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Historical biomonitoring of pollution trends in the North Pacific using archived samples from the Continuous Plankton Recorder Survey

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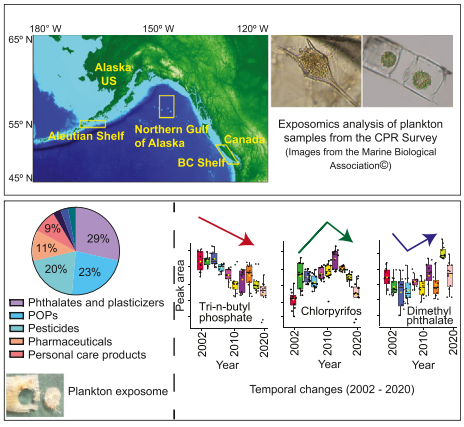
HIGHLIGHTS

- This study reports the use of archived plankton samples for ocean pollution monitoring.
- Samples from the Continuous Plankton Recorder (CPR) Survey were analyzed.
- Dozens of anthropogenic chemicals displayed distinct spatiotemporal trends.
- Phthalates from personal care products and plastics increased from 2002 to 2020.
- Plankton nearest to human population centers had more complex exposomes.

GRAPHICAL ABSTRACT



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ABSTRACT

First started in 1931, the Continuous Plankton Recorder (CPR) Survey is the longest-running and most geographically extensive marine plankton sampling program in the world. This pilot study investigates the feasibility of biomonitoring the spatiotemporal trends of marine pollution using archived CPR samples from the North Pacific. We selected specimens collected from three different locations (British Columbia Shelf, Northern Gulf of Alaska, and Aleutian Shelf) in the North Pacific between 2002 and 2020. Comprehensive profiling of the plankton chemical exposome was conducted using liquid and gas chromatography coupled with tandem mass spectrometry (LC-MS/MS and GC–MS/MS). Our results show that phthalates, plasticizers, persistent organic pollutants (POPs), pesticides, pharmaceuticals, and personal care products were present in the plankton exposome, and that many of these pollutants have decreased in

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amount over the last two decades, which was most pronounced for tri-n-butyl phosphate. In addition, the plankton exposome differed significantly by regional human activities, with the most polluted samples coming from the nearshore area. Exposome-wide association analysis revealed that bioaccumulation of environmental pollutants was highly correlated with the biomass of different plankton taxa. Overall, this study demonstrates that exposomic analysis of archived samples from the CPR Survey is effective for long-term biomonitoring of the spatial and temporal trends of environmental pollutants in the marine environment.

1. Introduction

Marine habitats have been under increasing threat due to the intensification of coastal human activities and the growing human population around the world. The release of anthropogenic chemicals, pesticides, and fertilizers has adverse impacts on marine ecosystems (Feng et al., 2022; John et al., 2022; Martinez-Varela et al., 2021). Therefore, in addition to reducing pollutant flows into oceans, it is essential to map and monitor ocean pollution to prioritize corrective measures, assess mitigation efforts for effectiveness and ensure a healthy and productive marine environment.

While a large body of data is available on the concentrations of many anthropogenic compounds in surface waters (Cunha et al., 2022; Sanganyado et al., 2021; Wilkinson et al., 2022), substantial gaps exist in our knowledge of the temporal changes of such exposures over decades. Long-term monitoring is an important approach for assessing the fate of contaminants in marine ecosystems. It can provide a first warning of the increase of potentially toxic compounds in the environment. In addition, multi-year temporal trend analysis may indicate whether regulatory actions aimed at reducing harmful chemicals in the oceans are proving successful. Long-term monitoring of persistent organic pollutants (POPs) in the marine environment has been established using Arctic marine biota (Riget et al., 2019), seabird eggs from the East Sea (Jang et al., 2022), and dolphins in the South Atlantic Ocean (de Oliveira-Ferreira et al., 2022).

Marine plankton, found in all ocean ecosystems, form complex communities of interacting organisms at the base of the food web and play essential roles in maintaining the health and balance of the ocean and influencing climate regulation (Field et al., 1998). Most planktonic species are short-lived and sensitive to environmental changes (Batten et al., 2022; Chaffron et al., 2021; Henson et al., 2021). Therefore, plankton may be an excellent bioindicator of contamination. Started in 1931, the Continuous Plankton Recorder (CPR) Survey is the longest-running and most geographically extensive marine plankton sampling program in the world (Richardson et al., 2006). Uniquely, CPR sampling has remained unchanged in terms of mesh size, weave, and fiber, throughout the Survey's history, making the archived CPR samples standardized candidates for long-term biomonitoring of marine pollution (Batten et al., 2019; Batten et al., 2003). For xenobiotics in marine plankton, information is typically only available for a small number of chemicals such as poly- and perfluoroalkyl substances (PFAS) (Zhang et al., 2019), Tetrabromobisphenol-A (Gong et al., 2021), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) (Peltonen et al., 2014). There is still a lack of comprehensive profiling of plankton chemical exposomes in the world's oceans.

Here, we selected archived plankton samples collected in three different locations in the North Pacific Ocean from 2002 to 2020. We quantified >1000 commonly used anthropogenic chemicals in plankton exposome using a broad-spectrum targeted approach. The targeted compounds were selected based on known occurrence in coastal marine environments and potential for biological impact. The objectives of this study were to (1) assess the temporal trends of the plankton chemical exposome in the North Pacific coastal environments over the last two decades; (2) compare the regional differences of the plankton exposome; (3) correlate the bioaccumulation of environmental pollutants within plankton with the biomass data of different plankton taxa and other ecological variables (Marine fishes abundance and GDP for agriculture, fishing, and hunting).

2. Materials and methods

2.1. CPR Survey apparatus and collection

The samples used in this study were collected by the Continuous Plankton Recorder (CPR) Survey, the longest, multi-decadal plankton monitoring program in the world. Detailed descriptions of the CPR device and its sampling characteristics over the lifespan of the Survey can be found online (https://www.cprsurvey.org/) and in the previous publication (Richardson et al., 2006). The CPR is a mechanical device that weighs about 85 kg and measures 106 cm × 43 cm × 37 cm. It is towed behind commercial ships in near surface waters (5–10 m depth) on their regular routes. Full details of the sampler and methodology are given in Batten et al. (2003) (Batten et al., 2003). Aspects relevant to the current study are provided here. The CPR is preloaded with a cassette containing two bands of filtering silk mesh on spools. The mesh is manufactured in China from natural silk, where it is produced for sifting flour. The mesh size is 270 μm. The width of the mesh band is 15 cm and the band length is determined by the distance of the transect such that 18.5 km (10 nautical miles) of transect requires about 10 cm length of mesh, such that 1 cm of mesh captures plankton from 1 nautical mile of ocean. A transect of up to 800 km (432 nautical miles) can be sampled with one cassette containing about 4.3 m of silk mesh. The CPR apparatus is delivered to the ship ready to be deployed and the ship's crew launch and recover the instrument from the stern when clear of port and traffic. Position, date, and time information for the sample is taken from ship's log and refers to the midpoint of each 10 cm long, 18.5 km sample. Constant speed between log entries is assumed. This method has proven to be quite accurate on the scale that the CPR is deployed since commercial ships tend to run at constant speed between ports.

During towing, seawater containing plankton enters through an aperture at the front of the CPR machine. The bands of mesh are pulled from their spools by the action of the propellor, one band is pulled across the face of the aperture so that the plankton are filtered from the water onto the band and then it is immediately covered by the second band forming a sandwich with the plankton in the center. The sandwich is rolled onto a third spool in a storage tank, which contains preloaded concentrated formalin. By the end of the transect the residual seawater entering the tank on the mesh has diluted the formalin there to approximately 4 %, sufficient to fix and preserve the plankton.

When the ship arrives in port after sampling, the CPR is returned to the laboratory, the cassettes are removed, and the mesh and plankton sandwich spool is unloaded from the cassette under a fume hood. The silk is unrolled and cut into individual sheets, 10 cm long x 15 cm wide, that each represent 18.5 km (10 nautical miles) of the transect and samples are randomly distributed to analysts in plastic boxes for taxonomic processing. Cross contamination between sample sheets from the sample silk spool is prevented by wrapping each sheet in plastic before it is placed in the boxes. During the processing and microscope analysis the samples may be sprayed with additional 4 % formalin to prevent drying and preserve plankton morphology. After analysis, the sample sheets are re-wrapped individually in plastic and archived in plastic boxes that contain a thin layer of 4 % formalin on the bottom to provide vapor phase hydration and preservation. The flat sheets of archived CPR samples are stored at room temperature (20–25 °C). Samples from one transect are archived together in numerical order. Each storage box may contain several repeat samplings of a single transect collected in the same year. Samples from different transects are

stored in physically separate containers. Blanks for exposome analysis were prepared from unused silk mesh and a pooled sample of 4 % formalin collected from the bottom of an archived sample box containing the CPR silk samples.

