



# Alkenones in oceanic odontocetes as a potential proxy of environmental water temperature

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## ABSTRACT

The alkenones  $C_{37:2}$  and  $C_{37:3}$  are produced exclusively by some haptophyte species. Their relative proportion ( $U^{k}_{37}$  index) may be used to infer the water temperature where the synthesising haptophyte lived. Alkenones have been analysed in sediments, planktonic communities and in the low trophic level, filter-feeding fin whales. However, it is unclear whether they can be detected in animals exploiting high trophic levels and used to infer the water temperature in which they live. Alkenones were analysed in blubber samples from three Mediterranean predatory species: the striped dolphin, an epipelagic species; the Risso's dolphin, a deep diver; and the bottlenose dolphin, a coastal species. Alkenones were detected in all striped dolphin samples and in most of the Risso's dolphin samples, but they were below detection limits in the bottlenose dolphin samples. The inferred temperature for the striped dolphins ( $16.4 \pm 3.3$  °C) was similar to the average water temperature of the region ( $16.9 \pm 3.9$  °C), but that for the Risso's dolphins was lower than expected ( $12.7 \pm 4.4$  °C). The small sample sizes and the large variance in the  $U^{k}_{37}$  index make it difficult to ascertain if the dissimilarity between the two oceanic species is real. Although further research is needed to calibrate this bio-indicator, we can conclude that alkenones are transferred through the trophic web and are found in oceanic cetaceans situated at a high trophic level.

## 1. Introduction

Alkenones are a group of molecules used in palaeoceanography to estimate past sea surface temperatures (Prah1 et al., 1988; Sikes and Sicre, 2002). The two most commonly used alkenones are  $C_{37:2}$  and  $C_{37:3}$ , which consist of chains of 37 carbons with one ketone group in the second carbon and two and three double bonds, respectively. These two molecules are synthesised exclusively by some haptophyte species, with *Emiliana huxleyi* being the major producer in most cases (Marlowe et al., 1984). When haptophytes die, alkenones are deposited on the sea sediment (Volkman et al., 1980a), where they remain relatively unaltered for thousands of years. Paleoceanographers have for long identified alkenones in sediments and used them to infer the temperature of the water at the time of alkenone deposition (Caissie et al., 2010; Knudsen et al., 2012; e.g. Kristjánssdóttir et al., 2017). Estimated temperatures are calculated based on the  $U^{k}_{37}$  index (i.e., the proportion of  $C_{37:2}$  concentration over the sum of the  $C_{37:2}$  and  $C_{37:3}$  concentrations; Prah1 and Wakeham, 1987), which is strongly correlated with sea surface temperature (Prah1 and Wakeham, 1987; Conte et al., 2006).

Despite the extensive use of these molecules, the fate of alkenones

when haptophytes are ingested by animals is unclear. There is evidence of coastal shrimp grazing on haptophytes (Ding and Sun, 2006), and alkenones have been detected in the excrements of anchovies and copepods (Volkman et al., 1980b; Wakeham et al., 1984; Harris, 1994; Grice et al., 1998). However, the occurrence of these molecules in the tissues of vertebrates has only been investigated in one species, the fin whale (*Balaenoptera physalus*) (Rita et al., 2020). Two factors may have contributed to the detection of alkenones in the tissues of this cetacean. First, fin whales feed at a low trophic level; therefore, the low number of trophic steps between molecule synthesizers and whales reduces the chances of alkenone degradation (McCaffrey et al., 1990; Conte et al., 1992). Second, fin whales obtain their food by filtering out small organisms from the water and, in doing so, they may incorporate alkenones directly from the water instead of through ingested krill, their main prey. Conversely, odontocetes are active predators and largely obtain the water they require from the prey consumed; seawater intake in these animals is low and mostly limited to occasional fasting periods (Hui, 1981; Costa, 2009; Ridgway and Venn-Watson, 2010). Thus, it was unclear whether alkenones could be found in odontocete species situated at the top of complex food webs.

