Marine Pollution Bulletin 102 (2016) 114–121

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Small-scale temporal and spatial variability in the abundance of plastic pellets on sandy beaches: Methodological considerations for estimating the input of microplastics

Fabiana Tavares Moreira [a], Alessandro Lívio Prantoni [b], Bruno Martini [c], Michelle Alves de Abreu [b], Sérgio Biato Stoiev [b], Alexander Turra [a,*]

[a] Oceanographic Institute, University of São Paulo, 05508-120, São Paulo, SP, Brazil
[b] Centro de Estudos do Mar, Universidade Federal do Paraná, 83255-000, Pontal do Paraná, PR, Brazil
[c] Núcleo de Pesquisa de Ciências (NUPESC), Rio de Janeiro RJ, Brazil

ARTICLE INFO

Article history:
Received 27 February 2015
Received in revised form 27 November 2015
Accepted 30 November 2015
Available online 8 December 2015

Keywords:
Solid wastes
Input
Tidal cycle
Transect
Strandline

ABSTRACT

Microplastics such as pellets have been reported for many years on sandy beaches around the globe. Nevertheless, high variability is observed in their estimates and distribution patterns across the beach environment are still to be unravelled. Here, we investigate the small-scale temporal and spatial variability in the abundance of pellets in the intertidal zone of a sandy beach and evaluate factors that can increase the variability in data sets. The abundance of pellets was estimated during twelve consecutive tidal cycles, identifying the position of the high tide between cycles and sampling drift-lines across the intertidal zone. We demonstrate that beach dynamic processes such as the overlap of strandlines and artefacts of the methods can increase the small-scale variability. The results obtained are discussed in terms of the methodological considerations needed to understand the distribution of pellets in the beach environment, with special implications for studies focused on patterns of input.

1. Introduction

Coastal environments are likely to accumulate much of the solid waste released by modern industrial and urban society to the oceans. Globally, millions of tons of plastic are produced every year and it is estimated that 10 to 20 million tonnes find their way into the world's oceans each year, costing an estimated US$13 billion per year in environmental damage to marine ecosystems (UNEP, 2014a). The industrial society chooses to take advantage of the light weight and durability of plastic and as a result it is found in nearly all modern products (Andrady and Neal, 2009). Nevertheless, these advantages also make plastic a serious environmental and health threat, as its widespread distribution and persistence in the ocean are favoured (Thompson et al., 2005; Oehlmann et al., 2009; Talsness et al., 2009; Rochman et al., 2013).

Among the wide spectrum of plastic debris, concern is growing over the threats posed by particles with an upper size limit of 5 mm in diameter, known as microplastics (Cole et al., 2011; UNEP, 2014b). Microplastics can originate from the fragmentation of discarded items or can be the industrial raw material for plastic products, such as virgin pellets, nibs or "mermaids' tears", which are categorized as the primary forms of microplastics (Cole et al., 2011). The sources of pellets found in the marine environment can be both marine and land-based and include losses during handling, transfer, transportation (Turner and Holmes, 2011) and possibly at port terminals (Manzano, 2009). Once lost, pellets and other microplastics reach coastal areas where tidal movements and alongshore drift currents carry these particles to the shoreline of habitats such as sandy beaches (e.g. Shiber, 1982; Moore et al., 2001; Kusui and Noda, 2003; Abu-Hilal and Al-Najjar, 2004; Ivar do Sul et al., 2009; Martins and Sobral, 2011; Liebezeit and Dubaish, 2012; Turra et al., 2014).

The loss of pellets represents a high cost to the environment and to industry, thus global efforts are in place to reduce losses along the production chain, such as 'Operation Clean Sweep' (OCS) created in the U.S. in 1992 and adopted in many countries (www.opcleansweep.org) and, the 'Declaration of the Global Plastics Associations for Solutions on Marine Litter' signed in 2011 (www.marinedebrissolutions.com/Declaration). Therefore, the need to understand patterns of distribution of plastic pellets and other microplastics across the beach profile is already established in the scientific community and industrial sector. Nevertheless, studies designed to evaluate the abundance and distribution of pellets and other microplastics on sandy beaches have been conducted in different tidal zones, using different sampling methods (see Hidalgo-Ruz et al., 2012), and usually not considering any possible artefacts caused by small-scale temporal and spatial variability associated with beach dynamics.

* Corresponding author: Biological Oceanography Department, Oceanographic Institute, University of São Paulo, 05508-900 São Paulo, SP, Brazil.
E-mail address: turra@usp.br (A. Turra).

http://dx.doi.org/10.1016/j.marpolbul.2015.11.051

Plastics arriving on beaches are firstly deposited in the intertidal zone, commonly on drift or strandlines (Thornton and Jackson, 1998), defined as the marking left by the high water of tidal action, and typically composed of debris left after a high tide (although additional lines may be produced due to oscillation during the descending tide, see Fig. S1). The distribution of pellets and other microplastics is not uniform across the beach profile (Heo et al., 2013), neither along the beach, nor with depth (Turra et al., 2014). In the across-shore direction, pellets are limited to the surface of the sediment in the upper beach intertidal zone, but are concentrated on the upper backshore where they can be found down to 2 m depth (Turra et al., 2014). Thus, the intertidal zone acts as a zone of transference of pellets from the sea to the backshore, and potentially to the dunes, where they accumulate. As a consequence, depending on the beach height and depth of the sediment sampled, different estimates of microplastic distribution and abundance can be obtained. Estimates using surface sediment samples taken from the intertidal zone would, therefore, be more appropriate to evaluate the input or load of microplastics to the beach system (i.e. the amount of litter arriving on a beach; Escardó-Boomsma et al. (1995)), while those taken from the backshore and dunes, and preferentially considering the depth distribution of these particles, would be meaningful to evaluate their standing-stock (i.e. the amount of accumulated plastics in the habitat). Such clear distinction is an important point for the design of sampling strategies, comparisons among beaches and the proper interpretation of data in time series. Although such rationale is already incorporated into protocols and studies for the evaluation of macro debris on beaches (e.g. Cheshire et al., 2009; Ribic et al., 2010), it is usually not evident in studies on microplastics (but see Claessens et al., 2011).