2.2. CPR sample site selection

To test the feasibility of using historical plankton samples for ocean pollution monitoring, we selected 30 CPR samples from 3 locations in the North Pacific: 10 from the British Columbia Shelf, 10 from the Northern Gulf of Alaska, and 10 from the Aleutian Shelf collected in August every other year from 2002 to 2020 (Fig. 1A and B).

2.3. Sample collection and taxa identification

One inch square (6.45 cm^2) samples of CPR silk mesh were cut from each of the 30 transects selected for exposome analysis. Each CPR sample represented about 10 nautical miles (18.5 km) of the tow and a volume of about 3 m^3 of seawater (Batten et al., 2003). Taxonomic analysis of marine plankton was performed by light microscopy, and up to 700 taxonomic entities were recorded in a quasi-logarithmic manner (Vezzulli et al., 2022) to the highest practical taxonomic resolution. Phytoplankton abundance was counted by viewing 20 fields of view (diameter 295 μm) across each sample under high magnification (× 450) (Batten et al., 2003), small zooplankton were counted from a 1/50 subsample and larger zooplankton were counted with no sub-sampling.

2.4. Plankton exposome extraction and analysis

The exposome extraction method was adapted from our previous work (Li et al., 2020). Briefly, a 6.3-mm punch (1/4 in.) of silk mesh with plankton samples from 0.15 m^3 of filtered sea water (Batten et al., 2003) was transferred to a 2 mL tube, and 40 μL of LC-MS grade water containing internal standards was added and incubated at 4 °C overnight. Two replicate punches were made and processed from each CPR silk mesh sample. One hundred and sixty μL of extraction buffer consisting of 80 % ethanol prechilled at −20 °C were then added, mixed thoroughly, and incubated on ice for 10 min. The mixture was centrifuged for 10 min at 16,000 x g, and 4 °C, and the supernatant was stored at −80 °C for further analysis. Silk that had undergone the same silk-preparation procedure but had not been used to sample the plankton, and a pooled sample of the 4 % formaldehyde solution present in an archived container were used as background blank controls and processed using the same protocol.

Exposome profiling of xenobiotic chemicals in plankton samples was performed by either high-pressure liquid chromatography or gas chromatography coupled with tandem mass spectrometry (LC-MS/MS or GC–MS/MS) depending on the chemical properties of the targets. Duplicate injections were performed from each extract. Briefly, LC-MS/MS analysis occurred using a Shimadzu LC-20AD UHPLC system coupled with a SCIEX Qtrap 5500 MS/MS. Ten μL of the 200 μL ethanol extract (5 %) was injected and separated using a Restek Raptor Biphenyl column. The MS/MS detection was operated using electrospray ionization (ESI) and advanced scheduled multiple reaction monitoring (MRM). For GC–MS/MS analysis, one μL of the 200 μL ethanol extract (0.5 %) was injected into an Agilent 8890 GC

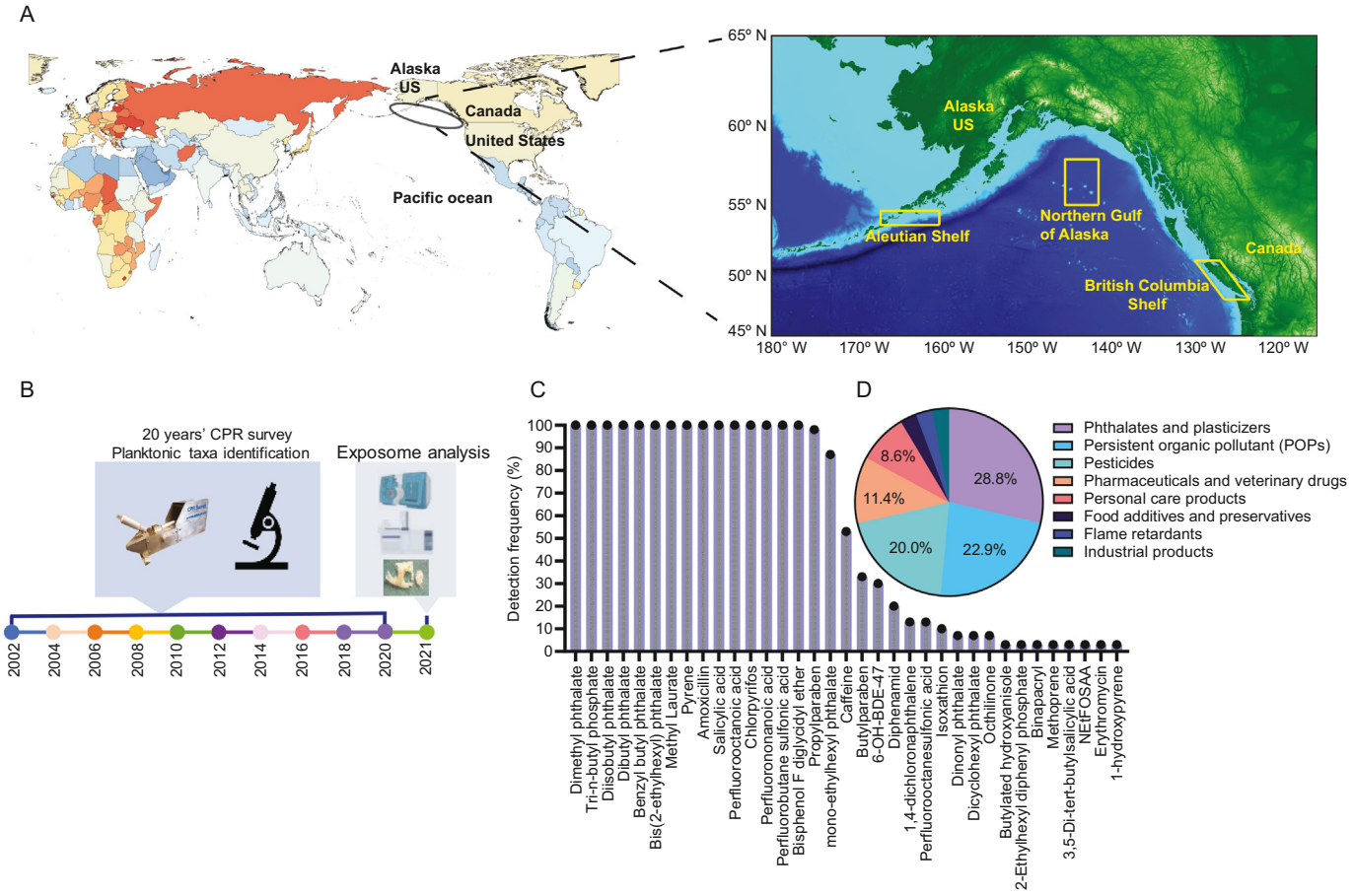

Fig. 1. Marine plankton sample collection and exposome analysis. (A) Map showing the plankton sampling sites. Three sites (yellow boxes) were collected. The samples are part of the Continuous Plankton Recorder (CPR) Survey, the world longest-running marine biological monitoring program. (B) Timeline for plankton sample collection and analysis. The samples were collected every other year between 2002 and 2020. The CPR Survey's sampling and analysis methods remained unchanged for all the trips. (C) Detection frequencies and (D) chemical classifications of anthropogenic compounds detected in plankton samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Anthropogenic chemicals in the North Pacific plankton exposome sampled from 2002 to 2020.

