



Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Plastic debris ingestion by marine catfish: An unexpected fisheries impact

Fernanda E. Possatto, Mário Barletta*, Monica F. Costa, Juliana A. Ivar do Sul, David V. Dantas

Laboratório de Ecologia e Gerenciamento de Ecossistemas Costeiros e Estuarinos, Departamento de Oceanografia, Universidade Federal de Pernambuco, Av. Arquitetura s/n, Cidade Universitária, 50740-550 Recife, Pernambuco, Brazil

ARTICLE INFO

Keywords:

South America
Goiana Estuary
Marine debris
Fishery
Nylon
Polyamide
Living resources conservation

ABSTRACT

Plastic marine debris is a pervasive type of pollution. River basins and estuaries are a source of plastics pollution for coastal waters and oceans. Estuarine fauna is therefore exposed to chronic plastic pollution. Three important catfish species [*Cathorops spixii* ($N = 60$), *Cathorops agassizii* ($N = 60$) and *Sciades herzbergii* ($N = 62$)] from South Western Atlantic estuaries were investigated in a tropical estuary of the Brazilian Northeast in relation to their accidental ingestion of plastic marine debris. Individuals from all three species had ingested plastics. In *C. spixii* and *C. agassizii*, 18% and 33% of individuals had plastic debris in their stomachs, respectively. *S. herzbergii* showed 18% of individuals were contaminated. All ontogenetic phases (juveniles, sub-adults and adults) were contaminated. Nylon fragments from cables used in fishery activities (subsistence, artisanal and commercial) played a major role in this contamination. These catfish spend their entire life cycles within the estuary and are an important feeding resource for larger, economically important, species. It is not yet possible to quantify the scale and depth of the consequences of this type of pollution. However, plastics are well known threat to living resources in this and other estuaries. Conservation actions will need to from now onto take plastics pollution into consideration.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

The accumulation of plastic and its debris in marine and coastal environments is the result of the intense and continuous release of this pollutant into the environment. Marine debris can significantly affect wildlife, for example, via entanglement and ingestion. Since the second half of the 20th century, the ingestion of plastic marine debris by seabirds, turtles and mammals has been widely reported and reviewed (Laist, 1997; Moore, 2008; Gregory, 2009; Colabuono et al., 2009; Tourinho et al., 2010; Guebert-Bartholo et al., 2011).

Fish are also affected by plastic marine debris (Boerger et al., 2010; Carpenter et al., 1972; Hoss and Settle, 1990; Kubota, 1990; Laist, 1997). Fish are one of the largest and most diverse animal groups on the planet, and of undisputable ecological and economic importance (Nelson, 2006), which increases the chance of contact with plastic marine debris and the development of further consequences. The ingestion of plastics by fish is a common fisher's anecdote, and has been scientifically long-known to occur (Boerger et al., 2010; Carpenter et al., 1972; Hoss and Settle, 1990; Kubota, 1990; Laist, 1997). The groups that are most frequently reported as being affected by marine debris ingestion are sharks and rays (Laist, 1997), but bony fish are also listed as being threatened (Boerger et al., 2010; Carpenter et al., 1972; Kubota, 1990; Laist,

1997). Laboratory experiments have demonstrated that this process is highly viable when plastics, especially those of smaller sizes, are available mixed with food items (Hoss and Settle, 1990; Browne et al., 2010), and should be considered in actions aimed at fish and aquatic environment conservation.

Reports on the ingestion of plastic marine debris by fish usually list sporadic, rare or infrequent events, showing no temporal or spatial trends. The identification of patterns is restricted to some entanglements reports, but, for ingestion, there is little indication of systematic or analytical data in the literature (Boerger et al., 2010; Carpenter et al., 1972; Hoss and Settle, 1990; Kubota, 1990; Laist, 1997) when compared to other vertebrate groups. There is no record of the quantitative assessments of this sort of pollution on well-known fish populations. Studies with a high level of detail (identification and explanation of spatial and temporal patterns and ecological and conservation consequences) remain to be done as a development of the early general diagnosis and basic reports of the existence of the problem of plastics ingestion by marine biota (Ivar do Sul and Costa, 2007). Coastal species living in reefs and estuaries (Carpenter et al., 1972; Kartar et al., 1976), as well as large pelagic fish (Boerger et al., 2010; Kubota, 1990), are cited in the literature as having ingested whole items and/or fragments of plastics. Catfish (Ariidae) specifically show no record in the literature for this sort of impact.

