Antarctic sea-ice low resonates in the ecophysiology of humpback whales

Susan M. Bengtson Nash [a,*], Jasmin Groβ [a], Juliana Castrillon [a], Maria Valeria Casa [a], Greta Dalle Luche [a], Justin Meager [b], Ruma Ghosh [a], June Eggebo [a], Pernilla Bohlin Nizzetto [c]

[a] Griffith University, Centre for Planetary Health and Food Security, Southern Ocean Persistent Organic Pollutants Program, Kessels Road, Nathan, QLD 4111, Australia
[b] Queensland Department of Environment and Science, 41 Boggo Road, Dutton Park, QLD 4102, Australia
[c] NILU - Norwegian Institute for Air Research, Kjeller 2007, Norway

HIGHLIGHTS

- Southern hemisphere humpback whales are sentinels of the Antarctic sea-ice ecosystem.
- 2017 was marked by multiple anomalous climatic events around the Antarctic region.
- Whale sentinel parameters were interrogated to gauge physiological response to ecosystem change.
- Five of six measures showed a negative or anomalous trend in 2017.
- One dietary tracer showed a lag response, likely indicative of adaptations at lower trophic levels.

GRAPHICAL ABSTRACT
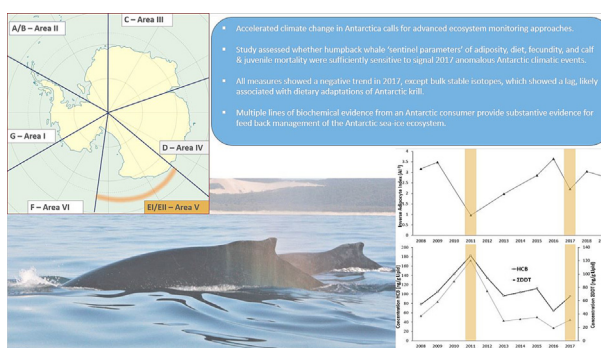
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ABSTRACT

The past six years have been marked by some of the most dramatic climatic events observed in the Antarctic region in recent history, commencing with the 2017 sea-ice extreme low. The Humpback Whale Sentinel Programme is a circum-polar biomonitoring program for long term surveillance of the Antarctic sea-ice ecosystem. It has previously signalled the extreme La Niña event of 2010/11, and it was therefore of interest to assess the capacity of existing biomonitoring measures under the program to detect the impacts of 2017 anomalous climatic events. Six ecophysiological markers of population adiposity, diet, and fecundity were targeted, as well as calf and juvenile mortality via stranding records. All indicators, with the exception of bulk stable isotope dietary tracers, indicated a negative trend in 2017, whilst C and N bulk stable isotopes appeared to indicate a lag phase resulting from the anomalous year. The collation of multiple biochemical, chemical, and observational lines of evidence via a single biomonitoring platform provides comprehensive information for evidence-led policy in the Antarctic and Southern Ocean region.

1. Introduction

The polar regions of the Earth are warming faster than anywhere else on the planet (IPCC, 2021). Antarctica is a continent covered by ice, holding 70 % of the world's freshwater reservoir. Melting of the Antarctic cryosphere is therefore expected to carry massive local and global ramifications (Bronselaer et al., 2018), and capturing the manifestation of climate change in this remote region is of critical importance. Quantifying the impacts of climate change in the Antarctic and Southern Ocean region requires sustained and standardised monitoring through time, and across a large spatial scale (Newman et al., 2019). The challenging conditions of work in the region render such observations expensive and difficult to obtain, and thus susceptible to missed observations as a result of: environmental factors; national political or scientific funding climates, and more recently, the global COVID-19 pandemic. Missed observations in a timeline dramatically reduce the power of the dataset to detect change (Bignert et al., 1993). These factors have led to widespread efforts to develop remote

observation systems and technologies for the Antarctic region, such as satellite imagery and ocean gliders (LaRue et al., 2022; Friedrichs et al., 2022). These approaches facilitate the collection of large volumes of biophysical data, which can enhance our understanding of ecosystem function and therefore our ability to detect change.

Capturing ecosystems' response to climate change is one of the most challenging, but most pressing needs for predicting the impact of accelerated climate change (Sukhotin and Berger, 2013). The Antarctic ecosystem is unique in that almost all Antarctic predators rely directly or indirectly on Antarctic krill (*Euphausia superba;* hereafter 'krill'). Krill are a sympagic species, with their life history inextricably linked to sea-ice and sea-ice dynamics. Krill eggs first descend before hatching at depth (Ikeda, 1984). The larvae must then ascend to the underside of the sea-ice by the time Calyptosis I, the first feeding stage of the krill life cycle (Ikeda, 1984), emerges to ensure access to food, the microbes associated with ice algae (Nicol, 2006). Throughout ontogeny, the sea-ice forms a refuge for the immature krill (Meyer et al., 2017) and the location of sea-ice at break-up appears to have significant importance for krill cohort survival, both in terms of food availability and inter age cohort competition (Ryabov et al., 2017).

The sympagic life history of krill leaves the species, and therefore dependent consumers, particularly vulnerable in a warming climate with ecosystem cascades predicted (Smetacek and Nicol, 2005). The keystone role of krill in the Antarctic sea-ice ecosystem underscores the need to understand temporal and spatial population dynamics of krill around the Antarctic region. This need has long been recognized by the Commission for Antarctic Marine Living Resources (CCAMLR), which takes an ecosystem approach to monitoring of krill, focusing on the success of dependent Antarctic consumers to obtain an integrated signal of krill productivity. To date the CCAMLR Ecosystem Monitoring Program uses penguins, seals, and flying seabirds as sentinels of the Antarctic sea-ice ecosystem (CCAMLR Ecosystem Monitoring Program Standard Methods, 2004). Measures of e.g. abundance, fledgling weight and foraging duration, however, necessitate in situ Antarctic observations, which are associated with high costs in terms of logistics and personnel hours, leaving sustained measurements vulnerable to societal stochasticity.