No.	Chemical	Median concentration across 30 samples (μg/mL)	Inter-quartile range (μg/mL)	Detection frequency out of 30 samples	Chemical class	Uses and sources
1	Amoxicillin	4.07E-05	2.8E-05 – 6.6E-05	100%	Antibiotics and antifungals	Widely used antibiotic
2	Octhilinone	2.23E-02	9.7E-03 – 2.7E-02	7%		Antimicrobial active ingredient and material preservative in coatings and building materials
3	Binapacryl	4.00E-01	1.0E-01 – 7.1E-01	3%		Fungicide and miticide
4	Erythromycin	3.86E-05	1.6E-05 – 6.4E-05	3%		Widely used antibiotic
5	Salicylic acid	3.03E-03	2.5E-03 – 3.7E-03	100%	Drug metabolite, natural product	Aspirin metabolite, natural product produced by plants and phytoplankton in response to abiotic stress
6	6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE-47)	1.03E-03	6.5E-04 – 1.7E-03	30%	Flame retardants	Flame retardants used in paints, plastics, foam furniture padding, textiles, rugs, curtains, televisions, building materials, airplanes, and automobiles
7	Caffeine	3.80E-04	3.0E-04 – 4.2E-04	53%	Food additives	Food additive and drug
8	Butylated hydroxyanisole	1.48E-03	8.2E-04 – 2.4E-03	3%		Used as an antioxidant and preservative in food, food packaging, animal feed, and cosmetics, and in rubber and petroleum products
9	3,5-Di-tert-butylsalicylic acid	6.76E-02	6.3E-02 – 7.2E-02	3%	Industrial chemicals	Main ingredient of charge control agents for toners
10	Dimethyl phthalate	1.40E-03	9.1E-04 – 2.5E-03	100%	Personal care products and plasticizers	Used in the manufacture of a variety of products including plastics, insect repellents, safety glass, and lacquer coatings
11	Diisobutyl phthalate	3.09E-02	2.2E-02 – 5.3E-02	100%		Plasticizer in nitrocellulose lacquers, elastomers, explosives, nail polish, and solid rocket propellants. Other uses include perfume fixative, textile lubricating agent, safety glass additive, printing inks, and adhesives
12	Dibutyl phthalate	7.58E-03	5.1E-03 – 1.2E-02	100%		Plastics, elastomers, lacquers, explosives, printing inks, resin solvents, perfume oil solvents, paper coatings, adhesives, and nail polish
13	Benzyl butyl phthalate	1.46E-03	8.7E-04 – 2.6E-03	100%		Plasticizer in adhesives and sealants, floor coverings, paints, and coatings, and use in plastic and rubber products
14	Bis(2-ethylhexyl) phthalate	4.51E-03	2.6E-03 – 9.2E-03	100%		Plasticizer for resins, in pesticides, and as a solvent for ink
15	Bisphenol F Diglycidyl Ether (BFDGE)	2.05E-03	1.8E-03 – 2.3E-03	100%		Widely used in the protective coatings of food and beverage cans and in paints and adhesives
16	Propylparaben	1.46E-04	1.1E-04 – 2.0E-04	98%		Antifungal and antimicrobial used in a variety of water-based cosmetics and personal-care products, and food additive
17	Mono-ethylhexyl phthalate	3.12E-03	2.6E-03 – 4.6E-03	87%		Widely used plasticizer
18	Butylparaben	4.75E-05	3.7E-05 – 7.8E-05	33%		Antimicrobial preservative used in many cosmetics, as a food flavoring agent and as a suspending agent for medications
19	Dinonyl phthalate	2.65E-03	2.1E-03 – 1.0E-02	7%		Plastic consumer products such as flooring, materials used in automobile interiors, wire, and cable insulation
20	Dicyclohexyl phthalate	4.18E-04	3.2E-04 – 9.8E-04	7%		Rubbers, resins, and polymers, including nitrocellulose, polyvinyl acetate, and polyvinyl chloride
21	2-Ethylhexyl diphenyl phosphate	2.96E-03	2.9E-04 – 5.8E-03	3%		Plasticizer, flame retardant, and a main component of non-flammable hydraulic fluids
22	Methyl Laurate	3.06E-03	1.9E-03 – 5.3E-03	100%	Pesticides	Detergent and larvicide in farming, spin finishes in textiles, metal working fluids
23	Chlorpyrifos	1.28E-02	7.3E-03 – 1.8E-02	100%		Widely used pesticide, banned in the US in 2021
24	Diphenamid	1.80E-04	1.3E-04 – 3.2E-04	20%		Selective herbicide for annual grasses and some broad-leaved weeds
25	Isoxathion	1.44E-02	1.0E-02 – 2.2E-02	10%		Insecticide for fruits, tea, tobacco, ornamentals, rice, sugarcane, vegetables, turf, and trees
26	Methoprene	2.17E-02	1.1E-02 – 3.9E-02	3%		Common ingredient found in flea treatments for dogs and cats, mosquito control products, insect baits, and home insect sprays or foggers
27	Tri-n-butyl phosphate	1.95E+01	5.6E+00 - 4.9E+01	100%	Plasticizer, drilling and hydraulic fluids	Aircraft hydraulic fluid, brake fluid, anti-foaming agent in detergent solutions, and in various emulsions, paints, and adhesives. It is also used in used in some consumer products such as herbicides, water-thinned paints, and tinting bases
28	Pyrene	1.41E-04	9.9E-05 – 2.1E-04	100%	Polyaromatic hydrocarbons	By-product of the pyrolysis of organic matter and is present in coal tar distillates, diesel exhaust, automobile exhaust, tobacco smoke, barbecue smoke, wood smoke, lake sediments, waste oils, and sewage
29	1,4-dichloronaphthalene	7.79E-06	4.7E-06 – 9.8E-06	13%		Engine oil additives, heat exchange fluids, flame retardants, cable insulation and wood preservatives
30	1-hydroxypyrene	8.75E-06	7.1E-06 – 1.1E-05	3%		By-product of the pyrolysis of organic matter and is present in coal tar distillates, diesel exhaust, automobile exhaust, tobacco smoke, barbecue smoke, wood smoke, lake sediments, waste oils, and sewage
31	Perfluorononanoic acid	1.87E-02	1.6E-02 – 2.3E-02	100%	Teflons, perfluoroalkyl substances (PFAS)	Fluoropolymer coatings and products that resist heat, oil, stains, grease, and water
32	Perfluorobutane sulfonic acid (PFBS)	2.06E-03	1.9E-03 – 2.2E-03	100%		Non-stick and stain-resistant consumer products, food packaging, fire-fighting foam, and industrial processes
33	Perfluorooctanoic acid (PFOA)	6.86E-05	5.9E-05 – 7.9E-05	100%		Non-stick and stain-resistant consumer products, food packaging, fire-fighting foam, and industrial processes
34	Perfluorooctanesulfonic acid (PFOS)	9.80E-05	6.0E-05 – 1.1E-04	13%		Widely used surfactant in fabric protectors, firefighting foams, and photolithographic chemical mixtures
35	2-(N-Ethylperfluoro octane sulfonamido) acetic acid (NEtFOSAA)	4.68E-03	4.1E-03 – 6.6E-03	3%		Non-stick and stain-resistant consumer products, food packaging, fire-fighting foam, and industrial processes

in splitless mode and separated with two 15-m fused silica HP-5MS UI capillary columns. The MS/MS analysis was run using a 7010B triple quadrupole mass spectrometer operated in electron ionization (EI) mode, and the dynamic MRM (dMRM) was used for data acquisition.

A total of 1001 anthropogenic compounds were targeted, including 209 analytes on the LC-MS/MS platform and 792 chemicals on the GC–MS/MS platform (Fig. S1A). MRM transitions and the retention time (RT) were optimized using the purified standards, and two MRMs transitions were used per target analyte. Normalization for plankton biomass across different CPR samples was performed by using the geometric mean AUC of four common phosphatidylcholine (PC) phospholipids as described in Supplementary Methods. The full targeted list and the associated MS/MS parameters and RT were listed in Tables S1 and S2. Absolute concentrations were calculated by comparison to purified chemical standards. Robust quality control measures were employed throughout sample preparation and analysis (Supplementary Materials and Methods).

2.5. Statistical analysis

Chromatographic peaks were processed using our in-house peak vetting scripts written in Python based on their peak area, retention time, signal-to-noise (S/N) ratios, and sample-to-blank ratios. Putative positive hits with the peak area at least 10-fold higher than the blank controls were manually inspected and verified by the purified standards (Table S3 and Fig. S1B-S1F). Compounds with >25 % missing values across the 30 samples were removed from correlation analysis. Probabilistic principal component analysis (PPCA) was used to impute values for two chemicals that had 2–13 % (1 to 4 samples out of 30) with missing values (Nyamundanda et al., 2010). The exposomic data were then log2 transformed and scaled by control standard deviations, and the resulting z-scores were used for random forest (RF) analysis (n = 500 trees) in MetaboAnalyst 5.0 (Pang et al., 2022). The exposomic richness and diversity of marine plankton were estimated by Chao 1 and Shannon indices (https://chao.shinyapps.io/SpadeR/). Canonical correspondence analysis (CCA), a multivariate data reduction technique, was employed to understand the relationships between plankton exposome and the biomass of different plankton taxa using PAST 4.11 software. All other general analyses including one-way ANOVA (or non-parametric Kruskal-Wallis test), Pearson and Spearman correlation were performed using GraphPad Prism 9.4.0 or Python. A 2-tailed $P < 0.05$ was considered statistically significant.