In South America (Western South Atlantic), the Ariidae family appears to be the most abundant in the estuaries (Araújo, 1988; Azevedo et al., 1999; Barletta et al., 2003, 2005, 2008, 2010). At

* Corresponding author. Tel.: +55 81 2126 7223.

E-mail address: mario.barletta@pq.cnpq.br (M. Barletta).

the Goiana Estuary (Northeast Brazil), the species of this family corresponds to 53% of the capture in number (~ 1600 individuals ha^{-1}) and 63% in weight (19 kg ha^{-1}) (Dantas et al., 2010). In this estuary there are eight species of marine catfish. The most representative were *Cathorops spixii* (density: 1340 individuals ha^{-1} and biomass: $14,203 \text{ g ha}^{-1}$), *Cathorops agassizii* (250 individuals ha^{-1} and 4226 g ha^{-1}), and *Sciades herzbergii* (9 individuals ha^{-1} and 270 g ha^{-1}) (Dantas et al., 2010).

The present study focuses on the ingestion of plastic marine debris by *C. spixii*, *C. agassizii* and *S. herzbergii* at three different ontogenetic phases (juvenile, sub-adult and adult) with the aim of quantifying the number of individuals from these three ecologically important species contaminated at the Goiana Estuary (Northeast Brazil).

2. Materials and methods

2.1. Study area

The Goiana Estuary (7.5°S – 34.5°W) is located in Northeast Brazil. It has 17 km of main channel and spreads across an area of $\sim 475,000 \text{ m}^2$, including the flood plain, which is dominated by mangrove forests (Fig. 1). Rainfall patterns define four seasons: early (March to May) and late rainy (June to August), and early (September to November) and late dry (December to February). Air temperature is almost constant ($27 \pm 2^{\circ}\text{C}$) throughout the year (Barletta and Costa, 2009). The mangrove forest, the estuary and the adjacent ecosystems still shelter large wild animals, such as capybaras, alligators, herons, hawks, manatees, porpoises, marine and freshwater turtles, and large reef fish such as groupers. There is also a rich fauna of fish, crustaceans, and molluscs that play an important role in the lives of traditional populations, determining the patterns, quality and quantity of their subsistence and artisanal fisheries' catches (Barletta and Costa, 2009). In addition, this estuary supports some small business-related (ferryboat, bars, and restaurants) activities and the presence of holiday homes used especially during the summer (Barletta and Costa, 2009).

However, the aquatic vegetation and mangrove forest that buffer the estuary are being rapidly reclaimed by sugar-cane plantations and unplanned urban development. The intense dredging of sand takes place at the upper estuary (Barletta and Costa, 2009), and sewage and domestic solid wastes receive little or no treatment from the local authorities. Fishing is an important activity in the area and encompasses subsistence, artisanal and commercial

activities. The main target group is lobster, which represents the main fuel for the local economy.

2.2. Fish samples

The fish used in this study were sampled using an otter-trawl net in the main channel of the Goiana Estuary from January 2006 to August 2008. The capture method that was used is described in detail by Dantas et al. (2010). Three ontogenetic phases were assigned to the main (density and biomass) catfish species: (i) *C. spixii* individuals were divided into juveniles (3 – 5 cm , $N = 20$), sub-adults (5.1 – 12 cm , $N = 20$) and adults ($>12 \text{ cm}$, $N = 20$); (ii) *C. agassizii* had individuals separated as juveniles (3 – 5 cm , $N = 20$), sub-adults (5.1 – 14 cm , $N = 20$) and adults ($>14 \text{ cm}$, $N = 20$); (iii) *S. herzbergii* individuals were classed as juveniles (5 – 10 cm , $N = 22$), sub-adults (10.1 – 16 cm , $N = 20$) and adults ($>16 \text{ cm}$, $N = 20$).