More recently, southern hemisphere humpback whale (*Megaptera novaeangliae*) adipose stores, diet, and fecundity, were shown to oscillate closely with environmental conditions in their Antarctic feeding grounds (Bengtson Nash et al., 2018; Druskat et al., 2019). Southern hemisphere humpback whales are high fidelity krill feeders (Groß et al., 2020; Waugh et al., 2012). They feed intensively in the Southern Ocean between the months of November and March, before undertaking extensive migrations to equatorial breeding grounds (Dawbin, 1966). Migrations are associated with voluntary fasting, with individuals incurring a massive energetic cost, particularly reproductive females that simultaneously carry the cost of pregnancy and nursing (Castrillon and Bengtson, 2020). Identification of key ecophysiological sentinel parameters in southern hemisphere humpback whales, and the development of novel chemical and biochemical tracers for their quantification, paved the way for implementation of the Humpback Whale Sentinel Programme (HWSP), a circum-polar biomonitoring program for long-term surveillance of climate change and chemical pollution in the Antarctic sea-ice ecosystem. The longitudinal monitoring record of the east coast of Australia migrating humpback whale breeding stock (E1 as designated by the International Whaling Commission (Bravington et al., 2007)), spans 15 years. Within this record, anomalously low adipose stores, female participation in the annual migration, and elevated lipophilic contaminant burdens were observed in 2011, corresponding with the extreme La Niña event of 2010/11 (Bengtson Nash et al., 2018; Druskat et al., 2019).

In 2017, the world observed some of the most dramatic changes recorded in Antarctica in modern history. July 2017 saw 10 % of the Larsen C ice-shelf cleave off as a massive iceberg. The year continued with the appearance of a 300,000 m^2 polynya in the winter sea-ice off east Antarctica, and summer sea-ice coverage was the lowest on record at the time (since exceeded in 2022) (Thompson, 2002); 27 % below the mean annual minimum (Turner and Comiso, 2017). As humpback whale foraging has been

shown to be associated with the marginal ice zone, the timing of sea-ice break-up (Andrews-Goff et al., 2018), and sea-ice concentration (Bengtson Nash et al., 2018), it was of interest to investigate whether an anomalous Antarctic sea-ice year would be a predictor of skewed sentinel parameters under the HWSP.

In order to investigate the capacity of existing biomonitoring measures under the program to detect the impacts of 2017 anomalous climatic events., this study targeted the established sentinel parameters of: i) adiposity, as measured by the Inverse Adipocyte Index (AI^{-1}) (Castrillon et al., 2017), an indicator of available blubber lipid stores, as well as outer blubber lipophilic, legacy persistent organic pollutants (POPs) burdens (Bengtson Nash et al., 2018; Bengtson, 2018), a tracer of whole-of-body lipid dynamics. ii) diet, was examined through blubber C and N bulk stable isotopes analysis (Bengtson Nash et al., 2018; Eggebo et al., 2023), and blubber fatty acid profiles (Groß et al., 2020; Waugh et al., 2012), whilst; iii) fecundity was gauged through the proxy measure of the migratory cohort sex ratio (Druskat et al., 2019). Finally, for this study we retrieved the humpback whale stranding records for the southeast Queensland region, some 1500 km south of the breeding grounds of this population, to compare sentinel parameter trends with calf and juvenile mortality, known to be linked closely with maternal provisioning (Christiansen et al., 2021; Coughran et al., 2013; Seyboth et al., 2015).

2. Methods

2.1. Study design

This investigation includes both previously published (2008–2013) (Bengtson Nash et al., 2018) sections of a long-term biomonitoring record, as well as new analyses performed to extend the record up to and including 2017. Data are presented as part of a single continuous record, whilst the full details of new analyses performed are provided in the supplementary information.

2.2. Biopsy sampling and study area

Blubber and skin biopsies were obtained as per Waugh et al. (2011). In brief, animals were sampled on their annual migration as they passed North Stradbroke Island, south-east Queensland, Australia (27°26S, 153°34E; Fig. 1). Samples were collected annually at one or two seasonal time points corresponding to: (i) the northward migration, i.e. individuals travelling from Antarctica to the breeding ground, sampled during the last two weeks of June/first two weeks of July, or (ii) the southward migration, i.e. individuals returning from the breeding ground sampled during the last two weeks of September/first two weeks of October. Only tissues from southward migrating cohorts were considered for adiposity and fecundity measures to standardise fasting and migration timepoint. By contrast, no differences were observed in dietary measures between timepoints, hence northward and southward cohorts were combined. Similarly, whilst only male animals were used for adiposity measures to control for variation introduced by female reproductive status, no sex differences were observed between dietary measures and so all animals sampled within a single year were combined. All sampling was conducted under relevant state and marine parks permits, and the approval of The University of Queensland and Griffith University Animal Ethics Committees.

2.3. Tissue analysis

2.3.1. Adiposity measures (AI^{-1} and lipophilic POPs)

2.3.1.1. The Inverse Adipocyte Index (AI^{-1}). The AI methodology is based on the principal that very early on in mammalian development, the number of adipocytes that an individual develops is set (Hopkin, 2008). Any fluctuations in energy stores can therefore be attributed to an increase or decrease in the volume of individual adipocytes, rather than adipocyte number. The AI^{-1} is quantified as the blubber adipocyte area relative to the
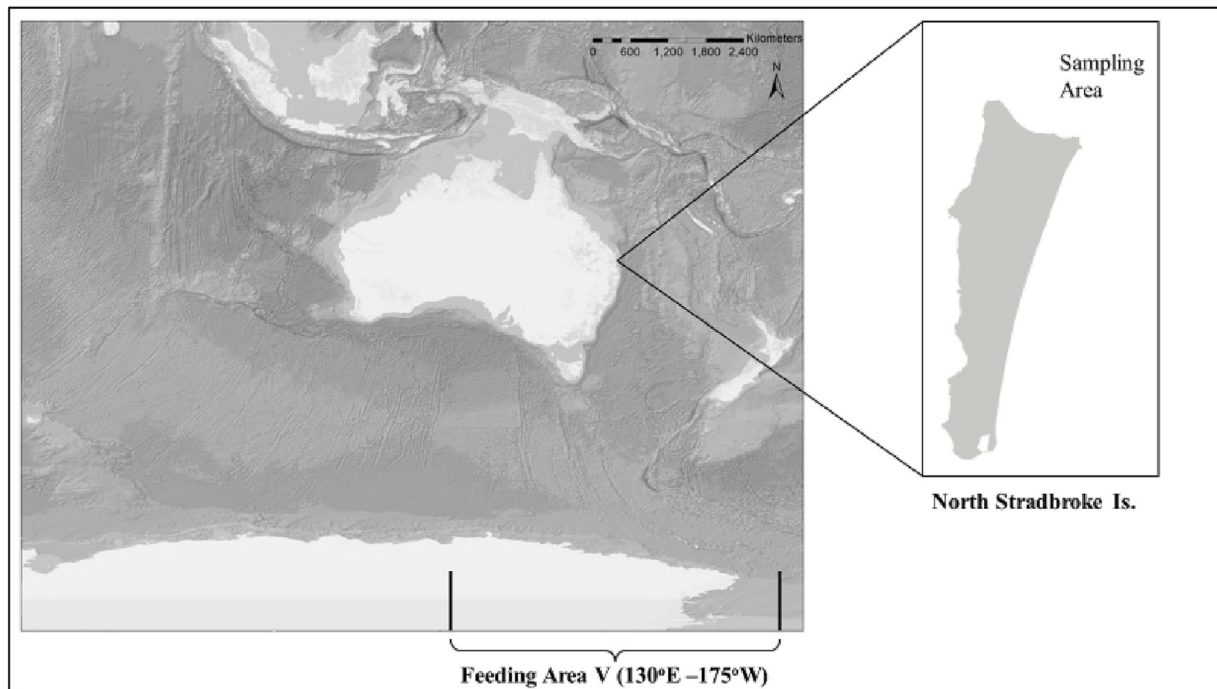
Fig. 1. Map depicting the Antarctic feeding grounds of the E1 humpback whale population (Area V), together with the eastern Australian sampling point of this study.

