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# Relative exposure to microplastics and prey for a pelagic forage fish

#### J M Chavarry<sup>1,5,\*</sup> , K L Law<sup>2</sup>, A D Barton<sup>1,3</sup>, N M Bowlin<sup>4</sup>, M D Ohman<sup>1</sup> and C A Choy<sup>1</sup>

Integrative Oceanography Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093, United States of America

<sup>2</sup> Sea Education Association, Woods Hole, MA 02543, United States of America

<sup>3</sup> Department of Ecology, Behavior and Evolution, University of California San Diego, La Jolla, CA 92093, United States of America

Southwest Fisheries Science Center, National Oceanic and Atmospheric Administration, San Diego, CA 92037, United States of America

<sup>5</sup> Present address: Integrative Oceanography Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla,

CA 92093, United States of America.

\* Author to whom any correspondence should be addressed.

E-mail: j4chavar@ucsd.edu

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### Abstract

LETTER

In the global ocean, more than 380 species are known to ingest microplastics (plastic particles less than 5 mm in size), including mid-trophic forage fishes central to pelagic food webs. Trophic pathways that bioaccumulate microplastics in marine food webs remain unclear. We assess the potential for the trophic transfer of microplastics through forage fishes, which are prey for diverse predators including commercial and protected species. Here, we quantify Northern Anchovy (Engraulis mordax) exposure to microplastics relative to their natural zooplankton prey, across their vertical habitat. Microplastic and zooplankton samples were collected from the California Current Ecosystem in 2006 and 2007. We estimated the abundance of microplastics beyond the sampled size range but within anchovy feeding size ranges using global microplastic size distributions. Depth-integrated microplastics (0-30 m depth) were estimated using a depth decay model, accounting for the effects of wind-driven vertical mixing on buoyant microplastics. In this coastal upwelling biome, the median relative exposure for an anchovy that consumed prey 0.287-5 mm in size was 1 microplastic particle for every 3399 zooplankton individuals. Microplastic exposure varied, peaking within offshore habitats, during the winter, and during the day. Maximum exposure to microplastic particles relative to zooplankton prey was higher for juvenile (1:23) than adult (1:33) anchovy due to growth-associated differences in anchovy feeding. Overall, microplastic particles constituted fewer than 5% of prey-sized items available to anchovy. Microplastic exposure is likely to increase for forage fishes in the global ocean alongside declines in primary productivity, and with increased water column stratification and microplastic pollution.

## 1. Introduction

Microplastic particles (<5 mm in size) enter marine food webs when animals directly and indirectly consume them [1, 2]. Direct consumption occurs when an organism ingests microplastic from the environment; indirect consumption occurs when an organism ingests prey containing microplastic. Currently, more than 380 marine species are known to consume microplastics including mammals [3], zooplankton [4], mollusks [5, 6], sea turtles [7], and fishes [8]. Direct and indirect microplastic consumption may exert a range of impacts on organism physiology and fitness, such as disruption to feeding, reproduction, and immunity [9, 10]. The extent of physiological and ecological consequences of microplastic consumption is not well known. More so, the relative contributions of direct and indirect microplastic consumption to the broader cycling of microplastics within marine food webs remain unknown.

Juvenile and adult Northern Anchovy (*Engraulis* mordax,  $\sim$ 1–4 years old) feed on prey in the same

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size range (0.287-5 mm) as microplastic particles [11, 12]. As anchovy grow the morphology of their feeding apparatus changes and limits their consumption of smaller particles and prey [12]. Filter-feeding forage fishes such as anchovy may be particularly susceptible to high levels of microplastic ingestion because they can filter multiple liters of water per minute [13, 14]. Microplastic consumption by anchovy has been examined with laboratory studies and fish gut content analyses [15-21]. However, none have examined the initial exposure of anchovies to microplastics relative to their natural zooplankton prev across their vertical habitat. More than 490 marine species feed on juvenile and adult forage fishes (including anchovies), such as seabirds (pelicans), marine mammals (sea lions), and commercial fish species (tuna) [22–25]. Given the wide range of predators that feed on anchovy, they are thus a likely trophic pathway for the cycling of microplastic particles within marine food webs.