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Most odontocetes are long-lived, resident though highly-mobile, top predators. Because of these traits, they integrate the heterogeneity of small-scale geographical variations and seasonal shifts and, consequently, some well-known species have been used as bioindicators of the environmental characteristics of the large water masses in which they live (Borrell and Aguilar, 2007; Bossart, 2011; Borrell et al., 2018). When determined in their tissues, the  $U_{37}^k$  index based on the C37 alkenones may be indicative of long-term, wide-scale temperature shifts in the oceans. However, at the same time, some species are elusive and difficult to study in the wild, and information on their biology and habitat may remain still unclear. For these species, alkenones can again be useful by providing information on the oceanographic characteristics of their habitat, such as the sea surface temperature.

Alkenones can also provide useful information on habitat because different types of alkenones are linked to different groups of haptophytes producers (I, II and III) that live in water masses with distinct salinity ranges (Weiss et al., 2020). Depending of their length (from C35 to C39), configuration and saturations, the detected alkenones can indicate feeding in fresh (group I), marine (group III) or brackish and hypersaline waters (group II) (Theroux et al., 2010). This may be especially useful for those species that inhabit the boundaries of the above-mentioned types of water masses, such as estuaries.

The present study aimed to investigate the presence of alkenones in three top predator odontocetes from the Balearic Sea (north-western Mediterranean) and investigate their potential use as water temperature indicators. The three species studied were the bottlenose dolphin (*Tursiops truncatus*), the striped dolphin (*Stenella coeruleoalba*) and the Risso's dolphin (*Grampus griseus*). In the Mediterranean, the three species live in similar temperature conditions but exploit different ecosystems. The bottlenose dolphin lives near the coast or over the continental slope (Gómez de Segura et al., 2008); it is a generalist species that feeds mainly on demersal fish and cephalopods (Giménez et al., 2018), and its diet widely varies according to area and season (Blanco et al., 2001; Bearzi et al., 2008). The striped dolphin is an oceanic species with adaptive feeding behaviour that in the Balearic Sea preys preferably on sardines (*Sardina pilchardus*) (Gómez de Segura et al., 2008; Gómez-Campos et al., 2011; Cardona et al., 2015). However, when sardines are scarce, striped dolphins can feed on a wide range of fish and cephalopod species from the epi- and mesopelagic zones (Gómez-Campos et al., 2011). Finally, the Risso's dolphin lives over the continental slope (Blanco et al., 2006), even though it may distribute further offshore to zones up to 1500 m deep (Gómez de Segura et al., 2008). The Risso's dolphin is the most specialist of the three species and feeds on squids of the meso- and bathypelagic zones (Blanco et al., 2006; Bearzi et al., 2011).

## 2. Materials and methods

Alkenones were analysed in the blubber tissue of dead stranded dolphins: 10 bottlenose dolphins, 10 striped dolphins and 10 Risso's dolphins. All were collected during the period 1993–2018 and they belong to the population that inhabits the Balearic Sea, this is, the mass of waters located between the eastern coast of the Iberian Peninsula and the Balearic archipelago. During necropsy, full-thickness blubber samples were excised from the dorsal region and were kept frozen at  $-20\text{ }^{\circ}\text{C}$  until analysis. Because alkenone concentrations are known to differ among different layers of whale blubber (Rita et al., 2020), in the current study the entire section of the blubber – from the epidermis to the muscle – was collected. Approximately 3 g of sample was freeze-dried for 42 h and, at the moment of the analysis, cut into small pieces and homogenized.

The extraction of alkenones from the blubber homogenate was carried out using established techniques (Rita et al., 2020). Briefly, 50  $\mu\text{l}$  of internal standard (2-pentatriacontanone; 20  $\text{ng}\cdot\mu\text{l}^{-1}$  in n-hexane; commercially available) was added to 1 g of dry weight samples. Then, samples were saponified in methanolic KOH solution (4 ml  $\text{H}_2\text{O}:\text{MeOH}$ ,