intervacuolar space (denominator), in such a way that a higher index indicates greater adipocyte area and therefore greater energetic reserves.

The AI methodology has previously been described In detail by Castrillon et al. (2017) and Druskat et al. (2019). In brief, histological slides are prepared with a Haematoxylin-Eosin stain, followed by microscope imaging, and image analysis using ImageJ software (1.53e).

2.3.1.2. Persistent organic pollutant analysis. Humpback whale outer blubber lipophilic POP concentrations have been shown to increase dramatically with diminishing energetic reserves (Bengtson Nash et al., 2013). As lipid stores are converted to energy during migration and fasting, the lipid-associated POP fractions are mobilised and recirculated to other body compartments (Cropp et al., 2014; Chevrier et al., 2000). The outer blubber layer represents a maintenance lipid (Klanjscek et al., 2007), the loss of which would jeopardize other physiological functions such as buoyancy and thermoregulation (Koopman et al., 2002). This layer is therefore more resistant to depletion and starts to take on an increasing POP fraction with loss of storage lipids, such as those in deeper blubber layers and visceral fat stores (Lockyer et al., 1985). The exposure of Southern Ocean foraging humpback whales to legacy POPs is expected to occur primarily from remote and diffuse sources, rather than point source pollution. Sudden fluctuations in outer blubber POP stores therefore primarily reflect individual lipid dynamics, rather than an increase in exposure.

The HWSP uses data associated with the two predominant legacy POP compounds known to accumulate in southern hemisphere humpback whales, namely hexachlorobenzene (HCB) and Dichlorodiphenyltrichloroethane (DDT) (Bengtson Nash et al., 2013), as tracers of lipid dynamics. Blubber extracts were analysed for HCB and six DDT congeners; o,p'- and p,p'- DDT, DDD, and DDE. The blubber extraction, clean-up, analysis and quality assurance procedures have been published elsewhere (Bengtson Nash et al., 2008). In brief, 0.3 g of blubber was solvent extracted on a cold column. The extract underwent further clean-up by gel permeation chromatography, carbon or alumina, and silica columns, before injection on high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) using an Agilent 6890 N gas chromatograph coupled to a Waters AutoSpec mass spectrometer (Micromass Waters, Manchester UK). ^{13}C-labelled isomers were used as internal standard for each group. Additionally, the recovery rates of the added internal standard compounds were determined. The average recovery of HCB and DDT congeners was 35 % and 53 % respectively, whilst the method achieved and average Level of Detection for HCB and DDT of 4 ng/g and 0.4 ng/g respectively. Reported results were blank corrected with the average laboratory blank value for the series. All HCB and DDT results are reported on a lipid weight basis where lipid fraction was determined on 30 mg of each blubber sample. Individual samples were extracted using a modified Bligh and Dyer methanol–chloroform–water extraction method as described in detail elsewhere (Groß et al., 2020; Bligh and Dyer, 1959; Waugh et al., 2014). Lipid class profiles were determined by an Iatroscan Mark V TH10 thin-layer chromatograph coupled with a flame ionization detector (Volkman and Nichols, 1991). The total blubber per cent lipid was calculated by summing the individual lipid class percentages.

2.3.2. Dietary measures (bulk stable isotopes and fatty acids)

2.3.2.1. Bulk stable isotope (C and N) analysis. Bulk stable isotope (BSI) analysis is used in ecology to trace elemental cycling in the biosphere (Fry, 2006). Carbon and nitrogen stable isotopes can provide valuable clues regarding food web connectivity, with N isotopes (δ^{15}N) increasing in a predictable manner along food chains (Fry, 2006). Carbon isotopes (δ^{13}C) are used to identify the primary C source, corresponding to the geographical origin of a food web. This is particularly useful in polar migratory biota as higher latitude environments typically have lower δ^{13}C values than those in temperate or tropical latitudes.

In BSI analysis, lipids confound analyses by decreasing the tissue ^{13}C/^{12}C, lowering the measured δ^{13}C values (DeNiro and Epstein, 1978). This can impact dietary interpretation (Carl and Ragnar, 2000). Thus, tissues with high lipid content, such as cetacean blubber, are commonly lipid normalised using a chemical lipid-extraction method or a mathematical lipid standardisation model (Ryan et al., 2012; Groß et al., 2021).

Bulk stable isotope analysis methods are published elsewhere (Groß et al., 2021; Eisenmann et al., 2016). In brief, lipids were chemically removed from blubber tissue prior to BSI using an overnight, modified Bligh and Dyer (1959) methanol-dichloromethane-water (MeOH/CH$_2$Cl$_2$/ H$_2$O) method (2:1:0.8 v/v/v). Extracted blubber tissue was oven-dried overnight at 58 °C and 1–2 mg of sample material was used for isotope

analysis. All stable isotope abundances were calculated in ‰ using the following formula:

$$\delta X = \left[\left(R_{sample} / R_{standard} \right) - 1 \right] \times 1000 \qquad (1)$$

where X = ^{13}C or ^{15}N, and R = the respective ratio ^{13}C/^{12}C or ^{15}N/^{14}N. The international reference standards for C and N are, respectively, Vienna Pee Dee Belemnite and N$_2$ in air. International standards IAEA-CH6 for C and IAEA N1 for N were used for calibration of laboratory standards KHP and (NH$_4$)$_2$SO$_4$ for sample runs. The preparation system was a Europa EA-GSL interfaced to a SERCON Hydra 20-20 isotope ratio mass-spectrometer (IRMS). Based on analysis of replicate standards, the standard deviation for δ^{13}C and δ^{15}N respectively averaged 0.1 ‰ and 0.15 ‰. A theoretical two dimensional (C and N axes) 'krill space', i.e. the C and N space expected of a consumer feeding solely on a primary consumer of the Antarctic ecosystem, for both tissues was determined using collated literature values as described in Eisenmann et al. (2016; 47).