Early surveys assessing meso-debris (2–20 mm fragments) loads (i.e. accumulation) demonstrated the importance of integrating estimates across the beach profile, sampling the surface sediment from transects running from the most recent high tide line up the beach to the storm strandline (Ryan and Moloney, 1990). In the intertidal zone, some studies evaluating the abundance and distribution of pellets were conducted using across-shore transects to integrate estimates across the beach profile, although the specific tidal zone varied or was not stated (Khordagui and Abuhilal, 1994; Abu-Hilal and Al-Najjar, 2009). Nevertheless, many studies on micro and macroplastics still use strandline areas to evaluate the abundance of debris (see Barnes et al., 2009) and again, the drift line considered, varied among studies (e.g. Browne et al., 2011; Martins and Sobral, 2011; Hidalgo-Ruz and Thiel, 2013; Dekiff et al., 2014) or was not stated (e.g. Ivar do Sul et al., 2009; Costa et al., 2010). Also, studies that have compared the abundance of microplastics among beaches, using samples from the intertidal zone, have either only sampled once per beach (Ivar do Sul et al., 2009; Martins and Sobral, 2011; Hidalgo-Ruz and Thiel, 2013) or repeated the sampling at each beach with largely spaced intervals (i.e. several months between samplings) (Khordagui and Abuhilal, 1994; Abu-Hilal and Al-Najjar, 2009). However, usually small-scale spatial and temporal variability is not considered. Moreover, sampling to compare abundance among beaches are usually not done contemporaneously, but samples for comparison have been taken from the different study beaches within a time window of several months (Khordagui and Abuhilal, 1994; Abu-Hilal and Al-Najjar, 2009; Ivar do Sul et al., 2009; Hidalgo-Ruz and Thiel, 2013). Only one study specified that the last high tide mark was used and also, that samples were taken at five beaches during equinoctial spring tides of a specific month (Martins and Sobral, 2011), thus controlling for any eventual consequences of not taking samples at the same time.

While studies have demonstrated that debris-stranding patterns are influenced by factors such as wave action (Thornton and Jackson, 1998), wind-driven water currents (Moore et al., 2001; Edyvane et al., 2004) and the direction of the prevalent wind (Browne et al., 2010), many other intrinsic factors may influence patterns of distribution on sandy beaches. These may be related to 1) the quantities of plastics commercialized in the nearby areas and local rain patterns, which may vary through the year or between years and influence the input of plastic debris into the marine system and onto sandy beaches; 2) the direction and morphology of the beach; and 3) barometric pressure, the cycle of the moon and the tidal stage, which together will influence tidal height. Moreover, the intertidal zone of sandy beaches is very dynamic and the distribution of the drift lines and the height of the shore reached by each high tide may vary on a daily or even on a tidal cycle basis. These variations are influenced by a combination of intrinsic factors affecting beach dynamics, such as the local tidal type (i.e. diurnal, semi-diurnal or mixed), the tidal stage (i.e. ebbing or flooding), height oscillations during descending tides, changes in wave action and wind forces (see Jackson et al., 2002; Ryan et al., 2009). Due to these factors, drift lines in the intertidal zone may or may not suffer an overlapping effect during subsequent tidal cycles and long-term debris may also be accumulated on storm strandlines in the backshore, thus potentially causing significant noise in estimates of microplastics based on point samples.

Hence, gathering knowledge about the influence of these factors is of central importance to the development of sampling methods and non-confounded sampling designs, with sufficient replication on appropriate temporal and spatial scales, to adequately quantify trends (Ryan et al., 2009) in terms of the input and accumulation of microplastics on sandy beaches. Nevertheless, factors such as the cycle of the moon, tidal stage and, especially, the history of the strandline are not usually considered in studies evaluating the distribution of microplastics in the intertidal zone of sandy beaches. These factors may increase temporal and spatial variability in the data, directly influence estimates of pellet abundance in the beach environment and possibly explain part of the high variability observed on estimates of micro-plastics distribution in data sets around the world (Gregory, 1978; Khordagui and Abuhilal, 1994; Abu-Hilal and Al-Najjar, 2009; Kershaw and Leslie, 2012; Dekiff et al., 2014).

The aim of this study was to investigate the small-scale temporal and spatial variability in the abundance of pellets in the intertidal area of a sandy beach and evaluate factors that can increase the variability in data sets from that coastal area. To achieve this aim, we estimated the abundance of pellets in drift lines during consecutive tidal cycles to test the specific hypotheses that the abundance of pellets sampled from drift lines would vary according to the tidal cycle and the sampling day (temporal scale; hypothesis 1) and that the number of pellets would be different among drift lines across shore (spatial scale; hypothesis 2). The underlying assumption of this study is that calculations of the abundance of plastic pellets in the intertidal of sandy beaches are highly influenced by environmental processes at a very small spatial and temporal scale and thus may not allow accurate and comparable estimates among areas and between studies without a strict sampling design.

2. Methods

2.1. Study area and sampling design

The study was conducted during 2010 at Pontal do Sul, a sandy beach located close to the mouth of the Paranaguá estuary, Paraná State, southern Brazil (Fig. 1). The selected study site was located in front of the Center for Marine Studies (Federal University of Paraná). Site selection was based on the abundance of natural debris, which was used as marker/indicator of the drift line position as well as the proximity to the Port of Paranaguá (located about 20 km inland), a possible source of pellets to the sampling area. The sampling strategy consisted of limiting the spatial scale (i.e. variability) and intensifying and refining the temporal and micro-spatial scale, considering a location with semi-diurnal tidal cycles, with diurnal inequality, during 12 subsequent tidal cycles.

Sampling started on the full moon on 30th January 2010 and finished in the waning moon on 4th February 2010. Tidal range during this period varied between 0.1–1.6 m (1st day) to 0.5–1.4 m (6th day) (Diretoria de Hidrografia e Navegação, 2010 — at Barra de