We assess the relative exposure of Northern Anchovy to microplastics compared to their primary zooplankton prey, which we refer to as anchovy microplastic exposure in this paper. Our study investigates anchovy microplastic exposure in the southern California Current Ecosystem (CCE). The CCE is a productive eastern boundary upwelling system that supports commercial fisheries, marine mammals, and protected species such as sea birds [26]. We analyze hydrographic, microplastic, and zooplankton data concurrently collected across four seasons in 2006 and 2007. Specifically, we quantify: (1) the relative exposure of Northern Anchovy to microplastics compared to their natural prey in the southern CCE; (2) spatial and temporal shifts in relative microplastic exposure; and (3) the role of anchovy body size on relative microplastic exposure. Our study provides a preliminary assessment of how, and to what degree, microplastics enter marine food webs through midtrophic level species.

## 2. Methods

Northern Anchovy (Engraulis mordax) are present throughout the mixed layer (~0-20 m depth) in the CCE (figure 1(a)) [27]. To quantify Northern Anchovy microplastic exposure in the CCE, we first performed a literature search to identify concurrently collected microplastic and zooplankton abundance data. We searched for articles with the keywords 'California' AND 'microplastic' AND 'zooplankton' in Web of Science. Our review produced a single applicable study region-the southern CCE. We incorporated historical data from the southern CCE collected through the California Cooperative Oceanic Fisheries Investigations (CalCOFI) and California Current Ecosystem Long Term Ecological Research programs (figure 1(a)). Microplastic and zooplankton samples were taken on lines 80 and 90 of the

CalCOFI sampling grid during four research cruises from 2006 (April, July, October) and 2007 (January) (figures 1(b) and (c)).

#### 2.1. Anchovy prey size estimation

Anchovy gill raker spacing limits the size of the smallest microplastic particle and prey an anchovy can consume [12]. To quantify shifts in anchovy exposure to microplastics and zooplankton within the relevant size ranges for anchovies of different ages, we used a historical dataset that documented increases in gill raker spacing with anchovy growth [12]. We estimated exposure for juvenile to adult anchovy,  $\sim$ 1–4 years old, ranging in size from 78–150 mm in standard length [29]. Juvenile to adult anchovy had corresponding increases in gill raker spacing from 0.287 to 0.493 mm (figure 2(a)). Both juveniles and adults preferentially feed on larger mesozooplankton [30] and derive a significant portion of their energy from zooplankton up to 5 mm in size [11]. Thus, we set the upper size limit of anchovy prey items to be 5 mm to correspond with the maximum established microplastic particle size range (figure 2(b)).

# 2.2. Surface microplastic collection and abundance estimates

Doyle *et al* (2011) provides a detailed description of how plastic particles were collected at sea [31]. Briefly, plastics from 0.714–15 mm in size were collected using a 505- $\mu$ m mesh manta net towed at the surface for approximately 15 min at ~1.5–2 knots (n = 79tows, figure 1(a)). We assumed that most microplastic particles are non-spherical with the potential to pass through the net dependent on particle orientation. Thus, only microplastics larger than or equal to the diagonal of the mesh opening (>0.714 mm) were likely reliably collected. The longest dimension of each microplastic particle was recorded by an independent laboratory as detailed in Doyle *et al* (2011) [31].

We applied the correction factor (*CF*) from Koelmans *et al* (2020) to the sampled plastic size range to estimate microplastic abundance within the feeding size range of anchovy [32] figure(2(b)). The sampled (or measured) plastic size minimum ( $x_{1M}$ ) and maximum ( $x_{2M}$ ) was 0.714 and 15 mm, respectively. The default (or estimated) plastic size minimum ( $x_{1D}$ ) and maximum ( $x_{2D}$ ) within the feeding size range of anchovy was 0.287 and 5 mm, respectively. The default plastic size minimum changed with anchovy growth, increasing from 0.287 to 0.493 mm for juvenile to adult anchovy. A fitting parameter, a = 1.6, was included and based on 14 size distributions of microplastics [32, 33]. The full equation is detailed below:

$$CF = \left(x_{2D}^{1-a} - x_{1D}^{1-a}\right) \left(x_{2M}^{1-a} - x_{1M}^{1-a}\right)^{-1}$$