1:9; 3 M KOH) for 60 min at  $80\text{ }^{\circ}\text{C}$ . The nonsaponifiable lipids were extracted three times using n-hexane (4 ml) each time. The n-hexane (from the three extractions) was combined with water KOH solution (12 ml  $\text{H}_2\text{O}$ ; 3 M KOH), the mix was vortexed and centrifuged, and the n-hexane phase was separated. Afterwards, clean n-hexane (12 ml) was added to the KOH/ $\text{H}_2\text{O}$  vials to remove any remaining nonsaponifiable lipids and separated. The two n-hexane extractions were mixed and passed through  $\text{Na}_2\text{SO}_4$  for the elimination of possible water remains. The n-hexane was evaporated under an  $\text{N}_2$  stream down to 1–2 ml. This volume was further purified using solid-phase extraction (Supelclean LC-NH2 SPE tubes; 3 ml). Two fractions of increasing polarity, i.e., hydrocarbons and ketones, were obtained by elution with n-hexane (4 ml) and n-hexane:DCM 3:1 (v/v; 6 ml), respectively. The first fraction was discarded, and the second was dried under an  $\text{N}_2$  stream and dissolved in 50  $\mu\text{l}$  n-hexane before gas chromatography (GC).

Chromatographic analysis was carried out on a Shimadzu GCMS-QP2010 equipped with a 30 m Sapiens-X5MS silica capillary column (0.25 mm ID, 0.25  $\mu\text{m}$  film thickness) and a mass spectrometer (MS) detector. Helium was the carrier gas with a flow rate of 1 ml/min. The GC temperature program was as follows: injection at  $60\text{ }^{\circ}\text{C}$ ; 1 min isothermal;  $60\text{ }^{\circ}\text{C}$  to  $310\text{ }^{\circ}\text{C}$  at  $40\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ ; and 28 min isothermal with a total run-time of 36 min. Peak identification of  $\text{C}_{37}$  alkenones was based on retention time and the comparison of the ion spectrum with those of pure alkenone standards. The concentrations of both alkenones were quantified using the area of the ion with  $m/z$  81 (Fig. 1).

$U_{37}^k$  was calculated as:

$$U_{37}^k = \frac{[\text{C}_{37:2}]}{[\text{C}_{37:2}] + [\text{C}_{37:3}]}$$

where  $[\text{C}_{37:2}]$  and  $[\text{C}_{37:3}]$  are the concentrations of each alkenone in the sample (Prah and Wakeham, 1987).  $U_{37}^k$  was later transformed to temperature (T) using the Conte et al. (2006) equation for the Atlantic region:

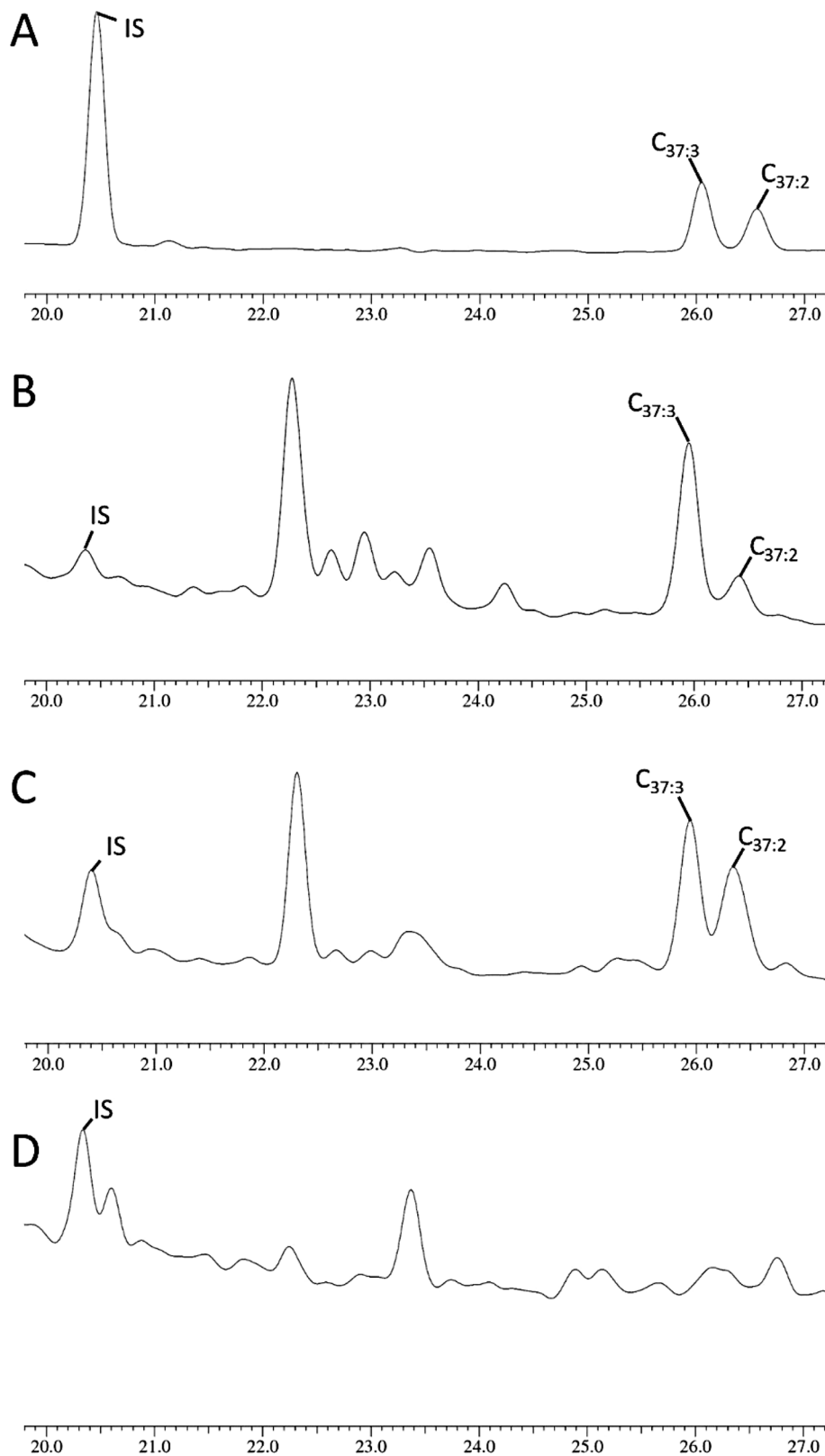
$$T = 48.673(U_{37}^k)^3 - 94.569(U_{37}^k)^2 + 80.716(U_{37}^k) - 5.977$$

Normality and homoscedasticity were checked with the Shapiro test and the Bartlett-test, respectively. Because the alkenone concentrations were not normal, they were log-transformed. T-student tests were used to establish the statistical significance of the difference between the two species in which alkenones were detected. The statistical analysis was performed with the program R (R Core Team., 2020).

## 3. Results and discussion

The habitat preferences of the odontocete species appeared to affect the presence of alkenones in their tissues. While alkenones were detected in the oceanic odontocetes (in 8 out of the 10 Risso's dolphin samples and in all the striped dolphin samples), they were below the detection limit in the bottlenose dolphin samples (Fig. 2). The concentrations of the alkenones were similar in the two oceanic dolphins (t-value =  $-0.036$ , d.f. = 17, p-value = 0.971) but more variable in the Risso's dolphin (mean  $\pm$  SD:  $581.7 \pm 683.2\text{ ng}\cdot\text{g}^{-1}$ ) than in the striped dolphins ( $406.5 \pm 283.2\text{ ng}\cdot\text{g}^{-1}\text{dw}$ ) (Fig. 2). C35, C36, C38 and C39 alkenones were not detected in these species, possibly due to the overall low concentration of alkenones and that all the samples came from the marine environment, where C37 alkenones are predominant (Prah and Wakeham, 1987). For further details on the results, see the Supplementary Table S1 online.