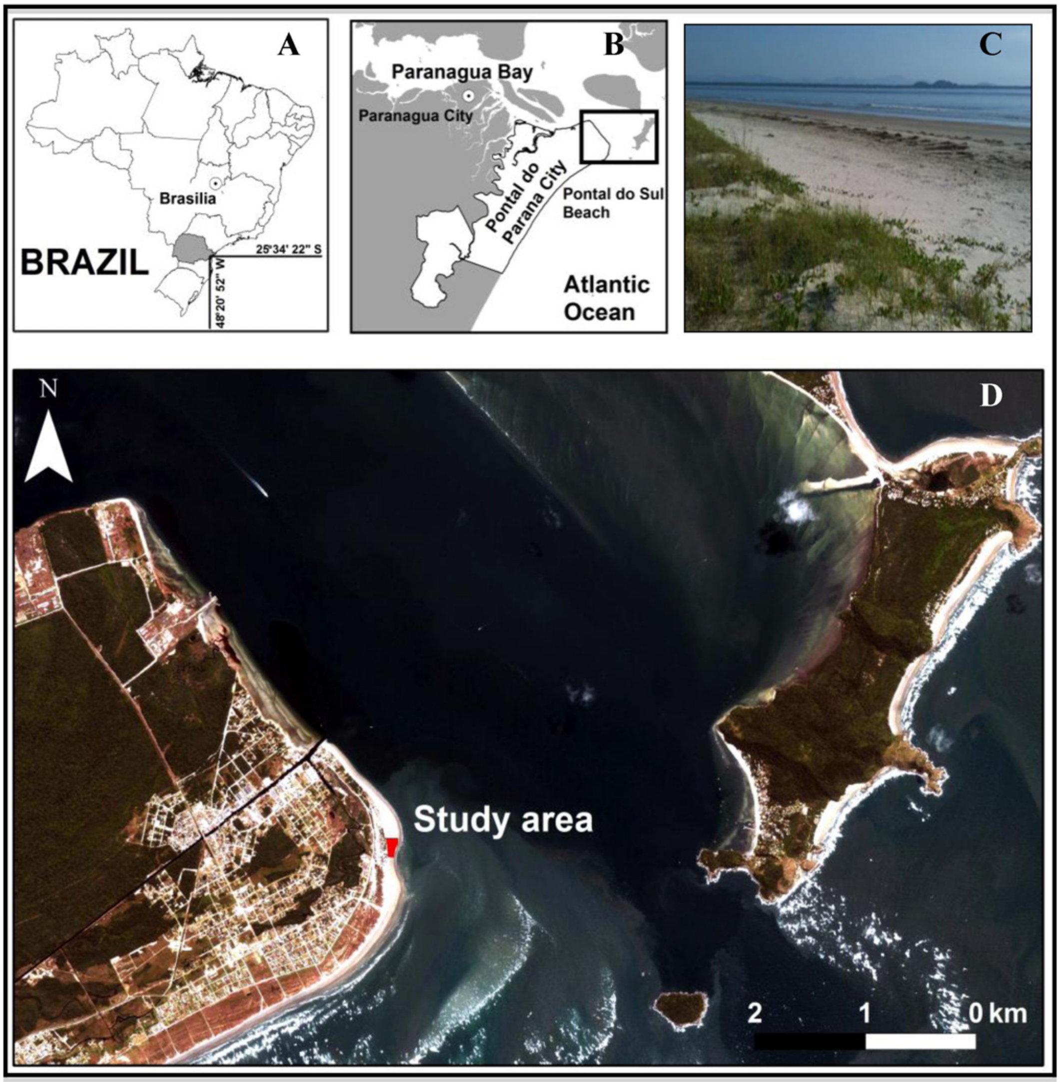

Fig. 1. Study area. A — Latitude and longitude of the study area and location of the Paraná State in Brazil. B — Geographic details of Pontal do Sul Balneary. C — Picture from the study area. D — Satellite image from the study area.

Paranaguá, Canal Sueste, Estado do Paraná). Prior to the sampling, there were 22 days of constant rain with stronger peaks on the 8th and 23rd January (215.4 and 124 mm/day, respectively) and during the 6 sampling days there were no rain events, apart from 1.2 mm of rain on the 31st January (as per data provided by the Instituto Tecnológico SIMEPAR, 2010). During the work, the factors related to the variability in the release of plastic pellets to the sea (direct losses or arrival of pellets by water flow after rain events) was diminished because this study was designed to be concentrated in a very limited period in which there was neither direct influence of rain events nor reported losses of cargo. Sampling took place twice a day, always during the slack ebb tide to consider the drift lines of the last tidal cycle. Cycle 1 was usually sampled during the morning and cycle 2 during the night. We used that approach to test the null hypothesis of very low variability in the amount of pellets deposited through time (i.e. days and/or tidal cycles).

A beach portion (100 m stretch) with very homogeneous morphodynamic characteristics was selected to reduce the effect of non-controlled parameters, i.e., the input of pellets was considered homogeneous along this beach portion. Ten transects (1 m wide) were traced extending along the whole drift band, defined as the cross-shore distance from the highest (landward) to the lowest (seaward) visible detritus left (only evident drift lines were considered) during the last tidal cycle (Fig. 1C). In each cycle sampled, the position of transects were defined randomly. Measurements of the distance (m) of the drift line from the frontal dune, width (m) of the drift line, and number of drift lines (1 to 3) were taken during each sampling. To identify drift lines and where the water reached in the last tidal cycle, perpendicular lines were also drawn in the sediment during the subsequent cycles. Therefore, if the high tide reached a lower level in a subsequent cycle and, there was a drift line located above the watermark of that specific cycle, that drift line was not sampled, as it would have been left by a previous cycle (e.g. Supplementary information Table SI — cycle 10).

Pellets were sampled from each drift line only along transects; therefore no previous cleaning of the entire sampling area was included in the sampling design. Collection of pellets was done through scratching the surface of the sand and therefore sampling included pellets buried subsuperficially (max. 5 mm) and those harder to identify due to similarity in colour and size to the sand grains. Scratched sand and debris were stored for subsequent laboratory sorting through two types of nets (10 mm, to separate larger debris, and 1 mm to separate sand grains) to identify and quantify pellets in each transect and sampling time. This approach also allowed an estimation of the number of pellets in each drift line.

2.2. Differences among days and tidal cycles

Analyses of variance (ANOVA) were used to test the null hypothesis of no difference in the number of pellets among tidal cycles and days.

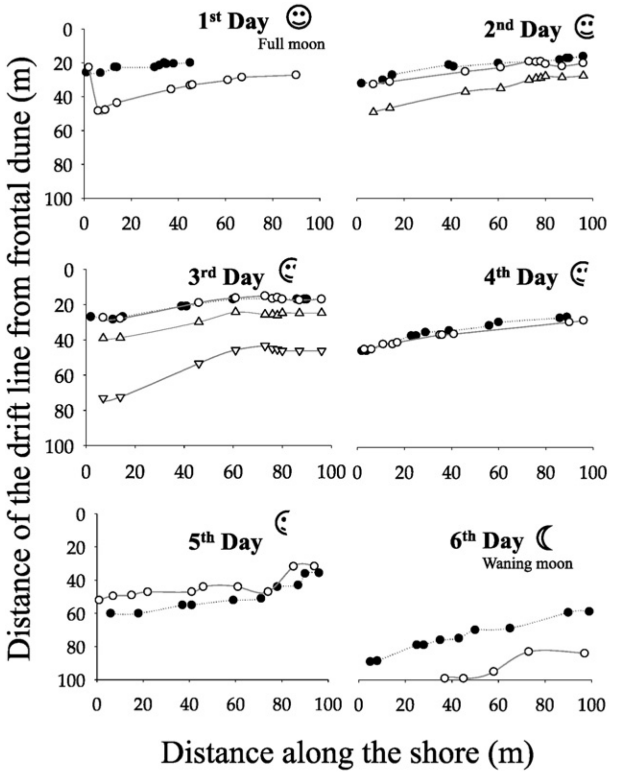

Fig. 2. Drift lines. Location and number of drift lines in each sampling day/cycle, as well as moon stage. Cycle 1 — dotted black line with filled symbol; Cycle 2 — solid grey line with white symbol. Line A — circle, Line B — triangle up, Line C — triangle down.