**Figure 1.** Methods and sampling region. (a) Sources and modeling techniques used to quantify microplastic and zooplankton abundances in the California Current Ecosystem. *Left*: Northern Anchovy (*Engraulis mordax*) largely forage from 0 to 20 m depth, where they encounter microplastics and their zooplankton prey. *Center: In situ* sampling of surface microplastics using a manta net (top) and depth-integrated sampling of zooplankton using a PRPOOS (Planktonic Rate Processes in Oligotrophic Ocean Systems) net (bottom). *Right*: Model estimation of positively buoyant plastics not sampled due to vertical mixing from wind stress. ((b) and (c)) CalCOFI (California Cooperative Oceanic Fisheries Investigations) sampling grid overlain on monthly mean wind speed (m s<sup>-1</sup>) at 10 m above the sea surface. Surface microplastics and depth-integrated zooplankton samples were collected and enumerated at all filled in stations (*n* = 20 stations per cruise). Wind speed data are from the Cross-Calibrated Multi-Platform Ocean Surface Wind Analyses for (b) October 2006 (c) and January 2007 [28]. Illustrations in (a) by K Lance.

# 2.3. Depth-integrated (0–30 m depth) microplastic abundance estimates

Wind speeds greater than 4 m s<sup>-1</sup> at 10 m above the sea surface  $(U_{10} > 4 \text{ m s}^{-1})$  drive near-surface vertical mixing that transports positively buoyant microplastics beneath the sea surface [34]. We used a simple model to estimate the total abundance of positively buoyant microplastics,  $N \ (\# \text{ m}^{-3})$ , including those not sampled due to wind-driven mixing and the downward transport of microplastics (figure 1(a)) [34]. See table A1 for a full description of model parameters and values. The depth decay model accounted for the abundance of microplastics collected in the net,  $N_{tow} \ (\# \text{ m}^{-3})$ , the immersion depth of the net,  $d \ (m)$ , the microplastic rise speed,  $w_b \ (m \ s^{-1})$ , and near-surface mixing due to breaking waves,  $A_o \ (m^2 \ s^{-1})$ :

$$N = N_{tow} * (1 - \exp(-dw_b A_o^{-1}))^{-1}$$

The rise speed of plastic,  $w_b$ , was not directly measured. Based on our particle size range, we selected a rise rate of 0.009 m s<sup>-1</sup> which corresponded with measured rise speeds of microplastics ranging from, 0.5–1 mm in size [35].

Following Kukulka *et al* (2012) [34], the degree of near-surface mixing,  $A_o$  (m<sup>2</sup> s<sup>-1</sup>), was estimated based on the drag coefficient,  $C_d$ , air density,  $\rho_{air}$ (kg m<sup>-3</sup>), the wind speed 10 m above sea surface,  $U_{10}$ (m s<sup>-1</sup>), water density,  $\rho_{water}$  (kg m<sup>-3</sup>), the von Karman constant,  $\kappa$ , gravity, g (m s<sup>-2</sup>), and wave age,  $\sigma$ :

$$A_o = 1.5 \left(\frac{C_d \rho_{air} U_{10}^2}{\rho_{water}}\right)^{\frac{1}{2}} \kappa 0.96 g^{-1} \sigma^{\frac{3}{2}} C_d U_{10}^2$$

Parameter values used to estimate  $A_o$  were taken from best available data. For April, July, and October 2006, we used underway ship data recorded at the time of the net tows. Underway ship data were not available for January 2007-we instead used hydrographic data recorded during a CTD cast on this same cruise at the same stations where the net tows occurred. Seawater density for all cruises was taken from hydrographic data collected at the same stations as the net tows. The *cdnlp* function from the seamat package in MATLAB was used to convert recorded air temperature and relative humidity into air density (for cruises in 2006) and recorded dry and wet temperature into relative humidity and then air density (for January 2007). For all cruises, the *cdnlp* function from the sea-mat package in MATLAB was used to convert recorded wind speed and ship anemometer height into wind speed 10 m above the sea surface  $(U_{10})$  and the drag coefficient  $(C_d)$ . A component of near-surface mixing, wave age,  $\sigma$ , was not readily available. To estimate wave age, we used available underway ship data to investigate wind speed and wind direction pre- and post-net tows. Winds were primarily unidirectional and steady surrounding the collection (figure A1). As such, we chose a wave age of 35 to correspond with a fully developed sea [34]. The instantaneous wind speeds during net tows are available in figure A2.