The stomach contents were analysed using binoculars ($45\times$) and the plastic items were separated from the organic food. The quantification of plastic debris ingestion followed three criteria: the number of individuals in which debris was found, or the frequency of occurrence; the number of debris elements in the stomach contents of each animal; and the weight (mass) of the debris in the stomach contents of each animal. The type of debris (nylon, hard and soft plastics) was also noted.

2.3. Statistical analysis

The original data describing the number and the weight of the debris were transformed (Box and Cox, 1964) to increase the normality of the distribution. The data were statistically tested by an analysis of variance (ANOVA). When significant differences between species or phase factors were detected, a *post hoc* Bonferroni test was applied to identify the sources of the difference (Quinn and Keough, 2002).

3. Results

The stomach contents of 182 individuals were analysed, and regarding the type of plastic found in the stomach of these three species of estuarine fish, blue nylon fragments (Fig. 2a) were found in 23% of the individuals (42 ind.). Hard plastics were also found (Fig. 2b) in some individuals. Stomachs containing plastic debris represent 18% in *C. spixii* (11 ind. : two juveniles, five sub-adults and four adults). In *C. agassizii*, these accounted for 33% (20 ind.).

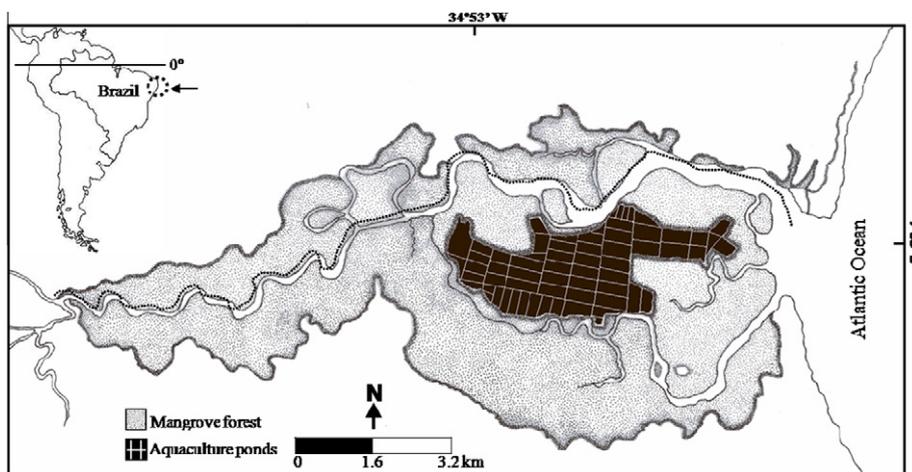


Fig. 1. Goiana Estuary, at the Brazilian Northeast. The samples were taken along the 17 km of main channel (.....).

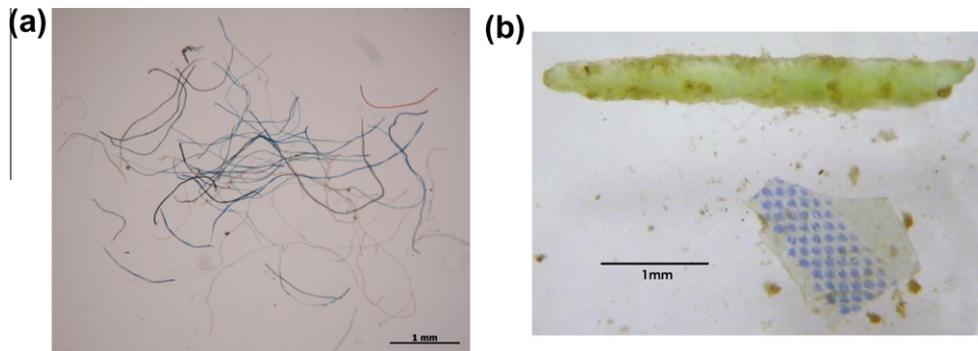


Fig. 2. (a) Example of nylon fibres (Polyamide) ingested by catfish from the Goiana Estuary; (b) hard plastic ingested by *C. agassizii*. Sources: A.R.A. Lima and F.E. Possatto.

three juveniles, eleven sub-adults and six adults) of the stomachs. For *S. herzbergii*, 17% (11 ind.: seven juveniles, two sub-adults and two adults) of the stomachs contained plastic debris.