As the number of drift lines varied from 1 to 3 among cycles and days (Fig. 2), two different analyses were done.

Firstly, the number of pellets present at one drift line (e.g. line A, located closer to the frontal dune and commonly sampled in the studies reported in the literature) from each tidal cycle was compared. A second analysis was done considering the total number of pellets in all drift lines in each cycle (i.e., number of pellets per transect), thus integrating estimates as recommended by Ryan and Moloney (1990). For both analyses, data from the two cycles during 6 consecutive days were used, considering the factors 'Cycle' (fixed, with 2 levels) and 'Day' (fixed and orthogonal, 6 levels, $n = 10$). Cochran's test for heterogeneity of variances was done prior to analyses and when necessary $\mathrm{Ln}(X + 1)$ transformation was applied to the data to meet the analysis assumptions. Student–Newman–Keuls (SNK) tests were done for a posteriori comparisons within significant factors (Underwood, 1997)..

2.3. Differences among drift lines

The null hypothesis of no difference in the number of pellets among drift lines was tested using ANOVA. As the number of drift lines varied from 1 to 3 among cycles and days (see Fig. 2), two different analyses were conducted.

Firstly, differences in the number of pellets among different drift lines (ABC) in the same cycle and day were evaluated using data from the 2nd cycle of the 3rd sampling day. The drift line 'A' was located closer to the frontal dune, while the drift line 'C' was located closer to the seawater. The factor considered was 'Line' (fixed, with 3 levels: A, B and C, $n = 10$). The second analysis compared the number of pellets between two lines (A and B), during the 2nd cycle of two sampling days (2nd and 3rd). The analysis considered the factors 'Line' (fixed, with 2 levels: A and B, $n = 10$) and 'Day' (random and orthogonal, 2 levels). Cochran's and SNK tests were performed as above.

3. Results

3.1. Distribution of drift lines

From the twelve consecutive tidal cycles sampled, only the 4th and 6th cycles had two and three drift lines, respectively, with all remaining cycles having only one line. The distance of the upper drift lines from the frontal dune (i.e. height on the shore) also varied among tidal cycles. From the 2nd to the 5th sampling days, there was overlapping of drift lines from subsequent tidal cycles, and drift lines reached or were even higher than the drift line from the previous cycle. Nevertheless, the overlapping effect was stronger during the 2nd and 3rd sampling days, when drift lines from subsequent tidal cycles reached the highest portions of the beach (Fig. 2, Supplementary information Table SI).

3.2. Differences among days and tidal cycles

The results of the ANOVA on the number of pellets in line A of all days and tidal cycles, indicated that there was significant interaction between Cycles (Day) (Table 1). SNK tests showed that numbers of pellets varied among days for both tidal cycles and also between tidal cycles in two out of the six sampling days, although in different directions (Fig. 3). When the number of pellets was compared considering all drift lines in each cycle, ANOVA also showed significant interaction between Cycles(Day) (Table 2). Nevertheless, numbers of pellets were similar among days for cycle 1 and varied for cycle 2. Differences between tidal cycles also occurred in two out of the six sampling days, but compared to the analysis considering only line A, these occurred on different days and, where they occurred, cycle 2 had greater numbers of pellets than cycle 1 (Fig. 4).

3.3. Differences among drift lines

There were significant differences among the three drift lines (ABC) sampled from the same cycle and sampling day (ANOVA: $C = 0.55$, $\mathrm{Ln}(X + 1)$, $F_{2,27} = 42.5$, $df = 2$, $p < 0.001$). The number of pellets decreased from line A (located closer to the frontal dune) to line B and C (located closer to the sea) (Fig. 5). Nevertheless, an ANOVA to evaluate differences among lines (A and B) during the second cycle of these two sampling days showed a significant interaction Lines x Days and the SNK test demonstrated that differences were not constant between lines nor through time (e.g. on the 2nd sampling day line B had more pellets than line A, while on the 3rd day the number of pellets was greater for line A) (Table 3 and Fig. 6).

Together, these results indicate the existence of high variability in the abundance of pellets during a short temporal and spatial scale. However, it would seem that some of that variability appears to be related to the evident overlapping effect between subsequent tidal cycles. When the distribution of drift lines (Fig. 2) is examined together

Table 1
ANOVA of the mean number of pellets in drift line A, among cycles and days. 'Cycle' (fixed, with 2 levels) and 'Day' (fixed and orthogonal, 6 levels, $n = 10$). Heteroscedasticity was tested with Cochran's test ($C = 0.20$, $\mathrm{Ln}(X + 1)$, *NS*).

Source	*df*	MS	F	*P*
Cycle Cy	1	2.67	4	< 0.05
Day Da	5	33.755	50.61	< 0.001
Cy × Da	5	5.3903	8.08	< 0.001
Residual	108	0.67		
SNK tests[a]				
Da (Cy)	Cy 1 = Day 3 = 1 ≫ 2 = 4 ≫ 5 = 6			
	Cy 2 = Day 3 ≫ 2 = 4 = 1 = 5 ≫ 6			
Cy (Da)	1st day = Cy 1 ≫ 2			
	5th day = Cy 1 ≫ 2			

[a] Student–Newman–Keuls (SNK) test is used for *post-hoc* comparisons within significant fixed factors (Underwood, 1997).