2.3.2.2. Fatty acid profiles. Fatty acids are a class of lipids that play an important role in physiological function and make up key constituents of plant and animal cells (de Carvalho and Caramujo, 2018). Some fatty acids are exclusively produced by primary producers and are transferred unchanged from one trophic level to another. These can be used as signature fatty acids and have been used in ecology to understand food web connection and predator-prey relationships (Jardine et al., 1804).

Fatty acid analysis has previously been described (Groß et al., 2020; Waugh et al., 2012), and the HWSP fatty acid temporal record for this period is published in Groß et al. (2020). The total lipid extract was obtained from blubber tissue through the lipid extraction procedure described above for BSI. The solvent phase, containing the lipid fraction, was evaporated to dryness via rotary evaporation. An aliquot of the total lipid extract was *trans*-methylated through the addition of MeOH/HCl/CH$_2$Cl$_2$ (3 mL 10:1:1 v/v/v) and heated, yielding fatty acid methyl esters. A C$_{21}$ methylated fatty acid internal injection standard was added to the final aliquot before analysing fatty acid methyl ester extracts by gas chromatography and flame ionization detection (GC-FID) using a gas chromatograph (Agilent Technologies 7890A) equipped with a Supelco Equity™-1 fused silica capillary column (15 m × 0.1 mm internal diameter, 0.1 μm film thickness (Alhazzaa et al., 2011). Individual fatty acids are expressed as percent of the total fatty acid area. Fatty acids that were present in trace amounts (<0.5 %) were excluded from results.

2.3.3. Fecundity measure (sex ratio)

2.3.3.1. Sex determination. The sex of individuals sampled through random encounters on the main E1 migratory corridor, on the southward migration each year, was used to calculate the sex ratio of the annual migratory cohort (Druskat et al., 2019). In the sex-ratio timeline, 2008 was excluded as field sampling actively avoided females with an accompanying calf, and therefore did not satisfy the requirement for random encounters. Use of the sex ratio of the annual migratory cohort as an indirect measure of fecundity is based on the evidenced link between the energetic reserves of female capital breeding mysticetes, and their reproductive output (Castrillon and Bengtson, 2020; Christiansen et al., 2014; Christiansen et al., 2018), and the assumption that of the mature, non-pregnant, female population, only those females that have acquired sufficient energy reserves will undertake a full migration and thereby commit to a new reproductive event (Druskat et al., 2019).

Genetic sexing of individuals has previously been described in (Druskat et al., 2019). Briefly, genomic DNA (gDNA) was extracted using a GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, Sydney, Australia) according to manufacturer's protocols. Approximately 80 ng of DNA was used per reaction for amplification with PCR (BioRad C1000™ Thermal Cycler). Each reaction included 1 μL of oligonucleotides sequences specific to the ZFY/ZFX and SRY DNA regions (10 mM) as previously published (Palsbøll et al., 1992). The amplified product was cut with

Taq1 restriction enzyme (ThermoFisher, Australia) at 60 °C for 1 h. Bands specific to ZFY (two bands, 621 and 439 bp long) and ZFX (one band 439 bp long) and SRY (170 bp) were compared with phiX174 *Hae*III digested lambda DNA marker on 2 % Agarose gel (Bio Rad Laboratories, Inc.) stained with 1 μL RedSafe (Nucleic Acid Staining Solution from Thermo fisher Scientific). The completed gel was viewed using a BioRad Molecular Imager® Gel Doc™ XR + with Image Lab™ Software.

2.3.4. Mortality (calf and juvenile stranding records)

2.3.4.1. Stranding records. Stranding records were obtained from the StrandNet Database, a marine wildlife stranding database maintained by the Queensland Government Department of Environment and Science. Only fatal strandings were included in the analysis, and age classes were based on body lengths where available (calves <8 m; immature: 8 to 10 m, and adults >11 m). Mortalities from known human causes (e.g. entanglement and vessel strikes) were excluded.

Maternal provisioning carries significant life history consequences for long-lived mammals (Villegas-Amtmann et al., 2015). Failure of a capital breeding female mysticetes to acquire sufficient energetic reserves during seasonal hyperphagia, may result in sub-optimal provisioning, calf mortality, premature birth, or aborted pregnancy (Holyoake et al., 2012). A humpback whale calf that has received sub-optimal maternal provisioning in turn will enter its first migration and fasting season with a lower energetic capital. This reduced capital in young animals is especially problematic, as a larger proportion of the energetic budget is dedicated to growth as opposed to maintenance (Christiansen et al., 2018).

Humpback whale stranding events near the WA and QLD breeding grounds are over-represented by calves and juveniles (Coughran et al., 2013). Among past necropsy efforts performed in response to unusual mortality events, evidence pointed to hypoplasia of neonate adipocytes, indicative of sub-optimal maternal provisioning (Holyoake et al., 2012). Stranding network data are therefore expected to encompass a number of population impacts, including: natural calf loss; infectious disease; ship strike; toxicity, as well as chronic energetic deficiencies in the population, or an abrupt change in food availability, or cumulative stress arising from any combination of the prior.

2.4. Statistics

Population adiposity measures are based on the mean metric obtained from adults sampled on the southward migration during the same four-week window of each year. Individual AI^{-1} and POP measures are presented in Tables S1 and S2.

Interannual differences between the means of the adiposity measures of AI^{-1} and blubber POP burdens were analysed by a one-way ANOVA with post-hoc Tukey HSD analysis, with the assumption of a normal distribution, although datapoints in the timeline were too few to verify this assumption.