Some individuals had ingested more than one plastic fragment. The number of fragments ingested by fish of the three species varied from one to ten fragments. The factors species and ontogenetic phases presented a significant interaction [$F_{(4171)} = 5.4096$, $p = 0.0004$] in relation to the number of plastic items ingested (Fig. 3a). This suggests that the ingestion of number plastic debris vary according the ontogenetic phase of each species. However, when the ingestion of plastic debris is analysed for each species independently, it was observed that *C. agassizii* showed significant differences among ontogenetic phases [$F_{(2,57)} = 3.9326$, $p = 0.02512$] in relation to the number of plastic fragments ingested. The highest number of plastic marine debris was ingested by the sub-adult phase (Fig. 3a). *S. herzbergii* showed significant differences among the ontogenetic phases in relation to number [$F_{(2,57)} = 7.6458$, $p = 0.00114$] and weight [$F_{(2,57)} = 3.3572$, $p = 0.04185$], and the juvenile phase ingested higher weights (Fig. 3 a and b).

4. Discussion

The ingestion of plastic marine debris probably happened during the fish's normal feeding activity. These species are epibenthophagous (Barletta and Blaber, 2007) and prey on small animals living on the surface of the sediment (Costa et al., 2004). Additionally, they are estuarine residents (Barletta and Blaber, 2007), which means that they only feed inside the estuary. Their realm extends to the continental platform during the rainy season, when salinity drops even in coastal waters. This is specially known to be so for the tropical estuaries of the Brazilian coast (Barletta et al., 2005,

2008; Dantas et al., 2010). This strongly indicates that plastic marine debris contamination spreads throughout the sediments of the whole system.

Although their preferred region of the estuary changes throughout the year and the different life stages these species spend their entire lifetime along the estuarine ecocline (Barletta et al., 2005, 2008; Dantas et al., 2010), and individuals of all three phases were found to be contaminated. Therefore, all of their life phases are exposed to plastic marine debris and can actually be impacted by it.

All three species feed into the mangrove forest and tidal creeks during high tide. These are the major sedimentation habitats within the estuary, where plastics can deposit together with settling particles (Browne et al., 2010). These habitats are low-energy waters with down current from tidal and river flows which facilitate the precipitation and settling of both organic and clay particles, as well as denser plastic fragments.

The individuals that we studied ranged from three to 30 cm of T_L ; therefore, their mouths are small, probably no bigger than 3 cm. So, the plastics that can be ingested are small items, or most likely fragments. The fragmentation of plastics at sea is a common phenomenon (Barnes et al., 2009) and increases the risk of ingestion and other consequences related to plastics pollution at sea. Plastic fragments of smaller sizes (1–10 and <1 mm) are more abundant than are larger items and fragments (Browne et al., 2010). This is also true for the abundance of plastic fibres (e.g., polyesters and polyamides such as nylon).

All species using the estuary are under the threat of ingesting plastic marine debris. It also includes indirect ingestion when some fish (e.g., *Cynoscion acoupa*, *Centropomus undecimalis*, *Dasyatis guttata*) preying on smaller fish that have been previously contaminated or entangled (Fig. 4). Catfish move up and down the estuary, and have the potential to be contaminated and to contaminate