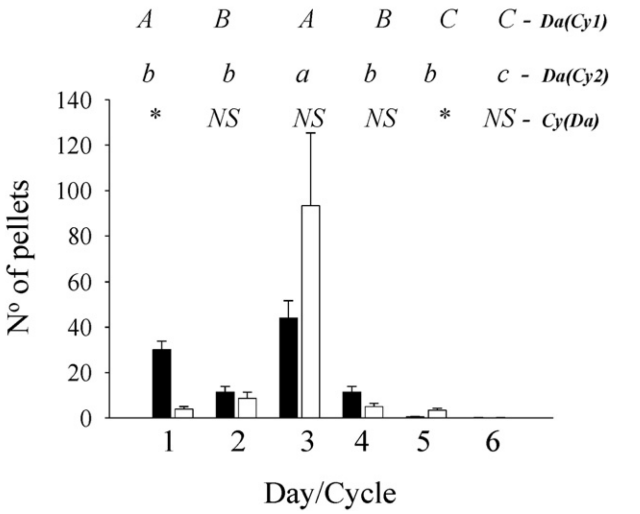

Fig. 3. Number of pellets at line A, between cycles and among days. Mean number of pellets (±SE) at Line A, between two tidal cycles, during six sampling days. Lower case letters denote differences in the number of pellets in the 2nd tidal cycle among sampling days, while capital letters denote differences in the 1st tidal cycle of the days sampled (as per SNK test).

with the data on the number of pellets among days and cycles, it is possible to see clearer patterns. Considering only drift line A from subsequent tidal cycles (Fig. 3), during cycles where drift lines reached lower heights on the shore than the line A from the previous cycles (i.e. no overlapping effect) there was a decrease in the number of pellets sampled in subsequent cycles (e.g. 1st to 2nd and 6th to 7th cycles), whereas when the line A only partially reached the line from the previous cycle, the number of pellets sampled tended to be similar (e.g. 2nd to 3rd and 3rd to 4th cycles). Conversely, when line A reached the same or higher portions of the beach face than the line A from the previous cycles, there was an evident increase in the numbers of pellets sampled (e.g. 4th to 5th and 5th to 6th cycles). Moreover, only two out of the 12 sampled cycles had more than one drift line (cycles 4th and 6th, Fig. 2), but the patterns of distribution of number of pellets among days and cycles were different between the analysis considering only line A (Fig. 3) and that considering all drift lines (Fig. 4).

4. Discussion

The main goals of this work were to investigate the small-scale temporal and spatial variability in the abundance of pellets in the intertidal zone of a sandy beach and to evaluate factors that can increase the variability in data sets from that coastal area. We have demonstrated that beach dynamic processes such as the overlap of strandlines and

Table 2
ANOVA of the mean number of pellets in all drift lines, among cycles and days. 'Cycle' (fixed, with 2 levels) and 'Day' (fixed and orthogonal, 6 levels, $n = 10$). Heteroscedasticity was tested with Cochran's test ($C = 0.20$, Ln(X + 1), *NS*).

Source	*df*	MS	F	*P*
Cycle Cy	1	2881.2	2.97	> 0.05
Day Da	5	13,399.76	13.82	> 0.05
Cy × Da	5	4126.7	4.25	< 0.05
Residual	108	969.91		
SNK tests[a]				
Da (*Cy*)	Cy 1	NS		
	Cycle 2	Day 3 ≫ 2 ≫ 4 = 1 = 5 = 6		
Cy (*Da*)	Day 1	NS		
	2nd day	Cy 1 ≫ Cy 2		
	3rd day	Cy 1 ≫ Cy 2		

[a] Student–Newman–Keuls (SNK) test is used for *post-hoc* comparisons within significant fixed factors (Underwood, 1997).

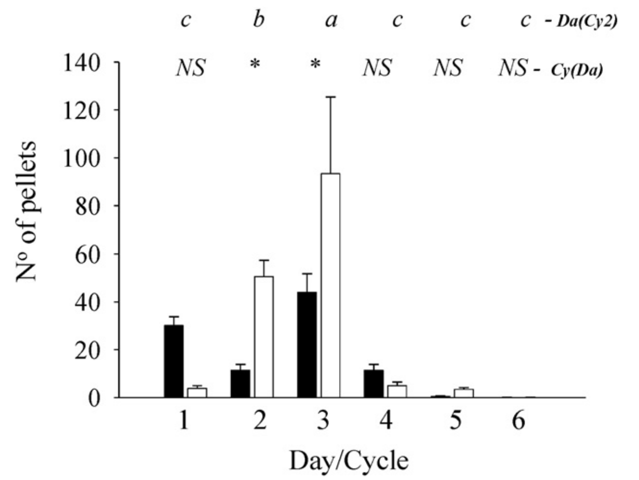

Fig. 4. Total number of pellets, between cycles and among days. Mean number of pellets (±SE) between two tidal cycles during six sampling days, considering the amount of pellets in all lines. Lower case letters denote differences in the number of pellets in the 2nd tidal cycle among sampling days (as per SNK test).

possible artefacts of the method traditionally employed in microplastics surveys on sandy beaches might increase the variability in data sets. The results obtained are discussed here in terms of the methodological considerations needed to understand the distribution of pellets and other microplastics in the sandy beach environment and have special implications for studies aiming to understand patterns of input (as increased variability in these estimates can hide long-term patterns), as well as for studies comparing multiple beaches with samples not taken at the same time. Our findings demonstrate that without a strict sampling design it may be difficult to obtain reliable estimates on the rate of entrance (input rate) and, indeed, quantitative comparisons among areas, periods and studies.

We used tidal cycle as the measured unit, sampling pellets at drift lines during 12 consecutive cycles and marking/delimiting on the shore the position of each drift line and tidal height between cycles. Statistical analyses of the abundance of pellets showed high variability in the number of pellets between tidal cycles, and among sampling days and drift lines in very small-scale temporal and spatial comparisons. Also, contrary to the general prediction, the amount of pellets deposited in the intertidal zone generally increased from the 1st towards the 3rd sampling day, decreasing thereafter. The temporal variation could only be observed as samples were taken during subsequent cycles and this clearly showed that if estimates were done using random point

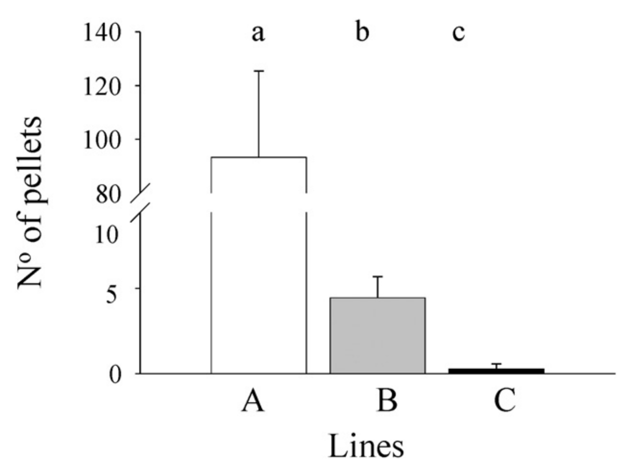

Fig. 5. Number of pellets among drift lines from the same cycle. Mean number of pellets (±SE) among three drift lines from the 2nd cycle of the 3rd sampling day. Small case letters (a–c) denote significant differences among lines (as per SNK test).