A canonical analysis of principal coordinates (CAP) was performed on fatty acid profiles in PRIMER v7 with PERMANOVA+ add-on (https//www.primer-e.com) on a Euclidean distance matrix of square-root transformed fatty acid percentages with the factor "Year".

3. Results

3.1. Adiposity

The Inverse Adipocyte Index (AI^{-1}), and lipophilic POP data (p,p′-DDT and HCB) indicated that 2017 southward migrating animals were the third and fourth leanest on record respectively (Figs. 2 and S1). The 2017 downturn in adipose stores recorded by both measures, followed the 2016 peak in energetic reserves as indicated by both measures (Fig. 2 and S1).

Initial screening indicated that one or more years of AI^{-1} data were significantly different (p = 0.0001, one-way ANOVA). Further analysis revealed that 2011 was significantly different from 2009, 2015, 2016, and 2018. In addition, 2013 was significantly different from 2015 and 2016
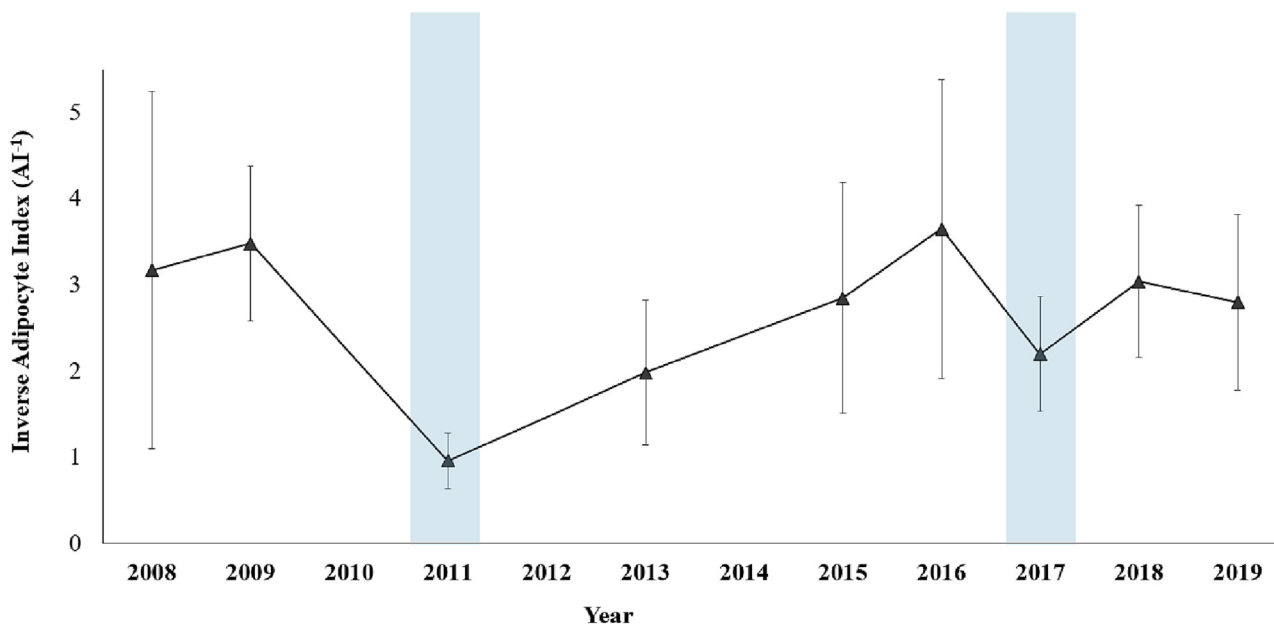
Fig. 2. Average (error bars indicate SD) population adiposity as measured by the AI^{-1} of adult males on their southward migration, where a higher value indicates a year of superior body condition. Shaded years represent those years associated with known sea-ice lows in the corresponding feeding ground, the summer immediately preceding sampling.

(post-hoc Tukey test). Despite a downturn in the mean AI^{-1} in 2017, it was not statistically significantly different from any other year in the record.

By contrast to AI^{-1} data, no significant inter-annual differences of neither ΣDDT nor HCB values were found (p = 0.2182 and 0.3978 respectively; one-way ANOVA), despite evident upturns in concentrations in both 2011 and 2017 (Fig. S1).

3.2. Diet

The HWSP dietary analysis timeline for blubber C and N stable isotopes largely adheres to the isotopic space predicted for a high-fidelity krill feeder, as indicated by the grey two-dimensional isotopic 'krill space' depicted in Fig. 3 (Volkman and Nichols, 1991). Interestingly, the years
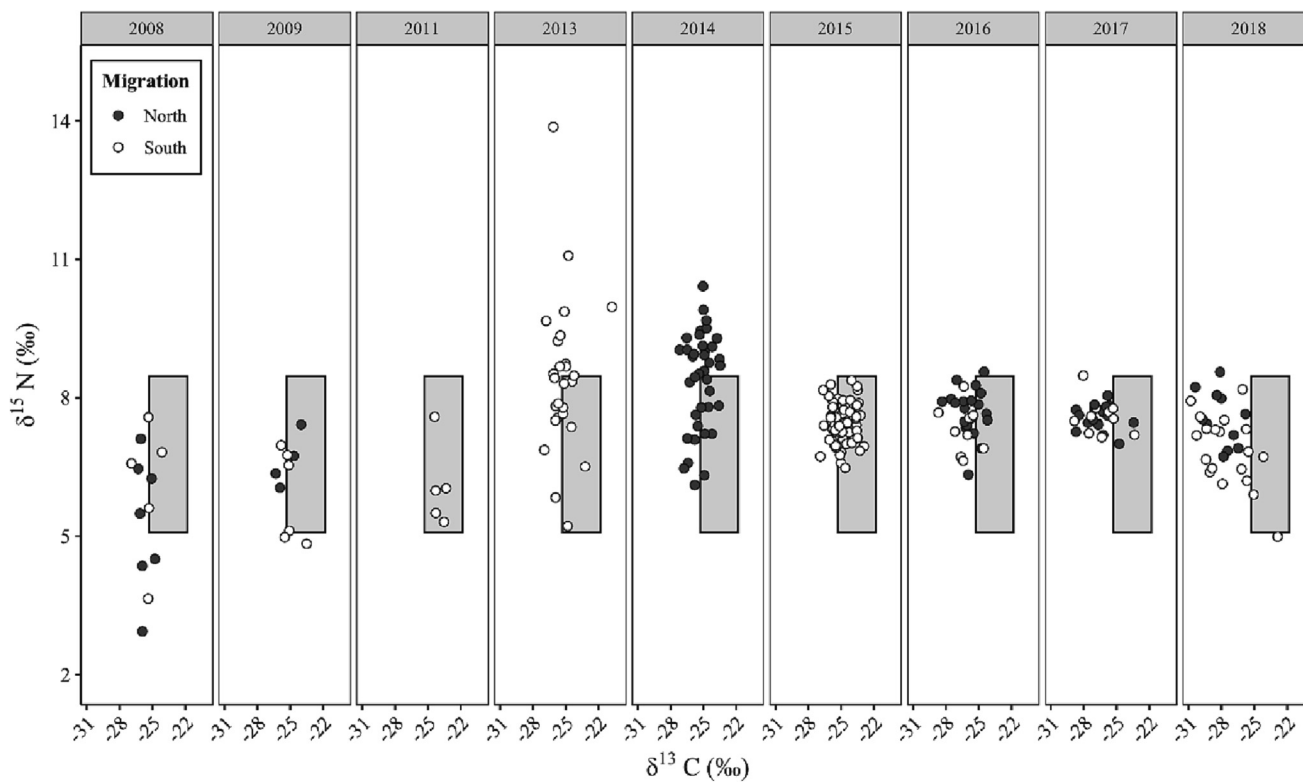

