Check for updates

Asian Journal of Water, Environment and Pollution, Vol. 18, No. 1 (2021), pp. 85-90.
DOI 10.3233/AJW210011

Possible Transoceanic Rafting of *Lepas* Spp. on an Unopened Plastic Bottle of Chinese Origin Washed Ashore in Victoria, Australia

Alexander Cooke and Huseyin Sumer*
Department of Chemistry and Biotechnology, Faculty of Science, Engineering and Technology
Swinburne University of Technology, John Street, Hawthorn VIC 3122, Australia
✉ hsumer@swin.edu.au

Received May 15, 2020; revised and accepted November 13, 2020

Abstract: Floating marine debris and litter act as a vector transporting various species across long distances. The present study reports possible transoceanic rafting of a small colony of barnacles on an unopened plastic bottle of Chinese origin found washed ashore on the Ninety Mile Beach in Victoria, Australia. The crustaceans attached were identified to be the goose barnacle *Lepas pectinata*. Based on the number and size of the colony the marine pollutant was estimated to adrift for several months. We hypothesised the origin of the flotsam, especially the barnacles and how it made its way from the Pacific to be washed ashore in Australia. Furthermore, we identified two types of microbes, *Vibrio alginolyticus* and *Vibrio parahaemolyticus*, associated with the *Lepas pectinata* growing on the bottle. This study appears to be the first report of possible transoceanic rafting on unused plastic pollutants and highlights the potential environmental threats caused by plastic.

Key words: Rafting, marine litter, *Lepas*, *Vibrio parahaemolyticus,* plastic bottle, species dispersal.

Introduction

Movement of organisms on natural marine debris have occurred for millions of years, however, in the past few decades, plastics and plastic debris have transformed marine rafting (Barnes and Miller, 2005). According to reports, floating debris and marine litter act as a vector for the transport of species across long distances (Rech et al., 2016). For example, >600 debris items landed on the shores of North America and Hawaii, due to the 2011 East Japan earthquake and tsunami, over the next 5 years (Carlton et al., 2017). The marine debris originating in Japan consisted of vessels, docks, buoys, totes (crates), wood, and many other objects carried various species rafting on them including invertebrates, protists and fish (Carlton et al., 2017). This transoceanic rafting on marine debris and pollution may intensify the spread of invasive species across the continents (Carlton et al., 2017; Miller et al., 2018).

This report studies a single piece of plastic pollution found in coastal Victoria, Australia and organisms it accompanies. A plastic bottle was found washed ashore on the Ninety Mile Beach, Victoria Australia on mid-January 2019. After a closer inspection, the bottle was unopened, had no English writing, however, it appeared to have only Chinese characters on it. Furthermore, a small colony of barnacles was growing on it (Figure 1). A survey of other washed-up debris such as drift wood, seaweed and cuttlefish bones along the same coast had no barnacles attached to them. This incidence poses some questions; what was the origin of the plastic pollution and how did it end up on Ninety Mile Beach?

*Corresponding Author

Figure 1: Marine pollutant found in coastal Victoria, Australia.
A. Unopened soft drink bottle containing no English writing found washed ashore on the 90 Mile Beach.
B. The small colony of barnacles attached to the unopened soft drink bottle.

The present study, therefore, determines the nature of the organisms on the marine pollutant and speculates the origins of the flotsam.

Materials and Methods

Barnacle Identification

The barnacles were carefully removed from the plastic bottle, counted and photographed. Genomic DNA was isolated, PCR was performed using 16S and COI primers in order to perform sequence analysis for the determination of species, however, the results were inconclusive. High-resolution images were sent to the Department of Aquatic Zoology at the Western Australian Museum for visual identification. The species was confirmed to be *Lepas pectinata,* the scutum length and capitulum length were measured from the shells as previously described (Gibson et al., 2005; Tsikhon-Lukanina et al., 2001) to determine the age of the barnacles so that the time the bottle was adrift could be estimated.

