi.org/10.1098/rspb.2006.0236).
- Kubodera T, Piatkowski U, Okutani T. 1998.** Taxonomy and zoography of the family Onychoteuthidae (Cephalopoda: Oegopsida). In: Voss NA, Vecchione M, Toll RB, Sweeney MJ, editors. Systematics and biogeography of cephalopods. *Smithsonian Contributions to Zoology* **586**(2):277–291.
- Lebrato M, Jones DOB. 2009.** Mass deposition event of *Pyrosoma atlanticum* carcasses off Ivory coast (West Africa). *Limnology and Oceanography* **54**(4):1197–1209 DOI [10.4319/lo.2009.54.4.1197](https://doi.org/10.4319/lo.2009.54.4.1197).
- Lu CC. 1977.** A new species of squid, *Chiroteuthis acanthoderma*, from the Southwest Pacific (Cephalopoda: Chiroteuthidae). *Steenstrupia* **4**:179–188.
- Lu CC, Ickeringill R. 2002.** Cephalopod beak identification and biomass estimation techniques: tools for dietary studies of southern Australian finfishes. *Museum Victoria Science Reports* **6**:1–65 DOI [10.24199/j.mvsr.2002.06](https://doi.org/10.24199/j.mvsr.2002.06).
- Lyrholm T, Gyllensten G. 1998.** Global matrilineal population structure in sperm whales as indicated by mitochondrial DNA sequences. *Philosophical Transactions of the Royal Society B: Biological Sciences* **265**:1679–1684 DOI [10.1098/rspb.1998.0488](https://doi.org/10.1098/rspb.1998.0488).
- Lyrholm T, Leimar O, Johannesson B, Gyllensten U. 1999.** Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Philosophical Transactions of the Royal Society B: Biological Sciences* **266**:347–354 DOI [10.1098/rspb.1999.0644](https://doi.org/10.1098/rspb.1999.0644).
- Martin AR, Clarke MR. 1986.** The diet of sperm whales (*Physeter macrocephalus*) captured between Iceland and Greenland. *Journal of the Marine Biological Association of the United Kingdom* **66**:779–790 DOI [10.1017/S0025315400048426](https://doi.org/10.1017/S0025315400048426).
- Mesnick SL, Taylor BL, Archer FI, Martien KK, Treviño SE, Hancock-Hanser BL, Medina SCMoreno, Pease VL, Robertson KM, Straley JM, Baird RW, Calambokidis J, Schorr GS, Wade P, Burkanov V, Lunsford CR, Rendell L, Morin PA. 2011.** Sperm whale population structure in the eastern and central North Pacific inferred by the use of single-nucleotide polymorphisms, microsatellites and mitochondrial DNA. *Molecular Ecology Resources* **11**(Suppl 1):278–298 DOI [10.1111/j.1755-0998.2010.02973.x](https://doi.org/10.1111/j.1755-0998.2010.02973.x).
- Milstein M. 2017.** *Researchers probe explosion of pyrosomes off the Northwest Coast*. Seattle: Northwest Fisheries Science Center.

- Mueanhawong K. 2012.** Dead sperm whale washes ashore in Phang Nga. *Phuket Gazette* 22 2012. Available at <https://thethaiger.com/news/phuket/Dead-sperm-whale-washes-ashore-Phang-Nga>.
- Mustika PLK, Hutasoit P, Madusari CC, Purnomo FS, Setiawan A, Tjandra K, Prabowo WE. 2009.** Whale strandings in Indonesia, including the first record of a humpback whale (*Megaptera novaeangliae*) in the Archipelago. *Raffles Bulletin of Zoology* 57(1):199–206.
- Norman MD, Nabhitabhata J, Lu CC. 2016.** An updated checklist of the cephalopods of the South China Sea. *Raffles Bulletin of Zoology* **Suppl 34**:566–592.
- Okutani T, Satake Y. 1978.** Squids in the diet of 38 sperm whales caught in the Pacific waters off northeastern Honshu, Japan, 1977. *Bulletin of Tokai Regional Fisheries Research Laboratory* 93:13–27.
- Okutani T, Satake Y, Ohsumi S, Kawakami T. 1976.** Squids eaten by sperm whales caught off Joban District, Japan, during January–February, 1976. *Bulletin of Tokai Regional Fisheries Research Laboratory* 87:67–113.
- Pace DS, Miragliuolo A, Mussi B. 2008.** Behaviour of a social unit of sperm whales (*Physeter macrocephalus*) entangled in a driftnet off Capo Palinuro (Southern Tyrrhenian Sea, Italy). *Journal of Cetacean Research and Management* 10:131–135.
- Ponnampalam LS. 2012.** Opportunistic observations on the distribution of cetaceans in the Malaysian South China, Sulu and Sulawesi Seas and an Updated Checklist of Marine Mammals in Malaysia. *Raffles Bull. Zool* 60(1):221–231.