Fig. 3. Blubber C and N stable isotope measurements by year, where the grey 'krill space' indicates the two-dimensional isotopic space predicted for an Antarctic krill consumer, where black markers indicate individuals sampled on the northward migration and white markers individuals sampled during the southward migration. Higher diversification in feeding is observed in blubber δ^{15}N values following 2011, and blubber δ^{13}C values following 2017.

following the known (2011), and predicted (2017), "poor years" appear to be associated with a greater degree of feeding diversification, with just 19.2, 18.4, and 6.7 % of recorded measures falling within the krill space in 2013, 2014, and 2018 respectively. The level of diversification was primarily observed in $\delta^{15}N$ values following 2011, and $\delta^{13}C$ values following 2017 (Fig. 3).

Unlike the BSI dietary measure that did not flag within-year anomalies for the 2011 and 2017 "poor years", both the 2017 and 2018 FA profiles of humpback whale blubber were distinct from any other year. Differences that characterised the 2017 blubber FA profile from other years included a low percentage of foraging signature fatty acid of krill, namely docosapentaenoic acid (DPA; 22:5ω3). In addition, 2017 blubber FA profiles demonstrated a comparatively high percentage (4.11 ± 0.13 %) of 21:5ω3, a FA that only appeared in trace amounts (<0.5 %) in other parts of the temporal record. By contrast, in 2018 the percentage of 20:4ω6 was three times higher than the average percentage of all other years. A cluster analysis on the FA profiles from 2008 to 2018 showed clear separation of both the 2017 and 2018 cohorts from other years (Fig. 4).

3.3. Fecundity

Fecundity, as indirectly inferred by the migratory sex ratio, revealed 2017 as having the second lowest proportion of females on record, behind the 2011 low (Fig. 5). Similarly to adiposity measures, 2016, alongside 2009 and 2014 were marked by ≥40 % female participation in the migratory cohort, well above the 32 % reported by Brown et al. for this population, at this timepoint (Christiansen et al., 2018).

3.4. Mortality

Records of presumed natural mortalities of E1 humpback whales displayed a similar trend to the inverse of AI^{-1}, with peaks in 2011 and 2017, and low rates of mortalities from 2006–2008 and 2014 to 2016 (Fig. 6). Whilst calves and immature individuals dominated stranding records, a significant number of individuals remained uncategorized.

4. Discussion

This study collated six chemical, biochemical, and observational measures of the four HWSP sentinel parameters of: adiposity, diet, fecundity, and mortality. Measures were collected across a timeline spanning nine, ten, or twelve years, depending on each measure, from humpback whales migrating along the east Australian coast. The response of sentinel parameters showed remarkable consistency in their temporal alignment. 2017 was a year of documented extreme climatic anomalies in the Antarctic feeding grounds of southern hemisphere humpback whales. Under the HWSP, 2017 was characterised by all sentinel measures, with the exception of BSI, as a year of a negative or anomalous trend in tracer signatures. By contrast, greater heterogeneity in BSI signatures emerged with an apparent lag.

The 2017 AI^{-1} adiposity measure was the third lowest across a 12-year timeline, whilst outer blubber POP burdens were the fourth highest across a 10-year timeline, indicating greater than average depletion of outer blubber lipid stores. The exclusion of females in POP measures will have underestimated this depletion of stores for the population as pregnant and nursing females carry the greatest energetic cost throughout migration. Notably, 2016 revealed the highest adiposity for the timeline under both measures. If whales encountered sub-optimal feeding in the Southern Ocean during the 2016/2017 austral summer, the energetic impact of this may have been buffered by the preceding 2016 peak in energetic reserves. Such inter-annual carry-over in energetic budgets is likely to be of amplified significance through consecutive years of sub-optimal feeding. Collation of the post-2017 HWSP timeline may therefore provide valuable clues as to ecosystem factors underlying the unusual humpback whale mortality events reported through 2021 in Brazil.