3. Results

3.1. CPR survey site selection

This study included sampling sites in the North Pacific Ocean subject to a broad suite of anthropogenic influences, spanning from the British Columbia Shelf (BC Shelf) near Vancouver, one of the most populated cities in Canada, to the Northern Gulf of Alaska (N Gulf Alaska), an ecologically productive area of the North Pacific (Fig. 1A). A site on the Aleutian Shelf was also selected, which is the home to some of the largest commercial fisheries in the United States but has a low local human population density. To characterize the historical changes in marine pollution in recent decades, we selected the plankton samples collected between 2002 and 2020 from each of the three sites (Fig. 1B).

3.2. CPR Survey plankton exposome analysis

Of 1001 anthropogenic compounds screened, 35 were detected in at least one plankton sample (Fig. S1A and Table S3). Fig. S1B-S1F show the

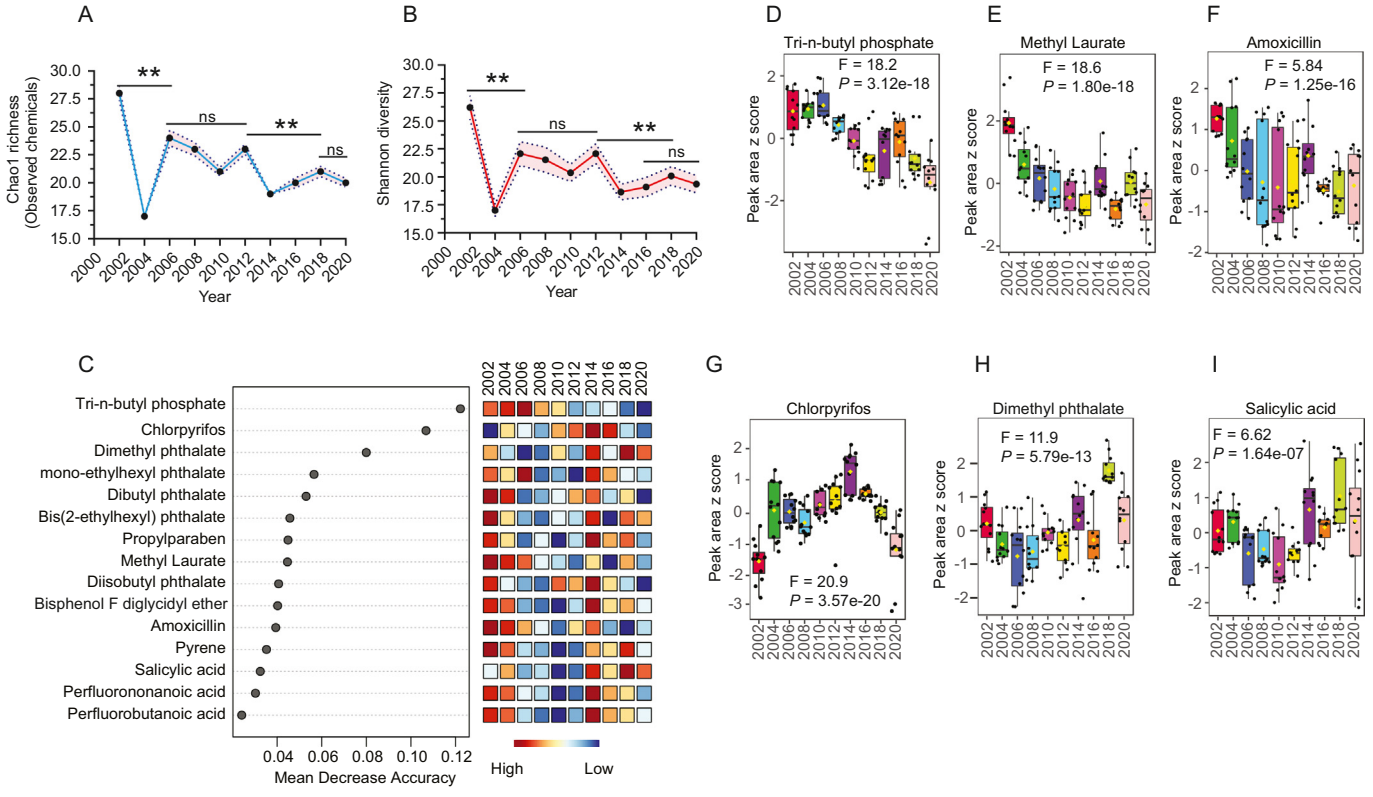

Fig. 2. Temporal changes of plankton exposome between 2002 and 2020. (A) The overall changes of plankton exposomic richness over 20 years revealed by the Chao 1 index. "ns" indicates not significant ($P > 0.05$), whereas the ** indicates a significant difference ($P < 0.01$) according to the Mann-Whitney U test (Same for Fig. 2B). (B) The exposomic diversity of marine plankton over the years revealed by the Shannon index. (C) Random forest (RF) analysis identified the top 15 anthropogenic compounds altered in plankton exposome between 2002 and 2020. The mean decrease accuracy quantifies the importance of a chemical to the prediction accuracy of the model (n = 500 trees). A higher value shows that the chemical has more importance to group separation. (D - I) The anthropogenic compounds that changed significantly over the years in plankton exposome by one-way ANOVA analysis.

representative chromatograms of anthropogenic compounds detected in plankton samples and background blanks. The mean sample AUC to blank ratio for the 35 detected chemicals was 374:1. The range was 21–5637:1 (Supplementary Table S3). The median concentrations in the 200 μL extracts varied from 7.8 pg/mL to 19.5 μg/mL (Table 1, Supplementary Table S4). The overall detection frequencies of anthropogenic compounds ranged from 3 % to 100 % (Fig. 1C). Fifteen (15) compounds were present in the chemical exposome of all plankton samples. These included dimethyl phthalate, tri-n-butyl phosphate, and methyl laurate. The most dominant chemicals in the plankton exposome were phthalates and plasticizers (28.8 %), followed by persistent organic pollutants (POPs) (22.9 %), pesticides (20.0 %), pharmaceuticals and veterinary drugs (11.4 %), and personal care products (8.6 %) (Fig. 1C).

3.3. Temporal changes of plankton exposome between 2002 and 2020

Zooplankton and phytoplankton follow a regular annual life cycle and therefore may serve as useful resources for monitoring the yearly changes in marine pollution. Here, we explored the temporal changes of the plankton exposome in the last two decades. Overall, both plankton exposomic richness (Chao1 index, Fig. 2A) and diversity (Shannon index, Fig. 2B) in the North Pacific decreased over the past 20 years. Random Forest analysis identified the top 15 anthropogenic compounds altered in plankton exposome between 2002 and 2020 (Fig. 2C), which was further confirmed by the univariate analysis (Fig. 2D-2I). For instance, there was a significant decreasing trend in tri-n-butyl phosphate, methyl laurate, and amoxicillin (Fig. 2D-2F). The bioaccumulation of chlorpyrifos in plankton increased between 2002 and 2014 and then decreased gradually from 2016 to 2020 (Fig. 2G). In contrast, dimethyl phthalate and salicylic acid decreased between 2002 and 2012 but had a trend of increase from 2014 to 2020 (Fig. 2H and I). Salicylic acid is both a plant and phytoplankton hormone produced in response to abiotic stress (Khan et al., 2015) and a metabolite of aspirin (Table 1).

3.4. The spatial differences in plankton chemical exposome in the North Pacific

The plankton samples collected from the BC Shelf were found to have significantly more anthropogenic chemicals than those from Aleutian Shelf and N Gulf Alaska ($P < 0.01$, Fig. 3A). Regarding the xenobiotic concentration in plankton exposome, Fig. 3B shows the top 15 differential xenobiotics among three sites revealed by Random Forest analysis. In general, a variety of pharmaceutical drugs, pesticides, POPs, and phthalates had higher levels in the plankton chemical exposome from the BC shelf and Aleutian Shelf compared to those in N Gulf Alaska. The significant differences in the concentration of exogenous compounds in plankton samples between the three sites were further confirmed by the univariate analysis (Fig. 3C-3E).