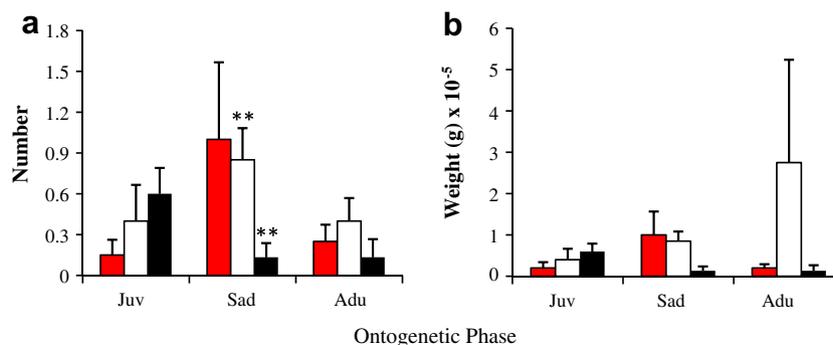


Fig. 3. Mean (+standard error) of the number (a) and weight (b) of plastic debris in *C. spixii* (■), *C. agassizii* (□) and *S. herzbergii* (■) in the three ontogenetic phases, Juv, juveniles; Sad, sub-adults; Adu, adults. Significant differences were identified by *post hoc* Bonferroni test (** $p < 0.01$) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).



Fig. 4. *Cathorops agassizii* captured still alive in the main channel of the Goiana Estuary entangled to a fragment of nylon monofilament fishing net (May 2006). Source: Laboratory of Ecology and management of Coastal and Estuarine Ecosystems (LEGECE: <http://legeceufpe.blogspot.com/>).

their predators along the entire ecocline and beyond it, especially when they are preyed by riverine or marine fish visiting the estuary (Dantas et al., 2010). Catfish are prey to larger fish of commercial importance (Dantas et al., 2010), and may be transmitting their plastic burden to their predators (Erikson and Burton, 2003).

Plastic marine debris is a pervasive type of pollution. However, close examination of the items found in the environment and/or the stomach contents of fish can reveal the most likely source of this pollution. Fishery activities, on a small or large scale, have some important items, such as polystyrene buoys, nylon ropes and fragments of lines or gill nets. These flag items and their fragments are expected to occur in greater abundance than other types due to the relative proportions of their manufacture, use and, in this particular case, discarding/loss (Browne et al., 2010). Therefore, fisheries may present different types of influence on fish populations: capture (overfishing), by catch, entanglement (ghost fishing) and plastics ingestion. In the case of the Goiana Estuary, the incidence of nylon fragments derived from the fragmentation of fishery ropes in the stomach contents of catfish is a clear example of the relationship between the source's proximity and the environmental consequences.

In the case of the Goiana Estuary, fisheries are responsible for a significant part of the marine debris, especially plastic, found on the sandy beaches, in the mangrove forests and in the main channel. The river basin and the riverine communities are the source of another large portion of debris (Ramos et al., 2011). This was confirmed by the results presented here where nylon fragments had predominance over other types of fragments found in the stomachs of catfish. This is a clear example of the development of initial contamination into more serious consequences: cables and fragments left or lost by fishermen will degrade in the estuarine environment and contaminate the biota. The physiological effects of nylon in these fish cannot be easily predicted (Hoss and Settle, 1990; Browne et al., 2010). However, it is well-known that other animals, when carrying plastics in their digestive tracts, suffer of a number of internal injuries, such as tumours and a false sensation of replenishment, which reduces their feeding drive and can kill the animal by starvation. Alternatively, weak/injured animals make easier, and less nutritious, prey.

If the plastics ingestion figures that are reported here are projected on the density of catfish found by Barletta and co-workers (2005, 2008) and Dantas and co-workers (2010), the size of the contamination problem can be quite worrying. The mean total density of catfish is 1600 individuals ha^{-1} (*C. spixii* = 83.6%; *C. agassizii* = 15.6%; *S. herzbergii* = 0.5%), and the biomass is 18.8 kg ha^{-1} (*C. spixii* = 75.4%; *C. agassizii* = 22.4%; *S. herzbergii* = 1.4%), so that 23% of these would be contaminated. Further consequences are still unknown, although, for an abundant species, it is possible that catfish are suffering from plastic marine debris inges-

tion. Although recognised as potential bio-indicators for estuarine contamination by trace metals (Costa et al., 2004), these species, especially *C. spixii*, have also proven to be suitable sentinel organisms for the state of the system with respect to other types of contaminants and environmental changes.