Table 3
ANOVA of the mean number of pellets between drift lines and days. 'Line' was fixed, with 2 levels: A and B ($n = 10$) and 'Day' (random and orthogonal, 2 levels). Heteroscedasticity was tested with Cochran's test ($C = 0.34$, Ln(X + 1), *NS*).

Source	*df*	MS	F	P
Line Li	1	1.381	0.03	> 0.05
Day Da	1	0.0125	0.01	> 0.05
Li × Da	1	51.4714	43.31	< 0.001
Residual	36	1.1884		
SNK tests[a]				
	2nd day = B ≫ A			
Li (*Da*)	3rd day = A ≫ B			
	Line A = 2nd day ≫ 1st day			
Da (*Li*)	Line B = 1st day ≫ 2nd day			

[a] Student–Newman–Keuls (SNK) test is used for *post-hoc* comparisons within significant fixed factors (Underwood, 1997).

sampling within this very short period, very different conclusions could be drawn (e.g. if sampled on the 3rd or 6th days of this study). This might be an important factor contributing to the great spatial variability in the abundance and distribution of pellets reported in the literature, which used point sampling strategies and compared samples taken at different sampling sites using long time intervals (e.g. Gregory, 1978; Khordagui and Abuhilal, 1994; Abu-Hilal and Al-Najjar, 2009). Moreover, it highlights the importance of including an assessment of small-scale temporal variation in the design of studies aiming to estimate the input of pellets, such as recently demonstrated for meso-debris (Ryan et al., 2014).

Our results also demonstrate that when multiple drift lines are present, calculations considering only the upper lines produced different results in terms of abundance of pellets than those considering all the lines present (or the transect data). Previous work has reported that the abundance of small plastic debris in the upper strandline was greater than at the cross-sectional line of the intertidal zone (Heo et al., 2013) and recently, Dekiff et al. (2014) observed no significant differences in the abundance of small plastics among the upper and the lower drift lines. These conclusions were, however, drawn from point observations and, our results have shown that when small-scale temporal variation is considered in the analysis, the number of drift lines and abundance of pellets among drift lines varied, although not in a consistent manner. We have also demonstrated that the upper drift lines do not necessarily concentrate most of the pellets, emphasizing that samples taken only at the upper drift lines should not be used as an index to represent the input of pellets.

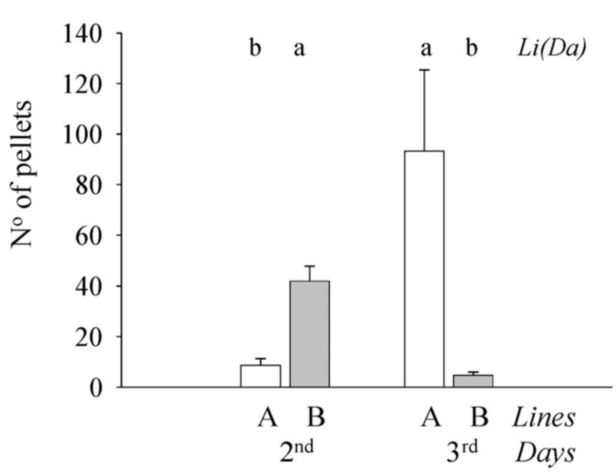

Fig. 6. Number of pellets between drift lines, cycles and days. Mean number of pellets (±SE) at two drift lines (A and B) during the 2nd cycle of the 2nd and 3rd sampling days. Small case letters (a and b) denote significant differences among lines in each sampling day (as per SNK test).

Part of the variability observed in our data set (i.e. between days/cycles and among drift lines) was clearly influenced by the overlapping effect of drift lines between consecutive tidal cycles, observed from the 4th to the 5th and 5th to the 6th cycles (both cycles of the 3rd sampling day). It was possible to observe the influence of this overlapping effect in the estimates of pellets from drift lines during subsequent cycles because the history of their distribution was also considered in the sampling design. This approach was important as it demonstrated that the distribution of drift lines is uneven between cycles and that the overlapping effect may cause important variation in the estimates, since it may aggregate pellets from previous tidal cycles with smaller amplitude/range and overestimate abundance.

This overlapping effect is very likely to occur in periods from neap to spring tides or even between subsequent tides within a lunar phase. Nevertheless, our results indicate a stronger overlapping effect for both cycles on the 3rd sampling day, thus from spring to neap tides, demonstrating that this effect may also occur during other lunar phases. Thus, the overlapping effect represents an intrinsic factor that can directly influence estimates of abundance of debris from the surface sediment of sandy beaches when using point sampling at drift lines or across-shore transects in the intertidal zone. Since, most previous studies examining the distribution of pellets and other microplastics in the intertidal zone of sandy beaches have not considered the history of the drift lines, the overlapping effect could represent an important artefact of the sampling methods, which may have greatly contributed to the variability in the estimates obtained.

Thus, intrinsic factors and methodological artefacts can increase small-scale temporal and spatial variability in estimates of microplastic abundance that can mask long-term trends. In addition, this variability can make it difficult to compare estimates of the abundance of debris from surface sediments of the intertidal zone of sandy beaches, as well as hamper quantitative comparisons among areas and time and between studies. Previous studies have demonstrated the importance of sampling using continuous transects to integrate small-scale spatial variability in estimates of the standing stock of meso and micro debris from sandy beaches (i.e. sampling debris accumulated at the back shore) (e.g. Ryan and Moloney, 1990; Turra et al., 2014). Similarly, to decrease such small-scale spatial variability, studies aiming to estimate the input rate of pellets, should sample using continuous transects, with samples taken from the surface sediment of the intertidal zone (i.e. from the highest to the lowest watermark). Nevertheless, due to the overlapping effect, estimates of input rates should also consider the history of the drift lines present in the intertidal zone at each tidal cycle.