Bacterial Growth and Characterisation

Bacterial cultures were established on Marine Agar containing 34.6g Marine Broth 2216 and 15g of Granulated Agar (Difco) per litre of deionised water. Swabs from the various samples were spread onto Marine Agar and pure isolates were obtained. Routine Gram-staining on glass slides was performed to verify the establishment of pure cultures. Pure cultures were then subjected to the Microbact™ 12A/12B Gram-Negative Identification System (Oxoid) according to the manufacturer's instructions for identification purposes. Following a series of biochemical tests, the results were logged into the Microbact™ database for species identification.

Profilometry and Wettability Analysis

Approximately 5×10mm pieces of the PET plastic bottle were cut for surface characteristic anaylsis. The 3D profiles were analysed using optical profilometry (Veeco, Bruker). Data analysis was performed on default settings on images taken under 50× magnification and reported using the average roughness (Ra) values. Wettability was determined using evaluation of the contact angles of 5 µL Milli-Q water droplets using the sessile drop method. The measurements were performed in air using an FTA1000 instrument (First Ten Ångstroms Inc.) and images were taken with a Pelco model PCHM 575-4 camera using FTA Windows Mode 4 software.

Results and Discussion

A small colony of barnacles attached to an unopened plastic bottle was found on the Ninety Mile Beach in Victoria at the approximate co-ordinates of 38°13′23.2″S 147°23′16.4″E. The writings inside the plastic bottle were translated by a bilingual Chinese colleague, which was identified to be 'Shanghai Soda Water' manufactured at Hengyi Road, West district, Industrial zone, Li Shui County in the eastern coastal province of Zhjiang, China. A survey conducted in the rural towns along the Ninety Mile Beach as well as approximately 20 Asian Grocery stores in the south eastern suburbs of Melbourne, Victoria revealed that no one stocked this product. We hypothesised that the unopened plastic bottle entered Australian waters either via transoceanic currents or by ship. On looking at global ocean currents, it was speculated that possibly the unopened plastic bottle entered the marine environment somewhere in China and made its way to the southeastern coast of Australia (Figure 2). Another possibility is that it entered

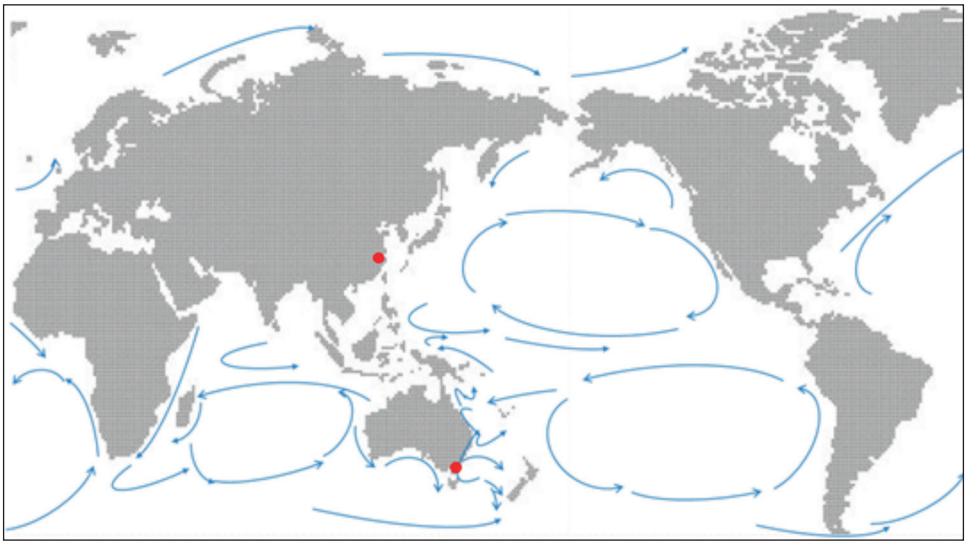

Figure 2: Global ocean currents and major currents along coastal Australia. Location of the Ninety Mile Beach in Victoria, Australia where the marine pollutant was found washed ashore as well as the possible origin are shown (red dots).