5. Conclusions

The size and depth of plastic marine debris ingestion by fish is actually unknown, but it might be affecting not only single individuals but also whole populations. This phenomenon is probably widespread in the tropical world and only well-designed experiments of stomach content examination can detect and possibly characterise and quantify it. Coastal communities depend upon fisheries at many different levels, but there is an urgent need to control and reduce the loss of plastic marine debris from this source. Control at the source is the only possible abatement for this sort of marine pollution. Special care with fishing gear and the use of more environment-friendly materials, at least at sensitive sites such as mangrove-lined estuaries, can be designed and tested using reliable bio-indicators, such as estuarine resident catfish.

Acknowledgements

The authors thank the financial support of FACEPE (APQ-0586-1.08/06), CNPq (474736/2004 and 482921/2007-2) and CNPq/CT-Hidro (552896/2007-1). FEP acknowledges CNPq for a two-year M. Phil. scholarship (130656/2008-9). MB and MFC are CNPq fellows.

References

- Araújo, F.G., 1988. Distribuição, abundância relativa e movimentos sazonais de Bagres Marinhos, Siluriformes, Ariidae no estuário da Lagoa dos Patos, RS, Brasil. *Revista Brasileira de Zoologia* 5, 509–543.
- Azevedo, M.C.C., Araújo, F.G., Cruz-Filho, A.G., Gomes, I.D., Pessanha, A.L.M., 1999. Variação espacial e temporal de bagres marinhos, Siluriformes, Ariidae na Baía de Sepetiba, Rio de Janeiro. *Revista Brasileira de Zoologia* 59, 443–454.
- Barletta, M., Blaber, S.J.M., 2007. Comparison of fish assemblage and guilds in tropical habitats of the Embely, Indo-West Pacific and Caeté, Western Atlantic estuaries. *Bulletin of Marine Science* 80, 647–680.
- Barletta, M., Costa, M.F., 2009. Living and non-living resources exploitation in tropical semi-arid estuary. *Journal of Coastal Research* SI 56, 371–375.
- Barletta, M., Barletta-Bergan, A., Saint-Paul, U., Hubold, G., 2003. Seasonal changes in density, biomass and diversity of estuarine fishes in tidal mangrove creeks of the lower Caeté Estuary, Northern Brazilian coast, East Amazon. *Marine Ecology Progress Series* 256, 217–228.
- Barletta, M., Barletta-Bergan, A., Saint-Paul, U., Hubold, G., 2005. The role of salinity in structuring the fish assemblages in a tropical estuary. *Journal of Fish Biology* 66, 1–28.
- Barletta, M., Jaureguizar, A.J., Baigun, C., Fontoura, N.F., Agostinho, A.A., Almeida-Val, V.M.F., Val, A.L., Torres, R.A., Jimenes-Segura, L.F., Giarrizzo, T., Fabrè, N.N., Batista, V.S., Lasso, C., Taphron, D.C., Costa, M.F., Chaves, P.T., Vieira, J.P., Correa, M.F., 2010. Fish and aquatic habitat conservation in South America: a