3.5. The associations between the biomass of different plankton taxa and anthropogenic compounds

The multivariate CCA ordination biplot was constructed to illustrate the combined effects of different plankton species and cell abundance on plankton chemical exposome. As shown in Fig. 4A, both the mesozooplankton and phytoplankton contributed to the bioaccumulation of anthropogenic compounds, within these groupings euphausiids, small copepods, and dinoflagellates were the main contributors. The Spearman's correlation matrix also indicated that the abundance of various plankton taxa correlated differently with different anthropogenic compounds (Fig. 4B and Table S5). The scatter plot graph reaffirmed a strong positive correlation between dinoflagellates and perfluorooctanoic acid (PFOA) ($P < 0.01$) (Fig. 4C). The abundance of euphausiids showed a strong positive relationship with the bioaccumulation of diisobutyl phthalate (Fig. 4D).

Similarly, total mesozooplankton positively correlated with amoxicillin (Fig. 4E), and within the mesozooplankton grouping, small copepod abundance had a positive correlation with the level of methyl laurate (Fig. 4F). A significant positive correlation was also observed between total phytoplankton abundance and salicylic acid concentration in the plankton exposome (Fig. 4G). In contrast, tri-n-butyl phosphate, a common plasticizer, was found to be negatively correlated with the abundance of pteropods (Fig. 4H).

4. Discussion

The North Pacific region features coastal and island ecosystems with spectacular marine life and commercially important fishing resources. Rapid coastal development, onshore and offshore industry, and tourism are taking an increasing toll on the North Pacific marine and coastal environmental health (Chen et al., 2018). Even though the existing untargeted methods of exposomic analysis are capable of capturing thousands of chemical features using high-resolution mass spectrometry (HRMS), fewer than 1 % of the spectral features in the HRMS chromatogram can be unambiguously identified due to the lack of exposome databases (Gao et al., 2022; Hsiao et al., 2022). In this study, we were able to measure >1000 exogenous compounds from over 13 different chemical classes using a broad-spectrum targeted approach on the triple quadrupole mass spectrometers (QqQ MS). Generally, compared to HRMS, targeted mass spectrometry methods using multiple reaction monitoring (MRM)-based triple quadrupole analysis offers increased selectivity, improved signal/noise, lower limits of quantitation, wider linear range, and improved accuracy (Li et al., 2017). In addition, our targeted list was built to specifically screen the heavily used pesticides, food additives, pharmaceuticals, personal care products, common environmental pollutants, and some of emerging contaminants.

4.1. Sources of marine pollution

Our results revealed that phthalates, plasticizers, POPs, pesticides, drugs, and personal care products predominated in the planktonic exposome of the North Pacific (Table 1). Phthalates and plasticizers are a group of man-made chemicals derived from plastics, and the North Pacific is one of the most polluted oceans by plastic debris, with >1 trillion floating pieces around the North Pacific Gyre (Eriksen et al., 2014; Isobe et al., 2019). Polycyclic aromatic hydrocarbons (PAHs) such as pyrene are persistent organic pollutants (POPs) produced by incomplete fossil fuel burning and accidental discharges of petroleum products from factories, vehicles, and ships. The presence of PAHs has been ubiquitously detected in the marine environment, even in plankton from polar regions (Pouch et al., 2022). Pesticides and personal care products have primarily been evaluated in rivers and coastal waters (Ccanccapa et al., 2016; Tsui et al., 2014). In this study, we reported, for the first time, the accumulation of pesticides and personal care products in the plankton of the North Pacific.

Our multi-year (2002 − 2020) analysis of the plankton exposome showed that legacy POPs and the antibiotic amoxicillin had generally decreased in the North Pacific Ocean during the last 20 years. Over the years from 2000 to 2010, there was a 2 % decrease in overall antibiotic use in the US and Canada (Van Boeckel et al., 2014). However, during the same time, there was an increase in usage in Russia and China. The sources of antibiotics found in the CPR samples from the North Pacific sites in this study are unknown, but may include marine aquaculture and terrestrial runoff. Antibiotic and nutrient pollution (Zhang et al., 2020), and the emergence of antibiotic resistance (Kraemer et al., 2019) are significant concerns in marine aquaculture. This has led to greater regulation and decreased antibiotic use in marine aquaculture in the US (EPA, 2006). However, international standards are not uniform and continued monitoring will be required. Decreasing trends have also been observed for most legacy organochlorine contaminants in Arctic air and marine biota (Hung et al., 2016; Riget et al., 2019). These downward trends are likely related to the restricted use of POPs and the voluntary phase-out of long-chain PFASs production in the EU and USA since the early 2000s (Bustnes et al., 2022). In addition, we found that the plankton exposome was impacted by regional forcings.

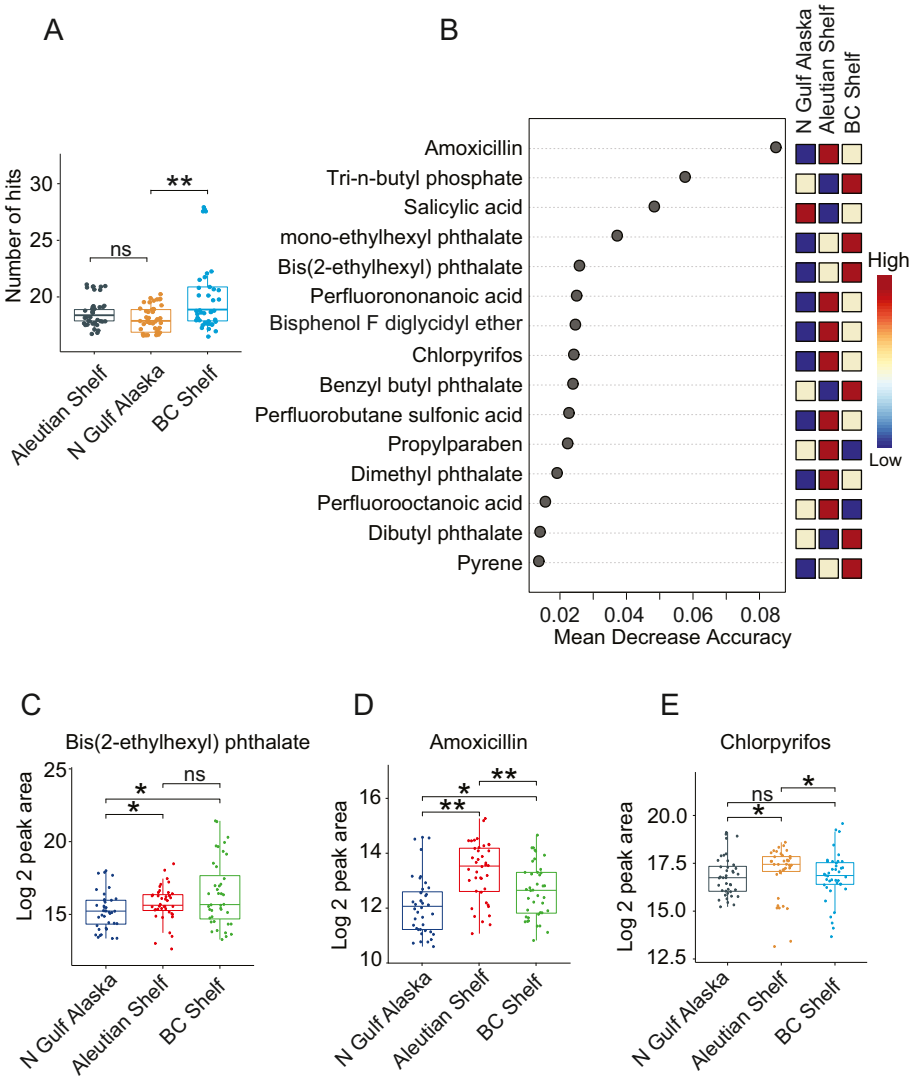

Fig. 3. The spatial differences of plankton exposome among three collection sites in the northern pacific. (A) The number of detected anthropogenic compounds in the exposome of plankton samples from different sites. Samples were collected from the Aleutian Shelf, Northern Gulf of Alaska (N Gulf Alaska), and British Columbia Shelf (BC Shelf). Kruskal Willis test followed by Dunn's post hoc was used for the comparison of group differences. "ns" indicates not significant ($P > 0.05$), whereas the ** indicates significant difference ($P < 0.01$). (B) Random forest (RF) analysis (n = 500 trees) identified the top 15 differential anthropogenic compounds in plankton exposome among three sites. The importance of an anthropogenic compound for group separation is ranked by mean decrease accuracy. The colour bar represents the concentration of anthropogenic compounds. (C - E) The anthropogenic compounds differed significantly in plankton exposome among three sites by one-way ANOVA analysis. "ns" indicates not significant ($P > 0.05$), *$P < 0.05$, and **$P < 0.01$.