To achieve this, we suggest that tidal cycle should be used as the measuring unit (marking the position of the high tide between cycles) and, prior to sampling, the sampling area should be cleaned (e.g. during the previous low tide). This could be done using manipulation techniques where parts of the beach(es) would be prepared to receive "new" debris using one of the methods suggested in previous protocols for monitoring marine debris loadings on sandy beaches (Cheshire et al., 2009; Ribic et al., 2010). Nevertheless, the amount of litter deposited can be greater on sandy beaches closer to urban centres (Barnes et al., 2009), which can prevent cleaning of large areas (Ryan et al., 2014). Thus, for the purpose of standardizing sampling methods, we suggest that future studies assessing pellet inputs in the intertidal area of sandy beaches should only clean the surface sediment in specific transects that will be sampled on the subsequent tidal cycle and so, evaluate the total input of pellets (i.e. delivered from the water and also those coming from lateral areas). Turra et al. (2014) demonstrated that pellets are only found in the surface sediment of the intertidal zone of sandy beaches. Therefore, cleaning of transects could be achieved by scraping the surface of the sediment with the aid of a hoe or a wooden rake (e.g. removing about 5 mm of the surface sediment) and sieving the removed sediment to recover debris for further sorting of pellets in situ or in the laboratory.

For the purpose of monitoring long-term changes in the input of pellets, the integration of small-scale temporal variability is also fundamental and thus, samples should be taken during consecutive tidal cycles to produce more reliable estimates. Nevertheless, this approach may not be logistically feasible if several sites are to be compared at the same time. In those situations, sampling on different beaches could be conducted during similar lunar phases (see Martins and Sobral, 2011), but ensuring that transects are cleaned during the tidal cycle prior to sampling. If only one beach is to be monitored, consecutive tidal cycles should be sampled for a longer time period (e.g. 30 days) with sampling repeated in different seasons for many years.

Here, we have demonstrated that estimates of the abundance of pellets in the intertidal zone can be highly influenced by intrinsic small-scale temporal and spatial sources of variability as well as artefacts of the sampling methods such as taking samples from drift lines and the overlapping effect of drift lines. These sources of variability are not usually considered in the sample design for studies evaluating the abundance and distribution of microplastics and might, in part, explain the high variability observed in estimates of microplastic abundance in many data sets around the world (Kershaw and Leslie, 2012). Our results also illustrate that quantitative comparisons among beaches (e.g. Hidalgo-Ruz and Thiel, 2013; Browne et al., 2011) and studies conducted in the intertidal zone of sandy beaches (e.g. Heo et al., 2013; Hidalgo-Ruz and Thiel, 2013) should be made with caution. This shows how difficult it is to evaluate patterns of distribution for microplastics from the available literature, as comparisons among works are hampered by the lack of consideration of the above factors and by artefacts of the sampling methods employed. However, understanding patterns of distribution and, particularly, the input of pellets is of great importance as these type of data can be used to evaluate the effectiveness of programmes aimed at helping the plastics industry reduce the loss of pellets to the environment, such as 'Operation Clean Sweep' (OCS), first adopted in the USA and latterly by several other regions around the world (e.g. Canada, European countries, Australia, New Zealand)..

In conclusion, if we are to understand the distribution of pellets and other microplastics on sandy beaches, standardized methods are needed to identify the existing patterns. Furthermore, sampling designs need to have a defined temporal and spatial scale and must incorporate intrinsic sources of small-scale variability, as well as distinguish and avoid the possible artefacts of sampling method. To achieve this, it is important in the future to have a greater integration between research activities dedicated to understand marine debris and the study of beach dynamics. Moreover, it is essential to distinguish if the aim is to evaluate the input of particles (i.e. sampling the surface sediment of the intertidal zone) or their standing stock (i.e. sampling in the backshore zone and considering the depth distribution of these particles). Suggestions on methods to evaluate patterns of accumulation (i.e. standing-stock) have been provided by (Turra et al., 2014). To understand patterns of the input of pellets and other microplastics on sandy beaches, we recommend integrating small-scale temporal variability by sampling during consecutive tidal cycles. Small-scale spatial variability can be reduced if samples are taken during the low tide from the entire intertidal zone using continuous transects. Finally to avoid artefacts of the sampling method such as the overlapping effect, we suggest that specific transects should be cleaned for sampling during the subsequent tidal cycle.

Author contributions

This study was conceived by A.T. Field samplings and laboratory works were done by AT, ALP, BM, MAA and SBS. Analysis and interpretation of the data were completed by FTM and AT. The paper was written by FTM and AT with contributions from BM and MAA.

Competing financial interests

The authors declare no competing financial interests.

Acknowledgements

We thank Plastivida (Instituto Sócio-Ambiental dos Plásticos) for the fellowship to FTM and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the fellowship to AT. Thanks to the laboratory facilities of Centro de Estudos do Mar of Universidade Federal do Paraná, and Prof. Dr. Cesar C. Martins for all arrangements to make this study viable. We thank Ian McCarthy (FAPESP 14/21804-3) for checking the English.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2015.11.051.