The two biochemical measures of diet revealed disparate trends. Whilst the BSI profiles in the 2011 and 2017 poor years adhered to the isotopic space predicted for a high-fidelity krill feeder, signatures indicative of feeding diversification appeared in the years following i.e. 2013, 2014 and 2018. Previously, such indication of dietary heterogeneity was hypothesised to result from one of two scenarios (Bengtson Nash et al., 2018), either: i) A deliberate change in whale foraging behaviour in years following a poor year, or ii) Unchanged feeding behaviour by the whales, but rather a change in movement and habitat, and therefore availability,
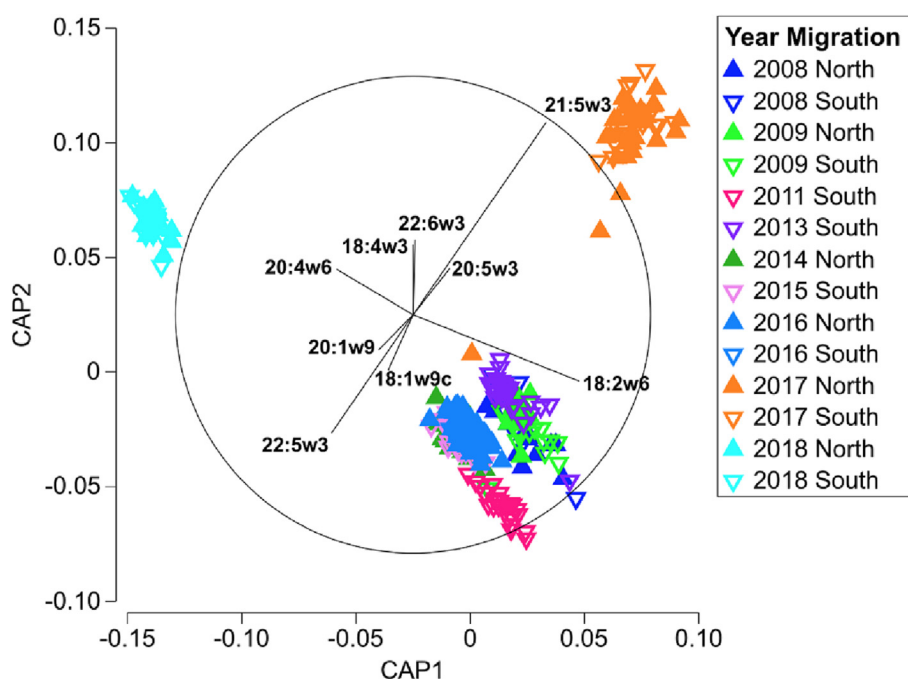

Fig. 4. Canonical analysis of principal coordinates (CAP) showing the fatty acid percentage composition of northward (closed upward arrow) and southward (open downward arrow) migrating east Australian humpback whales from 2008 to 2018 (n = 345). Graph reproduced from Groβ et al. (2020) (Thompson, 2002).
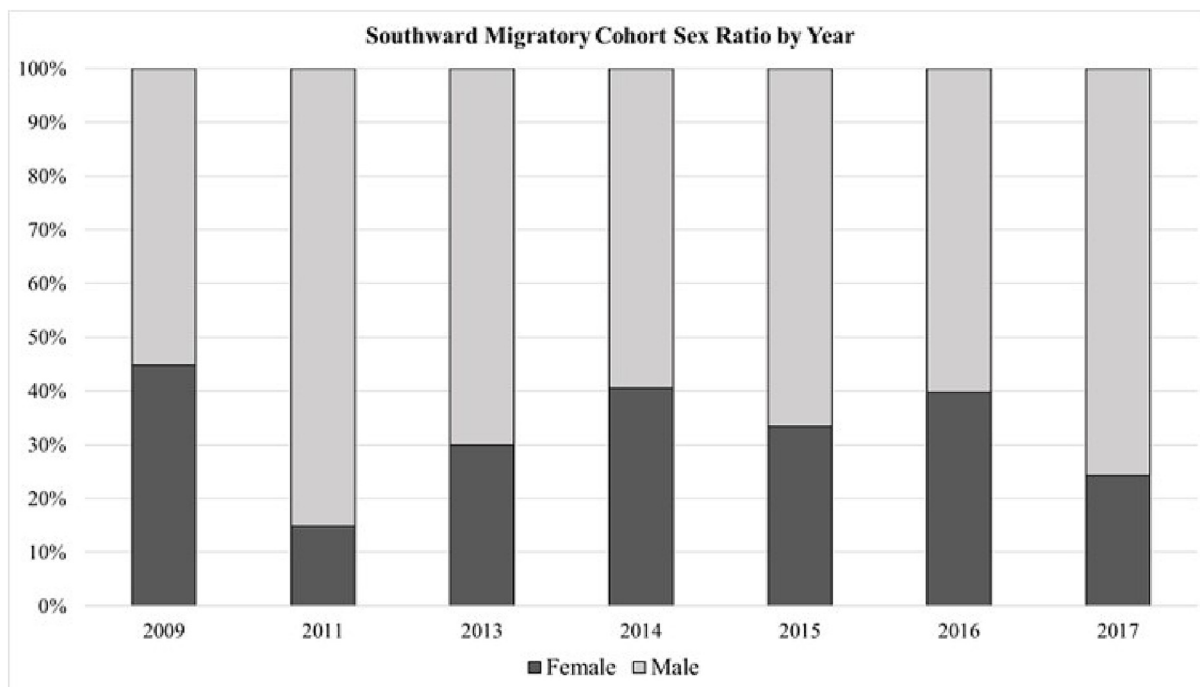
Fig. 5. Timeline of the sex ratio in southward migratory cohorts of humpback whales, revealing 2011 and 2017 as having the lowest female participation in the migration.

of krill during poor years. If such a change was accompanied by changed food intake by krill, the signature of such diversified feeding at the base of the food chain would then be carried forward to humpback whales in subsequent years, resulting in a lag in its appearance in the BSI dietary measure (Bengtson Nash et al., 2018). Notably, the latter hypothesis is supported by the observation of high interannual variability in the blubber fatty acid profiles of this population, without a significant change in signature fatty acids of krill feeding (Groß et al., 2020).

In contrast to BSI values, we observed a within-year anomaly in fatty acid profiles of 2017, via the appearance of higher-than-average percentages of the fatty acid 21:5ω3, which was previously only present in trace amounts in this population of humpback whales over the past decade of monitoring (Groß et al., 2020). The dissociation between the two dietary markers of BSI and fatty acids reveals a gap in our understanding of

metabolic fluxes, integration and turnover of biomolecules and isotopic pools (Poupin et al., 2014) in humpback whales.