4.2. Oceanographic factors

The oceanography of the wider northeast Pacific region is described in detail in Weingartner et al. 2005. There are coastal boundary currents around the North Pacific Ocean gyre which flow counterclockwise and transport water including its chemical and biological properties (the Alaska Current, the Alaskan Stream, and the Alaska Coastal Current or ACC). The ACC begins near the Washington-British Columbia border and carries precipitation and river runoff, including snow and glacial meltwater, around the rim of the region and eventually through the Aleutian Island passes into the Bering Sea. The Aleutian Island chain constricts the main North Pacific gyre and causes a recirculation as the Alaska gyre. Therefore, the water properties of the three sub-regions that our study focused on will be influenced by this oceanography with the Aleutian Shelf being "downstream" (by some distance) of the British Columbia (BC) shelf region, and both shelf regions are distinct from the oceanic Gulf of Alaska region in the Alaskan gyre. There are also differences in human population densities in proximity to each region. The Gulf of Alaska region is far from any human community and the BC Shelf region is close to southern BC and northern WA with several large cities nearby. The Aleutian Islands, near to the Aleutian shelf region, have smaller communities only. Both the number and the concentrations of environmental pollutants were higher in the plankton samples collected in the nearshore region of the BC Shelf, which indicated that most ocean pollution might begin on land as a result of runoff (Jambeck et al., 2015). This statement is also supported by our observation of positive correlations between the bioaccumulation of plasticizers in plankton and British Columbia GDP for agriculture, fishing, and hunting (Fig. 5).

4.3. Ecological and biological factors

Plankton is an important food source for other oceanic organisms such as fish, mammals, and squid. The concentration of anthropogenic pollutants in the plankton exposome may pose deleterious effects on the health of aquatic ecosystems. In a preliminary correlation analysis, we found negative associations between anthropogenic pollutants in marine plankton

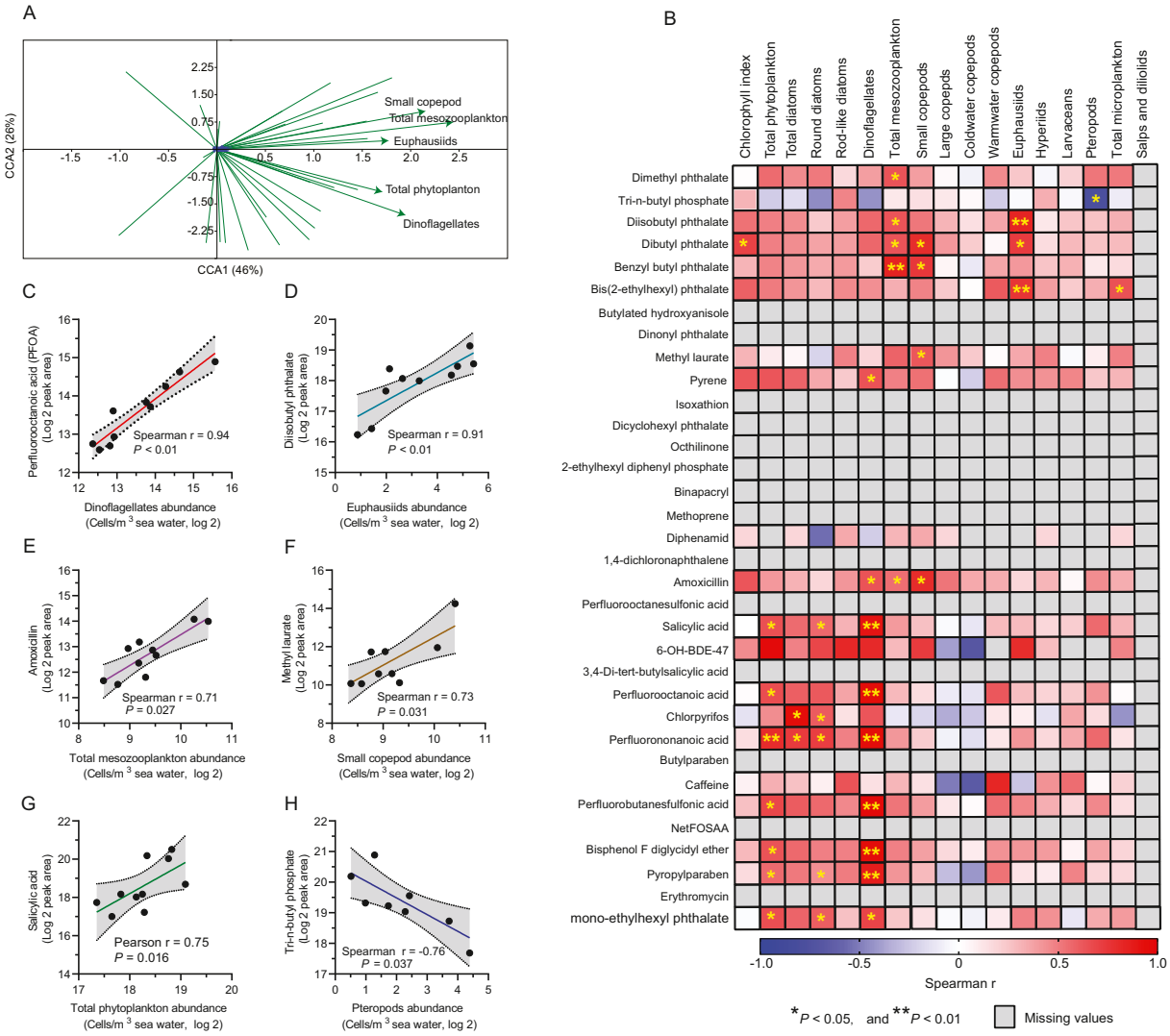

Fig. 4. The associations between plankton species abundance and exposome. (A) Canonical correspondence analysis (CCA) showing the relationships between the abundance of different planktonic species and anthropogenic compounds in the plankton exposome. In a CCA biplot, each arrow represents a plankton species, and the length of the arrow explains its relative importance for determining exposome variation. The longer arrows represent more important variables. The angle between an arrow and axis 1 indicates the correlations between plankton species abundance and exposome. An angle smaller than 90° indicates a positive correlation between the variables; the smaller the angle, the closer the positive correlation between the two variables. (B) The Spearman correlation heatmap showing the correlations of the plankton species abundance with the concentrations of anthropogenic compounds in plankton samples. $*P < 0.05$ and $**P < 0.01$. (C - H) The significant associations between plankton species abundance and the anthropogenic compound concentration were confirmed by linear correlation analysis.

and fish catch statistics from Aleutian and Gulf of Alaska (Fig. 5, Tables S6 and S7). We hasten to emphasize this was a pilot study and was not designed to examine cause and effect relationships. However, future studies can be designed using archived samples from the CPR Survey to answer some of these important questions.

Based on this preliminary analysis, we found that mesozooplankton such as copepods and euphausiids, tend to concentrate plastic-derived chemicals, methyl laurate, and amoxicillin. Zooplankton had been shown to consume the smaller size fractions of plastic (Botterell et al., 2020; Botterell et al., 2022), and a recent study found that zooplankton consumption of microplastic could decrease water column oxygen inventory by as much as 10 % in the North Pacific (Kvale et al., 2021). In addition, as a vital food source for life in the ocean, the biological uptake of environmental pollutants in the plankton may have significant downstream consequences on fish health and abundance (Hipfner et al., 2018). In contrast within the phytoplankton, diatoms and dinoflagellates are positively correlated with chlorpyrifos, salicylic acid, and perfluoroalkyl substances. The differences in the bioaccumulation of anthropogenic chemicals between zooplankton and phytoplankton could be due to differing physiological factors such as the abundance of intracellular lipid stores used for overwintering during diapause (Freese et al., 2015), intracellular pH and salinity, the bioavailability of the chemicals, and life stage, sex, and metabolic activity of zooplankton at the time of sampling (Kadiene et al., 2017; Pavlaki et al., 2017). For example, some phytoplankton species were reported to be resistant to phthalates (M'Rabet et al., 2019) and many metals have fast depuration rates in zooplankton (Chevrollier et al., 2022).