References

Abu-Hilal, A.H., Al-Najjar, T., 2004. Litter pollution on the Jordanian shores of the Gulf of Aqaba (Red Sea). Marine Environmental Research 58, 39–63.
Abu-Hilal, A.H., Al-Najjar, T.H., 2009. Plastic pellets on the beaches of the northern Gulf of Aqaba, Red Sea. Aquat. Ecosyst. Health Manag. 12, 461–470.
Andrady, A.L., Neal, M.A., 2009. Applications and societal benefits of plastics. Philos. Trans. R. Soc. B-Biol. Sci. 364, 1977–1984.
Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B-Biol. Sci. 364, 1985–1998.
Browne, M.A., Galloway, T.S., Thompson, R.C., 2010. Spatial patterns of plastic debris along estuarine shorelines. Environ. Sci. Technol. 44, 3404–3409.
Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179.
Cheshire, A.C., Adler, E., Barbière, J., Cohen, Y., Evans, S., Jarayabhand, S., Jeftic, L., Jung, R.T., Kinsey, S., Kusui, E.T., Lavine, I., Manyara, P., Oosterbaan, L., Pereira, M.A., Sheavly, S., Tkalin, A., Varadarajan, S., Wenneker, B., Westphalen, G., 2009. UNEP/IOC Guidelines on Survey and Monitoring of Marine Litter. In: N.I.T.S.N. (Ed.), UNEP Regional Seas Reports and Studies (xii + 120 pp.).
Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62, 2199–2204.
Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597.
Costa, M.F., Ivar do Sul, J.A., Silva-Cavalcanti, J.S., Araujo, M.C.B., Spengler, A., Tourinho, P.S., 2010. On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach. Environ. Monit. Assess. 168, 299–304.
Dekiff, J.H., Remy, D., Klasmeier, J., Fries, E., 2014. Occurrence and spatial distribution of microplastics in sediments from Norderney. Environ. Pollut. 186, 248–256.
Diretoria de Hidrografia e Navegação, D., 2010. Previsões de Marés [Online] http://www.mar.mil.br/dhn/chm/box-previsao-mare/tabuas/ (18February2010).
Edyvane, K.S., Dalgetty, A., Hone, P.W., Higham, J.S., Wace, N.M., 2004. Long-term marine litter monitoring in the remote great Australian Bight, South Australia. Mar. Pollut. Bull. 48, 1060–1075.
Escardó-Boomsma, J., O'Hara, K., Ribic, C.A., 1995. National Marine Debris Monitoring Program vol. 1-2. US EPA Office of Water, Washington, DC.
Gregory, M.R., 1978. Accumulation and distribution of virgin plastic granules on New-Zealand beaches. New Zeal. J Mar. Fresh. 12, 399–414.
Heo, N.W., Hong, S.H., Han, G.M., Hong, S., Lee, J., Song, Y.K., Jang, M., Shim, W.J., 2013. Distribution of small plastic debris in cross-section and high strandline on Heungnam Beach, South Korea. Ocean Sci. J. 48, 225–233.
Hidalgo-Ruz, V., Thiel, M., 2013. Distribution and abundance of small plastic debris on beaches in the SE Pacific (Chile): a study supported by a citizen science project. Mar. Environ. Res. 87-88, 12–18.
Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.
InstitutoTecnológicoSIMEPAR, 2010. Daily cumulative precipitation at Pontal do Sul [Website] http://www.simepar.br.
Ivar do Sul, J.A., Spengler, A., Costa, M.F., 2009. Here, there and everywhere. Small plastic fragments and pellets on beaches of Fernando de Noronha (Equatorial Western Atlantic). Mar. Pollut. Bull. 58, 1236–1238.
Jackson, N.L., Nordstrom, K.F., Eliot, I., Masselink, G., 2002. 'Low energy' sandy beaches in marine and estuarine environments: a review. Geomorphology 48, 147–162.
Kershaw, P.J., Leslie, H., 2012. GESAMP Working Group 40 — Sources, Fate & Effects of Micro-Plastics in the Marine Environment — A Global Assessment: Report of the Inception Meeting, 13–15th March 2012, Paris (45 pp.).

Khordagui, H.K., Abuhilal, A.H., 1994. Industrial Plastic on the Southern Beaches of the Arabian Gulf and the Western Beaches of the Gulf of Oman. Environmental Pollution 84, 325–327.
Kusui, T., Noda, M., 2003. International survey on the distribution of stranded and buried litter on beaches along the Sea of Japan. Mar. Pollut. Bull. 47, 175–179.
Liebezeit, G., Dubaish, F., 2012. Microplastics in beaches of the East Frisian Islands Spiekeroog and Kachelotplate. Bull. Environ. Contam. Toxicol. 89, 213–217.
Manzano, A.B., 2009. Distribuição, Taxa de Entrada, Composição Química e Identificação de Fontes de Grânulos Plásticos na Enseada de Santos, SP, Brasil. Instituto Oceanográfico. Departamento de Oceanografia Biológica. Universidade de São Paulo, São Paulo, Brazil.
Martins, J., Sobral, P., 2011. Plastic marine debris on the Portuguese coastline: a matter of size? Mar. Pollut. Bull. 62, 2649–2653.
Moore, S.L., Gregorio, D., Carreon, M., Weisberg, S.B., Leecaster, M.K., 2001. Composition and distribution of beach debris in Orange County, California. Mar. Pollut. Bull. 42, 241–245.
Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look, K.J.W., Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Philos. Trans. R. Soc. B-Biol. Sci. 364, 2047–2062.
Ribic, C.A., Sheavly, S.B., Rugg, D.J., Erdmann, E.S., 2010. Trends and drivers of marine debris on the Atlantic coast of the United States 1997–2007. Mar. Pollut. Bull. 60, 1231–1242.
Rochman, C.M., Hoh, E., Hentschel, B.T., Kaye, S., 2013. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: implications for plastic marine debris. Environ. Sci. Technol. 47, 1646–1654.
Ryan, P.G., Moloney, C.L., 1990. Plastic and other artefacts on South African beaches: temporal trends in abundance and composition. S. Afr. J. Sci. 86, 450–452.
Ryan, P.G., Moore, C.J., van Franeker, J.A., Moloney, C.L., 2009. Monitoring the abundance of plastic debris in the marine environment. Philos. Trans. R. Soc. B-Biol. Sci. 364, 1999–2012.
Ryan, P.G., Lamprecht, A., Swanepoel, D., Moloney, C.L., 2014. The effect of fine-scale sampling frequency on estimates of beach litter accumulation. Mar. Pollut. Bull. 88, 249–254.
Shiber, J.G., 1982. Plastic pellets on Spain's Costa-del-Sol beaches. Mar. Pollut. Bull. 13, 409–412.
Talsness, C.E., Andrade, A.J.M., Kuriyama, S.N., Taylor, J.A., vom Saal, F.S., 2009. Components of plastic: experimental studies in animals and relevance for human health. Philos. Trans. R. Soc. B-Biol. Sci. 364, 2079–2096.
Thompson, R., Moore, C., Andrady, A., Gregory, M., Takada, H., Weisberg, S., 2005. New directions in plastic debris. Science 310, 1117.
Thornton, L., Jackson, N.L., 1998. Spatial and temporal variations in debris accumulation and composition on an estuarine shoreline, Cliffwood Beach, New Jersey, USA. Mar. Pollut. Bull. 36, 705–711.
Turner, A., Holmes, L., 2011. Occurrence, distribution and characteristics of beached plastic production pellets on the island of Malta (central Mediterranean). Mar. Pollut. Bull. 62, 377–381.
Turra, A., Manzano, A.B., Dias, R.J.S., Mahiques, M.M., Barbosa, L., Balthazar-Silva, D., Moreira, F.T., 2014. Three-dimensional distribution of plastic pellets in sandy beaches: shifting paradigms. Sci. Rep. 4 (4435), 1–7.
Underwood, A.J., 1997. Experiments in Ecologoly: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge, p. 504.
UNEP, 2014a. Valuing Plastics: The Business Case for Measuring, Managing and Disclosing Plastic Use in the Consumer Goods Industry. p. 116.
UNEP, 2014b. Year Book: Emerging Issues in Our Global Environment. p. 71.