The difference between the bottlenose dolphin and the other two dolphin species may be explained by the fact that bottlenose dolphins live much closer to the coast than the other two species. While some coastal haptophytes can produce alkenones (Rontani et al., 2004), the major current alkenone producer, i.e., *Emiliana huxleyi*, is, mainly, an oceanic species (Ausín et al., 2018). The low abundance of coastal haptophytes (Baumann et al., 2005) likely produced low concentrations

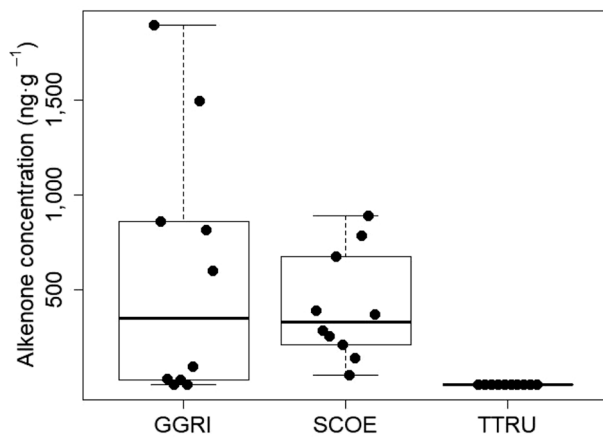


**Fig. 1.** Partial  $m/z$  81 chromatograms of pure alkenone standard (A), Risso's dolphin (B), striped dolphin (C) and bottlenose dolphin (D). The peaks corresponding to the internal standard (IS; 2-pentatriacontanone) and the two alkenones ( $C_{37:3}$  and  $C_{37:2}$ ) are also indicated.

of alkenones in the environment that could not be detected in the tissues of predators. Thus, the lack of alkenones in bottlenose dolphins may be reflecting the exploitation of a trophic web with scarce alkenone-producing haptophytes in its base. If true, alkenones could be used to

differentiate species exploiting oceanic ecosystems, which are more likely to contain abundant alkenone-producing haptophytes, from the coastal ones.

Unexpectedly, the concentrations of alkenones in the striped dolphin



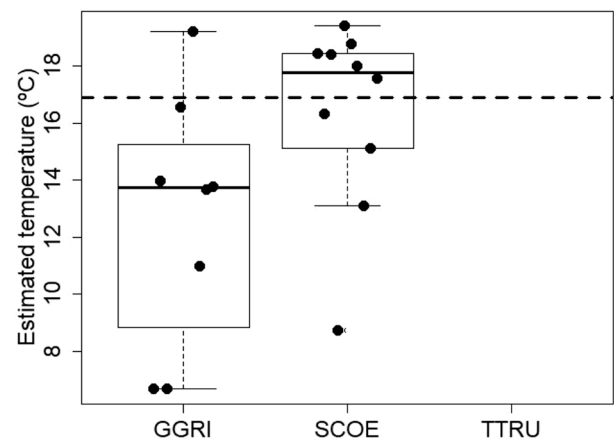
**Fig. 2.** Total alkenone concentrations in odontocete blubber samples. GGRI stands for *Grampus griseus* (Risso's dolphin), SCOE for *Stenella coeruleoalba* (striped dolphin), and TTRU for *Tursiops truncatus* (bottlenose dolphin).

and the Risso's dolphin were higher than those detected in the fin whale (Rita et al., 2020). The concentrations of alkenones in the fin whale were higher in the stomach content than in the blubber, which suggested that either the alkenones had been partially degraded (McCaffrey et al., 1990; Conte et al., 1992) or that they had not been completely absorbed by the whale's intestinal tract. In high trophic level species, alkenones have to go through many trophic steps, and the time from the synthesis of alkenones to their accumulation in the tissue is longer than in species situated at a low trophic level, thus increasing the chances of alkenone degradation. The unexpected finding of a higher concentration of alkenones in top predators as compared to those in low trophic level species may be explained by at least two reasons: the production of alkenones may be higher in the Mediterranean region where these odontocetes had fed than in the feeding grounds used by the fin whales, or there may have been alkenone biomagnification from prey to dolphin, contrary to what appeared to happen in fin whales, where biodilution was apparently observed (Rita et al., 2020).