4.4. Limitations

Our study has some limitations. First, even though the sample collection and handling protocols have been standardized and have not changed over several decades, the factors that may affect measurements of the plankton exposome remain largely unknown. One possible problem is the chance of physical cross-contamination *between* plankton samples from different transects, years, and ocean locations. Since the silk sheets from different

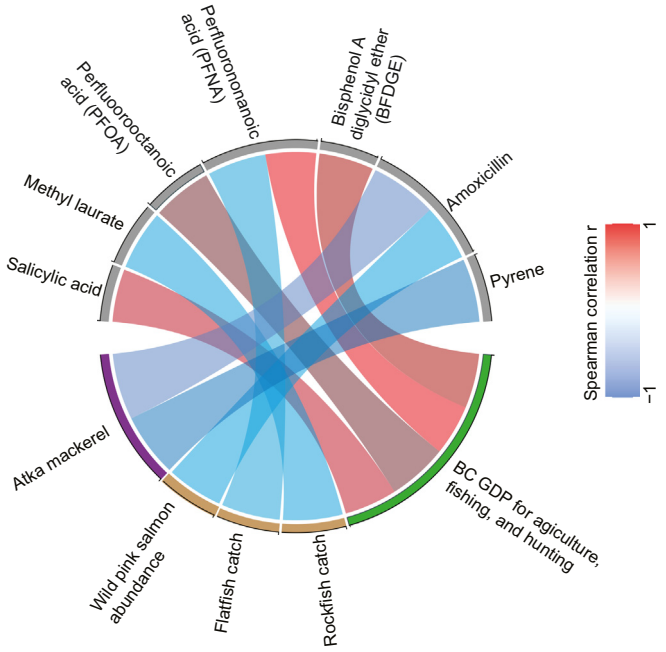

Fig. 5. Marine plankton exposome correlations with fisheries health. The CIRCOS correlation diagram shows significant associations between the accumulation of anthropogenic compounds in marine plankton and ecological indicators. Red linkage indicates a positive correlation, and blue is for the negative correlation. Both Spearman and Pearson correlations were conducted. $P < 0.05$ for both Spearman and Pearson correlations, and $q < 0.05$ are shown. British Columbia (BC) GDP data were obtained from Statistics Canada (https://www.statcan.gc.ca/en/start). Ecological data were obtained from annual marine ecosystem status reports released by National Oceanic and Atmospheric Administration (NOAA) Fisheries. Raw data are provided in Tables S6 and S7. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

transects are individually wrapped and stored in physically separate containers, there is a very low chance of contamination between samples from different years and locations. Another possible problem is that fixation in 4 % formalin upon initial plankton collection, and subsequent long-term storage in sealed containers of individually wrapped 10 cm long x 15 cm wide sheets of CPR silk mesh, may physically decrease the apparent concentration of chemicals in the samples by extraction in formalin. We controlled for possible chemical contamination present in the silk before use by extracting silk mesh samples that had not yet been exposed to plankton as blanks. We next controlled for possible contamination *within* single containers of CPR sample sheets by using a sample of pooled liquid 4 % formalin at the bottom of the container as a blank. This analysis showed that the chemical signal—the mean AUC ratio of sample to blank—in extracted plankton samples was 374:1, with a range of 21:1 to 5367:1 higher in samples than in the blanks. (Table S3, Fig. S1).

Another limitation is the relatively small sample size. Because the purpose of this pilot study was to determine the feasibility of using the plankton chemical exposome for biomonitoring the temporal and spatial trends of marine pollution, the sample size was just 30 samples collected from 3 sites over 20 years. Future studies will be needed to expand this. CPR sampling and laboratory protocols were not developed with biochemical assays in mind, and the laboratory is not contaminant free. However, the protocols in place are rigorous and consistent. This has allowed biochemical and DNA studies of archived samples which demonstrate the additional value that can be gained from the archive. For example, stable isotopes have been measured in CPR samples, which reveal patterns of ocean productivity in the Northeast Pacific over a 20-year period (Espinasse et al., 2020). Molecular methods have been applied to samples to amplify DNA and detect plankton species and potential pathogens that cannot be detected using light microscopy (Stern et al., 2018; Vezzulli et al., 2016; Vezzulli et al., 2015). Further validation studies will be needed to characterize the stability of different ocean pollutants in the CPR samples over time, to measure recovery efficiencies, and to quantify chemical susceptibility to losses due to chemical degradation or extraction in 4 % formalin prior to plankton exposome analysis.

4.5. Conclusions

This study demonstrates the feasibility of using archived plankton samples from the CPR Survey for historical biomonitoring of ocean pollution. Distinct temporal and spatial trends were observed in the exposomes of plankton samples collected over the past 20 years by comparing samples from three sites in the North Pacific. In the future, our approach could be expanded to the North Atlantic CPR Survey archive that have been collected since the 1930s. This would eventually allow for exploring the historical and spatial changes of pollution at sea in response to rapid industrialization and modernization after World War II and their impacts on marine ecosystems. The chemical exposome in plankton samples in the North Pacific was dominated by phthalates, plasticizers, POPs, pesticides, drugs, and personal care products. We observed a downward trend of many of these anthropogenic compounds in the plankton exposome over the last two decades, while phthalates associated with plastic debris were increased. The plankton samples from the nearshore region were more contaminated relative to other offshore areas. Our study reports the first use of archived samples from the Continuous Plankton Recorder Survey for reconstructing historical trends in the plankton exposome. These methods create an opportunity for follow-up studies designed to examine correlations between the plankton exposome, predator-prey relationships, and impacted fisheries around the world.

CRediT authorship contribution statement.

RKN, SB, and CO designed research; KL, JCN, SSL, LW, JMM, and RKN developed and performed the exposomic analysis; CO, SB, and CMT directed the selection of CPR samples for analysis; KL, RKN, JCN, SB, and CO analyzed data; KL wrote the paper; RKN, KL, JCN, CO, and SB edited the paper; and all authors approved the final version.

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Data availability

All data generated in this study are available in this article and the associated supplemental materials.

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.scitotenv.2022.161222.

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Supplementary Information

Historical biomonitoring of pollution trends in the North Pacific using archived samples from the Continuous Plankton Recorder Survey

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Fig. S1

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S2. Supplementary materials and methods

S2.1. Broad-spectrum targeted exposome profiling

Exposome profiling of xenobiotics in the plankton samples was conducted using both liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) in reverse phase mode (RP-MS/MS) and gas chromatography (GC)-MS/MS based on the chemical properties of the targets.

RP-MS/MS analysis was performed using a Shimadzu LC-20AD UHPLC system coupled with a SCIEX Qtrap 5500 MS/MS. Ten µL of ethanol extract was injected and separated using a Raptor Biphenyl column (150 × 2.1 mm, 2.7 µm) (Restek). The LC conditions were as follows: Mobile phase A: 90% H_2O with 0.1% formic acid and 10% MeOH, pH 4.0. Mobile phase B: MEOH-IPA (50:50, v/v) with 0.1% formic acid. The gradient was: 0 - 2 min 10% B, 2.1 - 4 min 40% B, 4 -12 min, linear ramping up to 100% B, 12 – 18 min 100% B, 19 - 24 min 10% B, 24.1 min stop. The flow rate was 250 µL/min, and the column temperature was controlled at 40 ºC. The MS/MS detection was performed using electrospray ionization (ESI) and by advanced scheduled multiple reaction monitoring (MRM). The ESI source conditions were set as follows: electrospray voltage of -4500V for negative mode and 5500V for positive mode, source temperature of 500 °C, curtain gas of 30, ion source gas 1, and gas 2 of 35 psi, respectively.

GC-MS/MS analysis was performed using an Agilent 8890 GC coupled with a 7010B triple quadrupole mass spectrometer. One µL of ethanol extract was injected in splitless mode with an ultra-inert inlet liner (Agilent, Catalog: 5190-2203), and the inlet temperature was kept at 280 ºC. The GC separation was performed using two fused silica HP-5MS UI capillary columns of 15 m × 0.25 mm (inner diameter), 0.25 µm (thickness) (Agilent) connected by a pneumatic switching device (PSD) to facilitate backflushing between injections. High purity helium (99.999%) was used as the carrier and quench gas, and nitrogen was used as the collision gas. The oven temperature was

programmed as follows: 60 ºC for 1 min; 40 ºC /min to 120 ºC, and then 5 ºC /min to 310 ºC. Retention time (RT) locking was performed using chlorpyrifos-methyl as a standard, and flow rates were adjusted to achieve a retention time lock of 18.1 min. The triple quadrupole was operated in electron ionization (EI) mode, and the temperature for the transfer line was set at 310 ºC. The EI ion source was operated at 280 ºC for source temperature and 150 ºC for quadrupole temperature. The dynamic multiple reaction monitoring (dMRM) was used for data acquisition with a gain factor of 10 and the solvent delay of 3 min. Backflushing was conducted after the analytical run at 310 ºC and 6 mL/min flow. The total run time was 40 min, followed by 5 min of backflushing, and 5 minutes for full oven and column cooldown. A total of 1001 anthropogenic compounds were targeted, including 209 analytes on the LC-MS/MS platform and 792 chemicals on the GC-MS/MS platform (Fig. S1A). MRM transitions and RT were optimized using the purified standards, and two MRMs transitions were used per target analyte. The full targeted lists and the associated MS/MS parameters and RTs are listed in Supplementary Tables S1 and S2. Robust quality control measures were employed throughout sample preparation and analysis.