Figure 2. The range in Northern Anchovy prey size in relation to the size of sampled microplastics and zooplankton. (a) Change in mean inter-raker gill spacing for juvenile and adult anchovy with fish body growth (n = 29). Vertical lines represent the approximate age of anchovy in years. Increase in gill raker spacing with increasing anchovy standard length taken from Rykaczewski (2009) [12]. Length-age relationship of northern anchovy taken from Baxter (1967), originally from Clark and Phillips (1952) [29]. Curve and shading represent a LOESS fit with a 95% confidence interval. (b) The range in anchovy prey size shifts from 0.287-5 mm to 0.493-5 mm for juvenile to adult anchovy due to shifts in gill raker spacing with anchovy growth. Microplastics from 0.714 to 15 mm in size were collected. Raw microplastic abundances were size-corrected based off Koelmans et al (2020) to estimate the abundance of microplastics within anchovy feeding size ranges (0.287-5 mm) [32]. Zooplankton from 0.286 to 5 mm in size were collected. (c) The proportional abundance of depth-integrated, size-corrected microplastics (blue) and zooplankton (orange) in relation to their size. Proportional abundance based on the median total abundance of microplastic particles and zooplankton individuals across all stations and sampling periods. Line type represents the size range of prey that all size classes of anchovy feed on (---) versus the size range of prey that only smaller anchovy can consistently feed on  $(\cdot -)$ .

The estimated maximum depth of microplastic particle transport was based on the same wind mixing model from Kukulka *et al* (2012) [34]; between the surface and this maximum depth is where 99% of microplastic particles were present [34]. The abundance of microplastic particles at a given depth, n(z) (# m<sup>-3</sup>), is a function of the microplastic particle abundance at the surface,  $n_o$  (# m<sup>-3</sup>), the depth, z (m), plastic rise speed,  $w_b$  (m s<sup>-1</sup>), and near-surface mixing,  $A_o$  (m<sup>2</sup> s<sup>-1</sup>):

$$n(z) = n_o \exp(zw_b A_o^{-1})$$

# 2.4. Zooplankton collection and abundance estimates

Zooplankton individuals with a longest dimension between 0.286 and 5 mm in size were collected using a 202-µm Planktonic Rate Processes in Oligotrophic Ocean Systems (PRPOOS) net (figures 1(a) and 2(b)). The PRPOOS net was towed vertically, ascending from  $\sim$ 210 m to the surface at a rate of 50 m per minute, during the day (n = 44 tows)and night (n = 35 tows) (figure A3). We again assumed most zooplankton are non-spherical, and thus that only zooplankton larger than or equal to the diagonal of the mesh opening were consistently collected (>0.286 mm). See Gorsky et al (2010) for a full description of zooplankton quantification and measuring methods [36]. Briefly, all individuals from a representative aliquot of zooplankton from each PRPOOS sample were digitally measured and enumerated using ZooScan imaging. The maximum dimension of each zooplankton individual was measured. We assumed that zooplankton were uniformly distributed throughout the vertical sampling region when estimating zooplankton abundance (#  $m^{-3}$ ). The primary constraint on anchovy prey selectivity is prey size, not taxonomy [37]. Therefore, we assumed all zooplankton with the longest dimension within the feeding size range of anchovy (0.287-5 mm) were available for foraging (figure 2(b)).

#### 2.5. Calculation of anchovy relative exposure

We assumed that microplastic particles and zooplankton prey had an equal likelihood of being consumed by anchovies. The relative microplastic exposure ratio of an anchovy, *ER*, was based on the abundance of microplastics, M (# m<sup>-3</sup>), and the abundance of their natural zooplankton prey, *Z* (# m<sup>-3</sup>) at each station. *ER* was calculated as follows:

$$ER = \frac{M}{Z}$$

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#### 2.6. Statistical analyses