It is also worth noting that the variability of alkenone concentrations was higher in the Risso's dolphin than in the striped dolphins. The reason for this difference is unknown and may be caused by dissimilarities in the habitat or in the diet of the two species. Further research is needed to elucidate the dynamics of alkenones in trophic webs to better understand how alkenones are transported from the producers to the final consumers and, thus, to correctly interpret the results of future studies.

The  $U^{k}_{37}$  index was slightly higher in the striped dolphin ( $0.5 \pm 0.12$ ) than in the Risso's dolphin (mean  $\pm$  SD:  $0.37 \pm 0.14$ ) although the differences were not statistically significant (t-value =  $-2.002$ , d.f. =  $13.8$ , p-value =  $0.065$ ). These index values correspond to temperatures of  $16.4 \pm 3.3$  °C for the striped dolphins and  $12.7 \pm 4.4$  °C for Risso's dolphin (Fig. 3). While the first temperature is consistent with the average sea surface temperature of the Balearic Sea ( $16.9 \pm 3.8$  °C) (Idescat, 2018), the estimated temperature for the Risso's dolphins was lower than expected.

The difference between the estimated temperature in the two dolphin species should be considered carefully. The proximity of the aforementioned p-value ( $0.065$ ) to the threshold value ( $0.05$ ), combined with the small sample size and the large data variability, reduced the confidence on the statistic. Clearly, given the wide variance observed, more samples are required to correctly assign temperatures to species and, in this case, to establish whether the Risso's dolphins estimated temperature is indeed lower than the environmental average or the dissimilarity is simply caused by limitations in sample size. However, we can envision three reasons that may explain the bias. First, although alkenones are typically produced in the first meters of water (Ausín



**Fig. 3.** Estimated temperatures calculated through  $U^{k}_{37}$  in odontocete blubber samples. The dashed horizontal line represents the annual average sea surface temperature in the Balearic Sea. GGRI stands for *Grampus griseus* (Risso's dolphin), SCOE for *Stenella coeruleoalba* (striped dolphin), and TTRU for *Tursiops truncatus* (bottlenose dolphin).

et al., 2018) and transported vertically (Harris, 1994), they can also be produced at or below the thermocline (Prahel et al., 1993; Herbert et al., 1998; Wolhowe et al., 2014) or produced in a colder water mass and transported horizontally (Benthien and Müller, 2000; Häggi et al., 2015); second, the  $U^{k}_{37}$  index may be biased in top predators due to an heterogeneous transfer of alkenones through the trophic web; and third, the Risso's dolphin may physiologically handle alkenones of different molecular structures in different ways and preferentially store in its blubber one group over the other.

Another factor that should be taken into consideration is the residence time of the alkenones in the dolphin's blubber. The information provided by the alkenones may change drastically depending on the scale of the residence time. If alkenones are stored in the dolphin blubber only for a certain lapse of time, the  $U^{k}_{37}$  index will likely present some variation that will be seasonal or interannual depending on the length of the lapse. If, on the contrary, alkenones are stored permanently, the  $U^{k}_{37}$  index will provide a signal that would average the entire life of the individual. Unfortunately, the age of the individuals studied was unknown and most of them were of adult size, and it was, therefore, impossible to test whether alkenones bioaccumulate or not.

In conclusion, alkenones are transferred through the trophic web and can be detected in the blubber of oceanic, top predator dolphins. This opens the possibility of using these molecules as habitat indicators since their presence seems to be related to the oceanic habitat. In this preliminary study, the alkenones seem to be able to reflect the environmental water temperature, especially in the case of the striped dolphins, although the low sample size prevents reaching firm conclusions. Further research should focus on analysing the intermediate trophic levels to better understand how alkenones are transferred through the trophic web.

#### 4. Data availability statement

The data underlying this article are available in the article and its online [supplementary material](#).

#### CRediT authorship contribution statement

**Diego Rita:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Asunción Borrell:** Funding acquisition, Supervision, Resources, Writing - review & editing. **Alex Aguilar:** Funding acquisition, Project administration, Resources, Writing - review & editing.

## Declaration of Competing Interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2020.107240>.

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