Australian waters by falling off a large tanker, shipping container or cruise ship where Australian ocean currents eventually washed it ashore. Another likely possibility is that a large vessel picked up the unopened plastic bottle from an Asian port as part of water ballast and discharged it either before arrival in Australian seas or between Australian ports. Ballast water is taken on board at a port by ships for stability, trim and manoeuvrability, and eventually discharged at another port; it is, therefore, a mechanism for transporting material from one region to another (Goldsmit et al., 2019). Ballast water may contain a range of species including bacteria, plankton, and fish introduced into the receiving environment. Billions of tons of ballast water are transported around the world each year leading to the distribution of many non-indigenous species, some of which may become invasive (David et al., 2015).

Further investigations on the organisms growing on the bottle was done to determine their probable origin. Initial investigation of the barnacles narrowed them down to goose barnacles within the order Pedunculata. Upon closer inspection, it appeared to be within the Family Lepadidae and a *Lepas spp.* High-resolution images were sent to the Department of Aquatic Zoology at the Western Australian Museum for visual identification whereby it was identified to be the barnacle *Lepas pectinata* based on its morphology including the shape of the side plates as well as the radial ridges. *Lepas pectinata* (Spengler, 1793) is the smallest of the gooseneck barnacles that is almost a triangular scutum with radial furrows, an almost triangular tergum with radial furrows. It has a notch to receive the scutum and has no space between the scutum and tergum (Darwin, 1851; Spengler, 1793).

Lepas pectinata does not float but is often found attached to floating debris (Minchin, 1996). *Lepas pectinata* species is reported to be attached on various floating debris such as fishing buoys and plastics in South Africa (Ryan and Branch, 2012; Whitehead et al., 2011), pieces of plastic, wood, bottles, floating cans and drug vials washed ashore in Ireland (Minchin, 1996), and even lumps of crude oil in the Mediterranean Sea and eastern North Atlantic Ocean (Horn et al., 1970). It is attached to floating substrata in the Northwest Pacific Ocean (Tsikhon-Lukanina et al., 2001) as well as the South Pacific off the Chilean Pacific coast (Hinojosa et al., 2006). *Lepas pectinata* has been found at oceanic depths of approx. 600-1000m in both the Mediterranean Sea and the North Atlantic Ocean (Conway et al., 1990; Di Geronimo, 2010). *Lepase pectincata* can truly be classified as a cosmopolitan species with a broad geographic range spanning approximately 50–55°N to 40–45°S, spanning both temperate and tropical regions of the oceans of the world (Schiffer and Herbig, 2016). Furthermore, genetic data show that northern and southern hemisphere populations of *L. pectinata* are not genetically distinct (Schiffer and Herbig, 2016), which makes the identification of the origin of the barnacles on the unopened water bottle difficult. Eighteen barnacles were found to be firmly attached to the neck of the plastic bottle of various sizes of which the largest had a scutum length of 10.5 mm and total a capitulum length

of 14mm. *Lepas pecinata* has a reported overall growth rate ranging from 1 mm (Horn et al., 1970) and up to 2.6 mm per week (Gibson and Atkinson, 2005; Tsikhon-Lukanina et al., 2001). The numbers and sizes of the barnacles suggest that the plastic bottle was adrift for a period of atleast 1-3.5 months. Taking into account the number and predicted age of the barnacles, global and Pacific Ocean currents (Figure 2), this data strengthens the argument of possible transoceanic movement that led the plastic bottle enter the marine environment somewhere in China or the Pacific Ocean from where Australian ocean currents eventually washed it ashore in Victoria, Australia.

To determine its role in the barnacles' attachment, the surface structure of the PET bottle was investigated. A new soda bottle was purchased in China and the surface of the PET plastic was analysed for wettability using Optical tensiometry and surface roughness using a 3D profiler. The static contact angle measurement was made by placing a droplet of water and imaging the static angle of the droplet. After the analysis of the two samples, a control surface on the body of the bottle, as well as the neck of the bottle to which the barnacles was found to be attached. The images show that the PET on body of the bottle surface has a high contact angle of 73° while the PET on the bottleneck had poorer spreading with a lower contact angle of 36° (Figure 3). This suggests that the PET around the neck of the bottle is more hydrophilic. Furthermore, the Bruker Contour GT 3D Profiler was used to explore the surface roughness of the bottle by 50× magnification imaging of the samples and comparing the average roughness (Ra) values. The body of the bottle was found to be smoother (Ra=53.23 nm) than the bottle neck (Ra=176.91 nm) which also had several parallel ridges (Figure 3b). This data suggests that the manufacturing process of injection moulding causes changes in the plastic neck of the bottle to become more hydrophobic and rough. These properties, along with the buoyancy of the unopened plastic bottle, may have provided a surface around the neck of the bottle for the barnacles to attach and grow.