- continental overview with emphasis on Neotropical systems. *Journal of Fish Biology* 76, 2118–2176.
- Barletta, M., Saint-Paul, U., Amaral, C.S., Corrêa, M.F., Guebert, F., Dantas, D.V., Lorenzi, L., 2008. Factors affecting seasonal variations in fish assemblages at an ecocline in a tropical–subtropical mangrove fringed estuary. *Journal of Fish Biology* 73, 1314–1336.
- Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society of London. Series B* 364, 1985–1998.
- Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Marine Pollution Bulletin* 60, 2275–2278.
- Box, G.E.P., Cox, D.R., 1964. An analysis of transformations. *Journal of the Royal Statistical Society–Series B* 26 (2), 211–252.
- Browne, M.A., Galloway, T.S., Thompson, R.C., 2010. Spatial patterns of plastic debris along estuarine shorelines. *Environmental Science and Technology* 44 (9), 3404–3409.
- Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P., Peck, B.B., 1972. Polystyrene spherules in coastal waters. *Science* 178, 749–750.
- Colabuono, F.I., Barquete, V., Domingues, B.S., Montone, R.C., 2009. Plastic ingestion by Procellariiformes in Southern Brazil. *Marine Pollution Bulletin* 58, 93–96.
- Costa, M., Barletta, M., Silva, O., 2004. Fish species as indicators of chemical pollution in a tropical estuary. In: Luis Val, Adalberto, MacKinlay, Don. (Eds.), *Advances in Fish Biology. International Congress on the Biology of Fish, Manaus – Brazil*.
- Dantas, D.V., Barletta, M., Costa, M.F., Barbosa-Cintra, S.C.T., Possatto, F.E., Ramos, J.A.A., Lima, A.R.A., Saint-Paul, U., 2010. Movement patterns of catfishes in a tropical semi-arid estuary. *Journal of Fish Biology* 76, 2540–2557.
- Erikson, C., Burton, H., 2003. Origins and biological accumulation of plastic particles in fur seals from Macquarie Island. *Ambio* 32, 380–384.
- Gregory, M.R., 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 of London. Series B* 364, 2013–2025.
- Guebert-Bartholo, F., Barletta, M., Costa, M.F., Monteiro-Filho, E.L.A., 2011. Using gut contents to assess foraging patterns of juvenile green turtles *Chelonia mydas* in the Paranaguá Estuary, Brazil. *Endangered Species Research* 13, 131–143. doi:10.3354/esr00320.
- Hoss, D.E., Settle, L.R., 1990. Ingestion of plastics by teleost fishes. In: Shomura, R.S., Codfrey, H.L. (Eds.), *Proceedings of the Second International Conference on Marine Debris*, 2–7 April 1989, Honolulu, Hawaii, 1990. US Department of Commerce, NOAA Technical Memorandum NMFS, Washington, DC, USA, pp. 693–709.
- Ivar do Sul, J.A., Costa, M.F., 2007. Marine debris review for Latin America and the Wider Caribbean Region: from the 1970s until now, and where do we go from here? *Marine Pollution Bulletin* 54, 1087–1104.
- Kartar, S., Abou-Seedo, F., Sainsbury, M., 1976. Polystyrene spherules in the Severn estuary – a progress report. *Marine Pollution Bulletin* 7 (3), 52.
- Kubota, T., 1990. Synthetic materials found in stomachs of longnose lancetfish collected from Suruga Bay, Central Japan. In: Shomura, R.S., Codfrey, H.L. (Eds.), *Proceedings of the Second International Conference on Marine Debris*, 2–7 April 1989, Honolulu, Hawaii, 1990. US Department of Commerce, NOAA Technical Memorandum NMFS, Washington, DC, USA, pp. 710–717.
- Laist, D.W., 1997. Impacts of marine debris: entanglement of marine life in marine debris including a comprehensive list of species with entanglement and ingestion records. In: Coe, J.M., Rogers, D.B. (Eds.), *Marine Debris: Sources, Impacts and Solutions*, pp. 99–139.
- Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environmental Research* 108, 131–139.
- Nelson, J.S., 2006. *Fishes of the World*, fourth ed. John Wiley and Sons, Inc., New York. 601pp.
- Quinn, G.R., Keough, M.J., 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- Ramos, J.A.A., Barletta, M., Dantas, D.V., Lima, A.R.A., Costa, M.F., 2011. Influence of moon phase on fish assemblages in estuarine mangrove tidal creeks. *Journal of Fish Biology* 78, 344–354. doi:10.1111/j.1095-8649.2010.02851.x.
- Tourinho, P.S., Ivar do Sul, J., Fillmann, G., 2010. Is marine debris ingestion still a problem for the coastal marine biota of southern Brazil? *Marine Pollution Bulletin* 60, 396–401.