S2.2. Peak vetting and verification

Peaks were initially screened using our in-house python peak vetting scripts according to their peak area, RT, signal-to-noise (S/N) ratios, and sample-to-blank ratios. The filtering criteria for peaks from LC-MS/MS were sample to blank ratios ≥ 10 for both MRM transitions. The cut-off criteria for peaks from GC-MS/MS were as follows: (1) S/N ratio ≥ 3 for MRM1; (2) S/N ratio ≥ 2 for MRM2; (3) RT difference ≤ 0.2 min; (4) Sample to blank ratio ≥ 10 for MRM1; (5) Sample to blank ratio ≥ 3 for MRM2; (6) Peak area for MRM1 ≥ 2000. Silk that had undergone the same silk-preparation procedure but not had been used to sample the plankton and 4% formaldehyde solution were used as background

blank controls and processed using the same protocol. Putative positive hits with the peak area at least 10-times higher than the blank controls were manually inspected and verified by the purified standards.

S2.3. Biomass normalization and missing value estimation

The plankton biomass was normalized using the geomean of four major phosphatidylcholine (PC) species, including PC(32:2), PC(16:0/18:1), PC(16:0/16:0), and PC(36:0). Raw exposome AUCs were then normalized by dividing each by the fraction: (PC geomean of the sample)/(Maximum PC geomean observed across all 30 samples). Compounds with > 25% missing values were removed for statistical analysis. Missing values in samples with fewer than 25% missing values were imputed by probabilistic principal component analysis (PPCA) using the same probabilistic distribution as the observed data (Nyamundanda et al., 2010).

S2.4. Quality Control (QC) and Assurance

The procedural blanks (Unused silk extract and pooled formalin) were analyzed in each batch for quality control. No quantifiable concentration of the targeted anthropogenic compounds was identified in the unused silk extract.

Additionally, two levels of reproducibility were checked daily before passing the exposomic results for data analysis: sample QC and platform QC. In the first level, internal standards (16:0-d31 ceramide, chlorpyrifos-methyl, p,p′-Methoxychlorolefin, and acenaphthylene) were added to every biological sample, extracted, and the peak shape, retention time shift, and peak area variability of spiked labeled internal standards were inspected in each sample. Samples that failed this QC analysis were reinjected the next day. In the second level of quality control, the stability of LC-MS/MS and GC-MS/MS instruments were assessed using 3 replicate injections per day of a standardized lot of pooled human plasma extract containing spiked purified standards of common

anthropogenic compounds (206 compounds for LC-MS/MS analysis at the concentration of 0.1 µM, and 222 compounds for GC-MS/MS at the final concentration of 0.5 µg/mL). The AUCs of 57 representative standards for LC-MS/MS and 96 for GC-MS/MS in the replicate QC samples were monitored daily for platform process control. The concentrations of chemicals found in the plankton exposome were calculated after subtraction of the highest blank AUC from pooled 4% formalin or unused CPR silk using Equation 1.

$$\text{Sample concentration (µg/mL)} = \text{(Concentration of the purified standard in µg/mL)} \times \text{(AUC of the sample - blank)} \div \text{(AUC of the purified standard – blank)} \quad (1)$$

Reproducibility was quantified by calculating the within-day and within-day plus between-day Pearson correlations and the median of the relative standard deviations (RSDs). The intra-day and inter-day Pearson correlations for QC injections were ≥ 0.999 and 0.998, respectively. The intra-day and inter-day median RSDs of replicated QC injections for GC-MS/MS on 3 days were 11% (IQR: 5.9% - 23.5%) and 21% (IQR: 16.5% - 28.0%), respectively. The intra-day and inter-day median RSDs of replicated QC injections for LC-MS/MS on 3 days were 9.2% (IQR: 5.8%-13.3%) and 11.8% (IQR 8.7%-17.2%), respectively.

Supplementary References

Nyamundanda G, Brennan L, Gormley IC. Probabilistic principal component analysis for metabolomic data. BMC Bioinform. 2010; 11: 571.

Supplementary Tables as a single Excel file

Table S1. List of common anthropogenic chemicals targeted by GC-MS/MS.

Table S2. List of compounds measured using LC-MS/MS.

Table S3. List of detected anthropogenic chemicals, raw peak areas under the curve (AUCs) and detection frequencies in plankton samples.

Table S4. Concentrations of anthropogenic chemicals and detection frequencies in the plankton exposome.

Table S5. Raw plankton biomass data for the correlation analysis with exposome (cells/m^3 sea water)

Table S6. Raw fisheries data used for exposome-wide association analysis.

Table S7. Pearson and Spearman correlations between marine plankton exposome and fisheries health indicators.

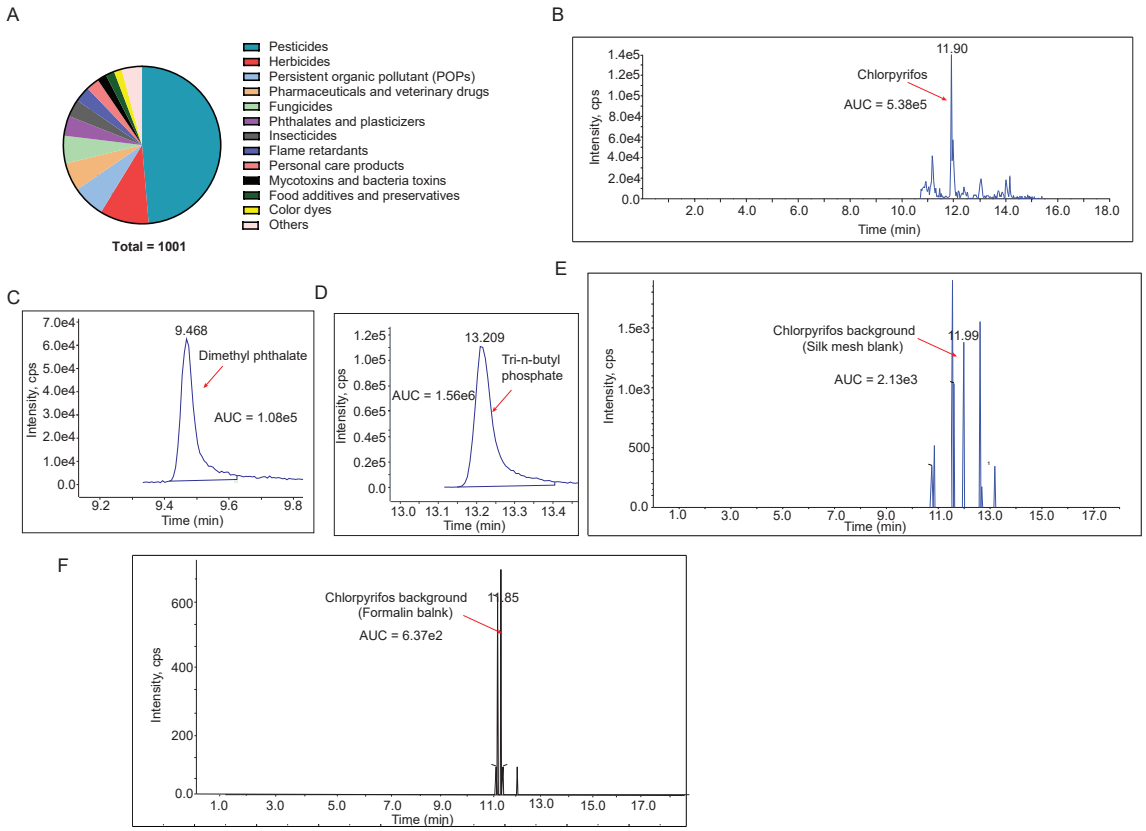

Fig. S1. The anthropogenic compounds screened in our broad-spectrum targeted exposomics platform (A), the representative chromatograms in plankton samples (B – D), a silk mesh background blank (E), and a 4% pooled formalin blank (F). The peak areas of anthropogenic chemicals found in the CPR plankton samples were 21 to 5,637 times greater than the highest blanks.