Finally, microorganisms found on the barnacles were investigated (on the marine pollutant) to determine whether it could be a potential biosecurity risk. Samples or swabs were taken from the sand on the Ninety Mile Beach, the ocean water and from the *Lepas pecinata* and were streaked onto marine agar plates and grown for 2 days at room temperature. Bacterial growth was observed from all samples, pure cultures were

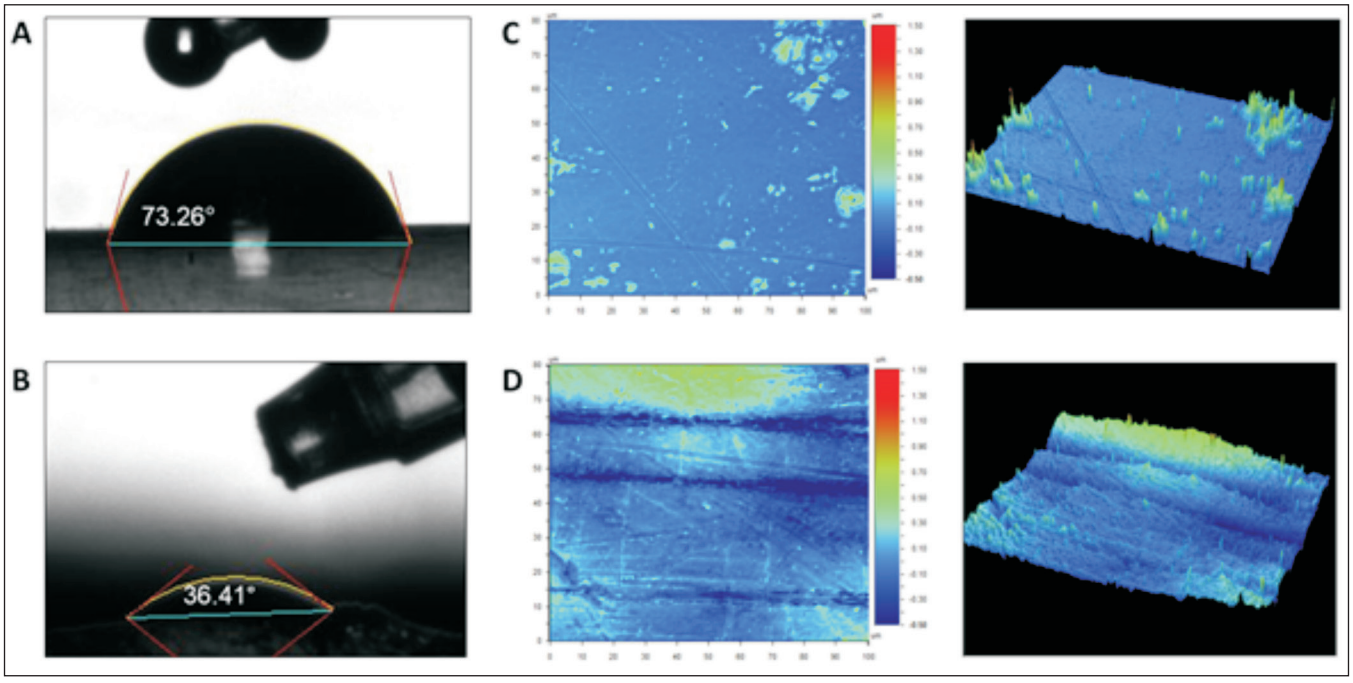

Figure 3: Surface Structure of Plastic Bottle.
Optical tensiometry static contact angle measurement of (A) control surface of the plastic bottle and (B) Neck of the plastic bottle. Surface roughness using a 3D profiler of (C) control surface of the plastic bottle and (D) Neck of the plastic bottle.