We tested for significant temporal and spatial differences in (1) microplastic abundance, (2) zooplankton abundance and, (3) anchovy relative exposure to microplastics. Non-parametric tests were used as our data were not normally distributed. The Spearman Rank correlation test, Kruskal-Wallis test, and Mann-Whitney U test were used to examine differences in abundances and the relative microplastic exposure with distance from shore, sampling month, and time of day, respectively. To visualize differences in microplastic particle and zooplankton abundances with distance from shore, we used a LOESS fit with 95% confidence intervals. Median abundance and exposure values for nearshore and offshore environments were calculated based on the median sampling distance from shore, 200 km. There were n = 40nearshore stations ( $\leq 200$  km) and n = 39 offshore stations (>200 km). The proportional abundances of different size classes of zooplankton and microplastics were based on the median abundance of microplastic particles and zooplankton individuals across all stations and sampling periods. All statistical analyses were done using R version 1.4.1106 [38].

## 3. Results and discussion

Microplastic and zooplankton samples were collected from the southern CCE in 2006 and 2007 across four seasons (figure 1(a)). There were changes in wind speed and near-surface mixing across and within sampling periods in the CCE (figures 1(b) and (c)). The abundance of microplastics and zooplankton available to foraging anchovy differed with particle and prey size as well as sampling location, resulting in differences in anchovy microplastic exposure (figures 2(a)-(c)). Anchovy microplastic exposure varied with distance from shore, time of day, sampling month, and fish body size. The median relative exposure was 1 microplastic particle for every 3399 zooplankton individuals. Across all sampling periods and anchovy sizes, microplastic particles constituted fewer than 5% of the prey-sized items that anchovy potentially encountered. The rate and prevalence of microplastic particle consumption by anchovies is likely related to factors such as anchovy feeding selectivity. The relatively low exposure to microplastics we find suggests that anchovy did not represent a significant trophic pathway for microplastics into marine food webs in the CCE during the 2006 and 2007 surveys. Our results corroborate recent findings of low trophic transfer from forage fishes to their predators [39-41]. Our analysis was limited to historical data due to constraints in co-collected microplastic, zooplankton, and environmental data, however future studies may benefit from assessing recent anchovy microplastic exposure. Further research is required to comprehensively understand the impact of microplastic consumption

by mid-trophic level species such as anchovy within marine food webs.

#### 3.1. Microplastic abundance in the southern CCE

Juvenile (~1 year old) anchovy can consume microplastic particles as small as 0.287 mm in size [12], which is smaller than the size range of plastic particles that were enumerated in CCE surveys (figures 2(a)and (b)). For this reason, we applied a size correction to estimate microplastic particles that occur within both the feeding size range of anchovy and the collection size range of their zooplankton prey (0.287-5 mm). The median surface size-corrected microplastic particle abundance was 0.198 microplastic particles m<sup>-3</sup> across all stations and sampling periods (table 1). The surface size-corrected microplastic particle abundances are within an order of magnitude of other studies in the CCE and North Pacific Subtropical Gyre (NPSG) [42-46]. No significant differences in surface size-corrected microplastic abundances were found with distance from shore (Spearman's rank correlation, p > 0.05) or day-night (Mann Whitney U test, p > 0.05) across all cruises (table 1). However, surface size-corrected microplastic abundances did vary significantly by month (Kruskal–Wallis test, p < 0.05; figures 3(a)– (d)). Surface size-corrected microplastic abundances were highest in January 2007 and lowest in October 2006, with medians of 0.507 and 0.078 particles  $m^{-3}$ , respectively (table 1, figures 3(c) and (d)).

Wind-stress generates near-surface vertical mixing in the ocean and transports positively buoyant microplastic particles below the surface [47]. Nearsurface mixing during the sampling period likely prevented the collection of some positively buoyant plastics by surface net tows. Accordingly, we applied a wind correction to estimate the total depthintegrated abundance of positively buoyant microplastic particles. The median size-corrected microplastic abundance increased from 0.198 to 0.466 particles m<sup>-3</sup> between surface and depth-integrated estimates across all stations and sampling periods, signifying the importance of microplastic particles mixed deeper into the water column (table 1). These depth-integrated microplastic abundances corroborate other regional studies investigating subsurface microplastic abundances [45, 48, 49]. The extent that depth-integrated estimates of microplastic particle abundances differed from surface estimates varied by month (figures 3(a)-(d)). For January 2007, depth-integrated, size-corrected microplastic particle abundances were as high as an order of magnitude greater than surface, sizecorrected measurements (figure 3(d)). This difference is due to increased winds speeds offshore in January 2007.