- Rice DW. 1989.** Sperm whale *Physeter macrocephalus* Linnaeus, 1758. In: Ridgway SH, Harrison R, eds. *Handbook of marine mammals, 4: River dolphins and the larger toothed whales*. Monterey, California: Academic Press, 177–234.
- Richard KR, Dillon MC, Whitehead H, Wright JM. 1996.** Patterns of kinship in groups of free-living sperm whales (*Physeter macrocephalus*) revealed by multiple molecular genetic analyses. *Proceedings of the National Academy of Sciences of the United States of America* 93:8792–8795 DOI 10.1073/pnas.93.16.8792.
- Roberts SM. 2003.** Examination of the stomach contents from a Mediterranean sperm whale found south of Crete, Greece. *Journal of the Marine Biological Association of the United Kingdom* 83:667–690 DOI 10.1017/S0025315403007628h.
- Rodhouse PG, Prince PA, Clarke MR, Murray AWA. 1990.** Cephalopod prey of the grey-headed albatross *Diomedea chrysostoma*. *Marine Biology* 104:353–362 DOI 10.1007/BF01314337.
- Santos MB, Pierce GJ, García Hartmann M, Smeenk C, Addink MJ, Kuiken T, Reid RJ, Patterson IAP, Lordan C, Rogan E, Mente E. 2002.** Additional notes on stomach contents of sperm whales *Physeter macrocephalus* stranded in the north-east Atlantic. *Journal of the Marine Biological Association of the United Kingdom* 82:501–507 DOI 10.1017/S0025315402005787.
- Sin TM, Ang HP, Buurman J, Lee AC, Leong L, Ooi SK, Steinberg P, Teo SL-M. 2016.** The urban marine environment of Singapore. *Regional Studies in Marine Science* 8(2):331–339 DOI 10.1016/j.rsma.2016.01.011.

- Smith SC, Whitehead H. 2000.** The diet of Galapagos sperm whales *Physeter macrocephalus* as indicated by fecal sample analysis. *Marine Mammal Science* **16**(2):315–325 DOI [10.1111/j.1748-7692.2000.tb00927.x](https://doi.org/10.1111/j.1748-7692.2000.tb00927.x).
- Southern SO, Southern PJ, Dizon A. 1988.** Molecular characterization of a cloned dolphin mitochondrial genome. *Journal of Molecular Evolution* **28**:32–42 DOI [10.1007/BF02143495](https://doi.org/10.1007/BF02143495).
- Tan KS, Koh KS, Ng JY, Goh L. 2016.** The Comprehensive Marine Biodiversity Survey Singapore Strait International Workshop 2013. *Raffles Bulletin of Zoology Suppl* **34**:1–7.
- Tay SHX, Kurniawan A, Ooi SK, Babovic V. 2016.** Sea level anomalies in straits of Malacca and Singapore. *Applied Ocean Research* **58**:104–117 DOI [10.1016/j.apor.2016.04.003](https://doi.org/10.1016/j.apor.2016.04.003).
- Todd PA, Ong X, Chou LM. 2010.** Impacts of pollution on marine life in Southeast Asia. *Biodiversity and Conservation* **19**(4):1063–1082 DOI [10.1007/s10531-010-9778-0](https://doi.org/10.1007/s10531-010-9778-0).
- Van Soest R. 1981.** A monograph of the order Pyrosomatida (Tunicata, Thaliacea). *Journal of Plankton Research* **3**(4):603–631 DOI [10.1093/plankt/3.4.603](https://doi.org/10.1093/plankt/3.4.603).
- Voris HK. 2000.** Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and tiem durations. *Journal of Biogeography* **27**(5):1153–1167 DOI [10.1046/j.1365-2699.2000.00489.x](https://doi.org/10.1046/j.1365-2699.2000.00489.x).
- Voss NA, Nesis KN, Rodhouse PG. 1998.** The cephalopod family Histiotteuthidae (Oegopsida): Systematics, biology and biogeography. In: Voss NA, Vecchione M, Toll RB, Sweeney MJ, editors. Systematics and biogeography of cephalopods. *Smithsonian Contributions to Zoology* **586**(2):293–372.
- Watwood SL, Miller PJO, Johnson M, Madsen PT, Tyack PL. 2006.** Deep-diving foraging behaviour of sperm whales (*Physeter macrocephalus*). *Journal of Animal Ecology* **75**:814–825 DOI [10.1111/j.1365-2656.2006.01101.x](https://doi.org/10.1111/j.1365-2656.2006.01101.x).
- Welch C. 2017.** Bizarre, glowing sea creatures bloom in the Pacific. Available at <http://news.nationalgeographic.com/2017/06/pyrosome-fire-body-bloom-eastern-pacific-warm-water/>.
- Wrobel D, Mills C. 1998.** *Pacific coast pelagic invertebrates: a guide to the common gelatinous animals*. Monterey: Sea Challengers, 108 pp.
- Xavier JC, Cherel Y. 2009.** *Cephalopod Beak Guide for the Southern Ocean*. Cambridge: British Antarctic Survey, 129 pp.