established and gram staining performed. Various microbial pure cultures that were established from the sand, water and crustacean were all found to be gram-negative. Microbial species identification was determined by a series of biochemical tests performed on the Oxoid Microbact™ Gram-negative system. The microbial species from the sand and water samples were identified as both *Vibrio alginolyticus* and *Shewenella putrefaciens.* These species are gram negative bacteria found in marine environments as well as marine animals which can infect both fish and animals (Fu et al., 2016; Vignier et al., 2013). The two types of microbes associated with the crustacean growing on the bottle were identified as *Vibrio alginolyticus* (also found in the marine water and sand samples) and *Vibrio parahaemolyticus*. The *Vibrio parahaemolyticus* species occur in marine environments and are found on a variety of shellfish which when consumed may lead to acute gastroenteritis (Su and Liu, 2007).

Our study appears is first to report the isolation of *Vibrio parahaemolyticus* on *Lepas* spp., therefore, more research is required to determine whether it is a common microbe on *Lepas pectinata* and/or whether it is a biosecurity risk.

Table 1: Microbial species identification using the Oxoid Microbact™ Gram-negative system

Sample	*Microbact™ number*	*Species ID*	*% Probability*
Barnacle 1	740370000	*V. alginolyticus*	94.19
Barnacle 2	570610000	*V. parahaemolyticus*	78.10
Barnacle 3	660750000	*V. alginolyticus*	99.94
Water 1	770530000	*S. putrefacieus*	99.99
Water 2	570120000	*S. putrefacieus*	77.88
Water 3	770250000	*V. alginolyticus*	99.64
Sand 1	770374000	*S. putrefacieus*	99.66
Sand 2	770340000	*V. alginolticus*	53.45
Sand 3	760374000	*V. alginolyticus*	99.68

In summary, we identified the small colony of barnacles attached to an unopened plastic bottle of Chinese origin found on the Ninety Mile Beach in Victoria to be *Lepas pectinata*. We determined that the flotsam was adrift for several months due to the size and number of the barnacles as *Lepas pectinata* does not produce its own float. Furthermore, we found some microbes associated with the crustacean which has not been previously reported. This study appears to be the first to report the transoceanic movement of a rafted unopened plastic bottle and highlights the potential environmental risks plastic pollutants cause.

Acknowledgements

The authors would like to thank Adem, Aydin, Leyla and Sonia Sumer for sample collection, Mr Jinchao Gu for translation and control bottle sample, and Dr Andrew Hosie and Dr Lisa Kirkendale, Department of Aquatic Zoology at the Western Australian Museums for visual identification of the crustascea. Mr Raghu Badari and Mr Errol Phuah for technical support.

Authorship Contributions

Alexander Cooke: Acquisition of data, Methodology, Analysis and/or interpretation of data.

Huseyin Sumer: Conceptualization; Project administration, Acquisition of data, Methodology, Analysis and/or interpretation of data; Writing-original draft preparation, Writing-reviewing and editing.

Declaration of Conflicting Interest

The authors declare that they have no known conflicting financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Barnes, D.K.A. and P. Milner (2005). Drifting plastic and its consequences for sessile organism dispersal in the Atlantic Ocean. *Marine Biology*, **146(4):** 815-825.

Carlton, J.T., Chapman, J.W., Geller, J.B., Miller, J.A., Carlton, D.A., McCuller, M.I., Treneman, N.C., Steves, B.P. and G.M. Ruiz (2017). Tsunami-driven rafting: Transoceanic species dispersal and implications for marine biogeography. *Science*, **357(6358):** 1402-1406.

Conway, D.V.P., Ellis, C.J. and I.G. Humpheryes (1990). Deep distributions of oceanic cirripede larvae in the Sargasso Sea and surrounding North Atlantic Ocean. *Marine Biology*, **105(3):** 419-428.

David, M., Gollasch, S., Leppäkoski, E. and C. Hewitt (2015). Risk assessment in ballast water management. *In:* Global Maritime Transport and Ballast Water Management: Issues and Solutions, pp. 133-169.