A maximum wind speed of  $14 \text{ m s}^{-1}$  in January 2007 resulted in an estimated transport of microplastics down to 30 m depth. During these high wind **Table 1.** Microplastic and zooplankton abundances, and anchovy relative microplastic exposure. Statistical significance and median values for (1) the abundance of size-corrected microplastic particles and zooplankton individuals, sizes 0.287–5 mm, and (2) the ratio of microplastic particles to zooplankton individuals. Size-corrected and depth-integrated microplastic abundance estimates were based on Koelmans *et al* (2020) [32] and Kukulka *et al* (2012) [34], respectively. Samples were designated as 'nearshore' or 'offshore' based on the median sampling distance to shore (200 km), resulting in n = 40 nearshore samples ( $\leq 200$  km) and n = 39 offshore samples (> 200 km).

	Surface microplastic (# m <sup>-3</sup> )	Depth-integrated microplastic (# m <sup>-3</sup> )	Depth-integrated zooplankton (# m <sup>-3</sup> )	Microplastic abundance: zooplankton abundance		
Location						
Nearshore	0.224	0.369	1918	1:4973		
Offshore	0.164	0.565	536	1:714		
Statistical test		Spearman's Rank correlation				
Statistical results	$\rho = -0.1823693$	$\rho = 0.1152287$	$\rho = -0.8016336$	ho = -0.4997017		
	p = 0.1077	$p = 0.3119$ $p = < 2.2 \times 10^{-16}$		$p = 2.753 \times 10^{-6}$		
Month						
April-06	0.269	0.492	1590	1:3399		
Jul-06	0.114	0.459	1165	1:3951		
Oct-06	0.078	0.104	607	1:5941		
Jan-07	0.507	3.273	1066	1:407		
Statistical test		Kruskal–Wallis				
Statistical results	$\chi^2 = 43.947$	$\chi^2 = 37.746$	$\chi^2 = 4.5861$	$\chi^2 = 19.771$		
	$p = 1.549 \times 10^{-9}$	$p = 3.199 \times 10^{-8}$	p = 0.2047	$p = 1.893 \times 10^{-4}$		
Time of day						
Day	0.162	0.493	824	1:3667		
Night	0.254	0.462	932	1:3103		
Statistical test	Mann–Whitney U test					
Statistical results	W = 637	W = 787	W = 714	W = 767		
	p = 0.1909	p = 0.8706	p = 0.5858	p = 0.9803		

All samples (n = 79)



914

0.466





**Figure 3.** Relative exposure of anchovy to microplastics and their natural zooplankton prey. (a)–(d) Abundances of zooplankton ( $\bigcirc$ ), depth-integrated microplastics ( $\square$ ), and surface microplastics ( $\triangle$ ), sizes 0.287–5 mm, from collections in (a) April 2006 (n = 19), (b) July 2006 (n = 20), (c) October 2006 (n = 20), and (d) January 2007 (n = 20). Curves and shading represent a LOESS fit with 95% confidence intervals. For cases where wind speeds were relatively weak, the depth-integrated and surface microplastic abundances were similar, and the curves overlap. Distance offshore estimated from the CalCOFI stations to the closest point of land. (e) Zooplankton abundances and wind-corrected (depth-integrated) microplastic abundances (sizes 0.287–5 mm) across all stations and years (n = 79). Point colors represent *in situ* wind speed (m s<sup>-1</sup>) at 10 m above the sea surface during microplastic collections. Polygons represent convex hulls for April 2006 (yellow), July 2006 (orange), October 2006 (blue) and January 2007 (red). Curves represent the ratio of microplastic particles to zooplankton individuals, 1:100 (top) and 1:1000 (bottom). Plastic size correction factors were taken from Koelmans *et al* (2012) [32]. Wind-corrected, depth-integrated microplastic abundances of microplastic abundances for Kukulka *et al* (2012) [34]. Model estimates of microplastic abundances may be inaccurate during high wind conditions (wind speed at 10 m above the sea surface >12 m s<sup>-1</sup>). Data collected during these high wind periods are marked with a '+' in panels (d) and (e).