2017 saw the second lowest representation of females in the southward migratory cohort across the monitoring period at just 25 % compared to the low of 14 % in 2011 and high of 44 % and 40 % in 2009, 2014 and 2016, compared to the historically recorded 32 % for the same migration time point in this geographical location. Female participation in the migration is hypothesised to be driven by feeding success encountered both in the feeding season immediately preceding migration (of importance to females Preparing for Pregnancy; PP), as well as the feeding season the year prior (females now in Late Pregnancy; LP) that are now motivated to migrate for parturition in warmer waters. The 2017 drop in female participation in the migration following the 2016 peak in body condition therefore likely represents a drop in the PP females that failed to attain sufficient energy
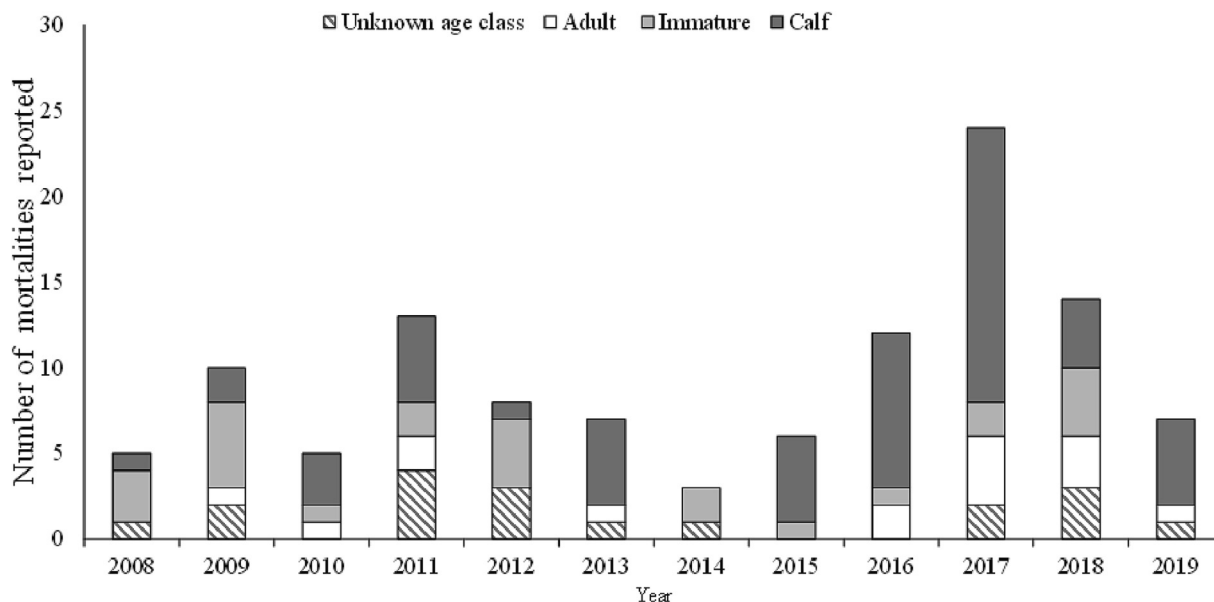

Fig. 6. Number of E1 humpback whales reported dead in the StrandNet database from 2008 to 2019.

reserves during the 2016/17 feeding season, rather than LP females migrating. This appears supported by the sharp increase in calf mortality in 2017. Further, it may be considered that the absence of a similarly high level of calf mortality in 2011 was attributed to a drop in LP females migrating. As the extreme La Niña event hypothesised to be a contributing factor in the observed 2011 'poor year' commenced already in July 2010, consecutive years of poor feeding would result in both a drop in LP and PP female participation in the migration. Pregnancy determination via steroid hormone analysis (Dalle Luche et al., 2019; Dalle et al., 2020) commenced for this population in 2015 and will provide further insight into this aspect of southern hemisphere humpback whale ecology now also shown to co-vary with krill abundance in other Antarctic sectors (Pallin et al., 2023) and will provide an added measure of fecundity under the HWSP, although as yet too few years of data are available for meaningful temporal trend analysis.

Finally, stranding incidence represented a new line of investigation that revealed evidence consistent with patterns observed in the healthy, free-roaming E1 population. Elevated calf and juvenile mortalities were observed during both poor years of 2011 and 2017. This is an important finding that demonstrates the value of stranding networks, particularly in remote regions lacking the funding or infrastructure for sustained, proactive monitoring programs. It also reveals the potentially significant population consequences of a single, or consecutive years of sub-optimal foraging for this capital breeding species. Critical for further research in this area is detailed investigation of the anomalous humpback whale mortality events observed in Brazil during 2021. The HWSP expanded to incorporate Brazil (Population A), Reunion Islands (Population C), Western Australia (Population D), New Caledonia (Population E2), Colombia and Ecuador (Population G) between 2017 and 2022. These inclusions will provide an invaluable reference for both interpopulation comparison, and also the comparison of stranding mortality incidence and necropsy findings, with biochemical health measures in the free-roaming populations. Studies such as these respond to the International Whaling Commission priorities outlined at the Climate Change workshops of 2021 (IWC, 2021).

5. Conclusions

The ability to measure and capture ecosystem change provides the strongest evidence of the planetary health impacts of climate change. Bio-monitoring of sentinel species can provide an integrated signal of temporal and spatial ecosystem change (Bossart, 2011). The effectiveness of any bio-monitoring system in delivering quality assured data for guiding policy and management decision-making processes, however, hinges on two key features; i) the identification of sensible sentinel parameters indicative of ecosystem change, and ii) the ability to robustly measure these identified sentinel parameters through simple and cost-effective approaches, ensuring sustained and coordinated monitoring through time and socio-political change.

This study revealed high consistency in the temporal trends observed between multiple chemical, biochemical and observational measures captured under the HWSP. HWSP Sentinel parameters of adiposity, diet, fecundity, and mortality showed strong temporal alignment, revealing a negative shift in 2017, although the shift was not as pronounced as that re (Brown et al., 1995)corded in response to the 2010/11 La Niña event. These findings lend further support for the use of migratory Antarctic krill feeders as powerful and practical sentinels of the Antarctic sea-ice ecosystem. The HWSP is the primary surveillance activity of the Antarctic Monitoring and Assessment Programme (AnMAP), an endorsed United Nations Ocean Decade Activity. Since 2017, the HWSP has expanded to incorporate additional breeding stocks around the southern hemisphere, allowing monitoring of the circum-Antarctic region. Sustained, coordinated, and spatially comprehensive efforts, such as those under the HWSP will offer the ability to investigate the ecosystem impacts of climate anomalies, such as recent heatwaves in Antarctica (González-Herrero et al., 2022), unusual humpback whale mortality events in Brazil, as well as capture directional ecosystem and pollution trends.

CRediT authorship contribution statement

Susan M. Bengtson Nash: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jasmin Groβ:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Juliana Castrillon:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Maria Valeria Casa:** Formal analysis, Writing – review & editing. **Greta Dalle Luche:** Formal analysis, Writing – review & editing. **Justin Meager:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Ruma Ghosh:** Formal analysis, Writing – review & editing. **June Eggebo:** Formal analysis, Writing – review & editing. **Pernilla Bohlin Nizzetto:** Data curation, Formal analysis, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Susan Bengtson Nash reports financial support was provided by The Ocean Foundation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164053.

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