Darwin, C. (1852). A monograph of the sub-class cirripedia, with figures of all the species: The Lepadidae; or Pedunculated Cirripedes. London, Ray Society, pp. 400.

Di Geronimo, R. (2010). Cirripeds from deep-water coral mounds off S. Maria di Leuca, Apulian Plateau Bank

(Mediterranean Sea). *Deep-Sea Research Part II: Topical Studies in Oceanography*, **57(5-6):** 487-492.

Fu, K., Li, J., Wang, Y., Liu, J., Yan, H., Shi, L. and L. Zhou (2016). An innovative method for rapid identification and detection of *Vibrio alginolyticus* in different infection models. *Frontiers in Microbiology*, **7:** 651.

Gibson, R.N., Atkinson, R.J.A. and J.D.M. Gordon (2005). Oceanography and Marine Biology: An Annual Review, pp. 1-534.

Goldsmit, J., Nudds, S.H., Stewart, D.B., Higdon, J.W., Hannah, C.G. and K.L. Howland (2019). Where else? Assessing zones of alternate ballast water exchange in the Canadian eastern Arctic. *Marine Pollution Bulletin*, **139:** 74-90.

Hinojosa, I., Boltaña, S., Lancellotti, D., Macaya, E., Ugalde, P., Valdivia, N., Vásquez, N., Newman, W.A. and M. Thiel (2006). Geographic distribution and description of four pelagic barnacles along the south east Pacific coast of Chile – A zoogeographical approximation. *Revista Chilena de Historia Natural*, **79(1):** 13-27.

Horn, M.H., Teal, J.M. and R.H. Backus (1970). Petroleum lumps on the surface of the sea. *Science*, **168(3928):** 245-246.

Miller, J.A., Gillman, R., Carlton, J.T., Murray, C.C., Nelson, J.C., Otani, M. and G.M. Ruiz (2018). Trait-based characterization of species transported on Japanese tsunami marine debris: Effect of prior invasion history on trait distribution. *Marine Pollution Bulletin*, **132:** 90-101.

Minchin, D. (1996). Tar pellets and plastics as attachment surfaces for lepadid cirripedes in the North Atlantic Ocean. *Marine Pollution Bulletin*, **32(12):** 855-859.

Rech, S., Borrell, Y. and E. García-Vazquez (2016). Marine litter as a vector for non-native species: What we need to know. *Marine Pollution Bulletin*, **113(1-2):** 40-43.

Ryan, P.G. and G.M. Branch (2012). The November 2011 Irruption of Buoy Barnacles *Dosima Fascicularis* in the Western Cape, South Africa. *African Journal of Marine Science*, **34(1):** 157-162.

Schiffer, P.H. and H. Herbig (2016). Endorsing Darwin: Global biogeography of the epipelagic goose barnacles *Lepas* spp. (Cirripedia, Lepadomorpha) proves cryptic speciation. *Zoological Journal of the Linnean Society*, **177(3):** 507-525.

Spengler, L. (1793). Beskrivelse over tvende nye Arter af Lepas, tilligemed nogle Anmærkninger over Lepades, som findes i Naturhistorie-Selskabets Skrivters. *Skrivter af Naturhistorie-Selskabet*, **2(2):** 103-110.

Su, Y. and C. Liu (2007). Vibrio parahaemolyticus: A concern of seafood safety. *Food Microbiology*, **24(6):** 549-558.

Tsikhon-Lukanina, E.A., Reznichenko, O.G. and G.G. Nikolaeva (2001). Ecology of invertebrates on the oceanic floating substrata in the Northwest Pacific Ocean. *Oceanology*, **41(4):** 525-530.

Vignier, N., Barreau, M., Olive, C., Baubion, E., Theodose, R., Hochedez, P. and A. Cabie (2013). Human Infection with *Shewanella putrefaciens* and *S. algae*: Report of 16 cases in martinique and review of the literature. *American Journal of Tropical Medicine and Hygiene*, **89(1):** 151-156.

Whitehead, T.O., Biccard, A. and C.L. Griffiths (2011). South African pelagic goose barnacles (Cirripedia, Thoracica): Substratum preferences and influence of plastic debris on abundance and distribution. *Crustaceana*, **84(5-6):** 635-649.