periods, the model estimated the depth-integrated microplastic abundance to be 40 times greater than the surface microplastic abundance. The wind mixing model may be unreliable when wind speed exceeds 12 m s<sup>-1</sup> as it may incorrectly predict the transport of positively buoyant microplastics beneath the mixed layer. Higher microplastic densities are associated with surface collections during calm, low-wind conditions [34]. Therefore, although the true depth-integrated microplastic abundances may not have been more than 40-fold higher than surface abundances, it is still likely that the majority of microplastics in areas of high wind were not collected. Depth-integrated microplastic abundances in regions where winds speeds were from 4 to 12 m s<sup>-1</sup> were approximately five times greater than measured surface values. Wind speeds were lower than 4 m s<sup>-1</sup> at a third of our stations (figure A2). For these locations, we assumed negligible surface mixing and that all positively buoyant microplastics were concentrated at the surface. Some evidence exists that wind mixing models underestimate the abundance of subsurface microplastics in low wind conditions [35].

The wind correction used to estimate depthintegrated microplastic particles is a simple model based on a suite of assumptions. Our approach does not account for physical and/or biological mechanisms that transport neutrally and negatively buoyant plastics throughout the water column [50]. In the CCE, microplastics are generally more abundant at the sea surface [31, 45, 48]. Smaller microplastic particles (0.100-5 mm in size) are most abundant below the mixed layer, peaking in abundance at 200 m depth within the central CCE [51]. As anchovy primarily forage from  $\sim$ 0–20 m depth [27], we did not consider microplastics deeper than 30 m in our analysis. We assumed that near-surface mixing was the primary driver of the vertical transport of microplastic particles, which disregards the potential role of seawater density differences. Finally, microplastic rise velocity determines the rate at which a vertically mixed, positively buoyant microplastic particle will return to the surface. The rise velocity of microplastic particles decreases with decreasing particle size [35, 47]. In the absence of available direct measurements of microplastics abundance in the smallest size class (0.287-0.5 mm) and their associated rise velocities, there may be errors in our estimated particle abundance.

Ocean circulation influences microplastic distributions and likely influenced microplastic particle abundances within our sampling region [50, 52]. There were no visible trends from nearshore to off-shore for depth-integrated, size-corrected microplastic particle abundances within the April, July, and October 2006 sampling periods (figures 3(a)-(c)). However, microplastic abundances did increase

slightly with distance from shore during January 2007 (figure 3(d)). This increase could be associated with plastic entrainment into the subtropical gyre, as observed in previous studies [45, 53]. Across our study region, depth-integrated, sizecorrected microplastic abundances ranged from 0 to 23 microplastic particles per m<sup>-3</sup>. Small-scale variations in microplastic abundances may be due to patchiness caused by physical drivers such as plastic laden runoff following a storm or the development of surface slicks [44, 54]. Depth-integrated microplastic abundances significantly varied by month (Kruskal–Wallis test, p < 0.05) and were highest in January and lowest in October, aligning with surface microplastic abundances (table 1, figures 3(c)) and (d)).

# 3.2. Depth-integrated zooplankton abundance in the southern CCE

Depth-integrated zooplankton abundances varied according to distance from shore, sampling period, and time of day, with a median of 914 zooplankton individuals per m<sup>3</sup> across all stations and sampling periods (table 1). Zooplankton abundances significantly varied with distance from shore (Spearman's rank correlation, p < 0.05), decreasing from nearshore to offshore waters within our sampling grid (table 1, figures 3(a)-(d)). In the CCE, coastal winds drive upwelling of nutrient-rich waters from beneath the mixed layer to the surface, boosting primary production and affecting zooplankton abundances [55]. As the effects of coastal upwelling decrease with distance from shore, increased abundances of zooplankton are expected, particularly in larger zooplankton, within nearshore waters [56]. In the region and during the time of this study, greater abundances of zooplankton and a greater relative abundance of larger individuals (equivalent circular diameter > 3.8 mm) were reported in nearshore waters [57]. We were unable to account for small-scale changes in the spatial distribution of zooplankton. Topographic features in the CCE (e.g. seamounts, canyons, and shelf breaks) may aggregate zooplankton, creating zooplankton 'hotspots' and increasing zooplankton availability to anchovy [58-61]. Upwelling in the CCE is strongest from March to August [62]. This peak in upwelling corresponded with the highest zooplankton abundances in April and July of 2006 (table 1, figures 3(a) and (b)). Similarly, upwelling is limited during the fall, corresponding with the lowest zooplankton abundances reported in October 2006 (figure 3(c)). It should be noted that the differences in zooplankton abundance across sampling months were not significantly different (Kruskal-Wallis, p > 0.05; table 1). Finally, zooplankton undergo diel vertical migrations, descending hundreds of meters below the foraging habitat of anchovies during the day [27, 63]. Zooplankton were collected during the day (n = 44 tows) and night (n = 35 tows) allowing us to investigate diel differences in zooplankton abundance (figure A3). Day and night median zooplankton abundances were 824 and 932 individuals per m<sup>3</sup>, respectively. Although these day-night differences were not significant during the time of our surveys (table 1, Mann–Whitney, p > 0.05), the diurnal vertical shift in the distribution of zooplankton prey may still decrease anchovy prey availability during the day. Overall, anchovy prey were most abundant nearshore, during the spring, and at night.

# 3.3. Relative exposure of northern anchovy to microplastic particles vs. zooplankton prey in the southern CCE

The median relative exposure for anchovies across their vertical habitat, and within their feeding size range (0.287–5 mm), was 1 microplastic particle for every 3399 zooplankton (table 1). Across all stations and years, zooplankton were more than an order of magnitude more abundant than microplastics (figure 3(e)).

Anchovy microplastic exposure varied with distance from shore, sampling period, and time of day. Ocean circulation and productivity patterns in the CCE should result in higher relative microplastics exposure offshore and with closer proximity to the NPSG. Nearshore-offshore differences in microplastic exposure were significant (Spearman's rank correlation, p < 0.05), increasing by an order of magnitude from nearshore to offshore (table 1). The reported median offshore exposure (1 microplastic particle: 714 zooplankton individuals) is approximately three orders of magnitude greater than exposure reports for planktivorous fishes near the central NPSG [43]. Our work differs from previous research by estimating the abundance of positively buoyant microplastics not captured by surface collections due to near-surface mixing. Thus, it is reasonable for us to report higher microplastic exposure across the vertical habitat of a planktivorous fish compared to studies that focus their analyses on the surface. We expect anchovy microplastic exposure to continue increasing with increased proximity to the central NPSG, but our available microplastic sampling data did not cover this full spatial gradient. Adult anchovies are primarily found nearshore in the CCE, and were likely not subject to increased microplastic exposure in offshore waters [64]. Across our sampling period, the lowest and highest median microplastic exposure occurred in October 2006 and January 2007, respectively, aligning with the overall lowest and highest microplastic abundances (figures 3(c) and (d)). Finally, anchovy microplastic exposure was higher during the day than at night (table 1), likely due to daily zooplankton migration patterns. Taken together, anchovy had the highest relative microplastic exposure in offshore waters, during the winter, and during the day.

# 3.4. Implications of anchovy morphology and behavior on their relative exposure

Anchovy morphology and behavior affect microplastic exposure regardless of microplastic and zooplankton prey abundances. Globally, microplastic particles increase in abundance with decreasing size [32]. Therefore, the smaller the microplastic particle a fish can consume, the higher their likelihood for increased microplastic exposure. The gill rakers of juvenile anchovy, ~1 year old, retain microplastic particles down to 0.287 mm in size. Conversely, adult anchovy,  $\sim 4$  years old, have wider gill raker spacing, preventing the consistent retention of microplastic particles smaller than 0.493 mm [12, 29] (figures 2(a) and (b)). The maximum exposure for juvenile to adult anchovy decreased from 1 microplastic particle: 23 zooplankton individuals to 1:33. Mahara et al (2022) [65] found that juvenile herring, a similarly zooplanktivorous species, had higher microplastic exposure than zooplankton and larval herring. This finding was similarly a product juvenile herring prey size and the size distribution of available particles and prey [65]. In the case of anchovy, feeding on large prey is more energetically favorable. Therefore, anchovy of any size may be less likely to seek out and ingest the smaller more abundant microplastic particles [30, 37, 66]. Anchovy typically filter feed during the day and particulate feed at night [66]. The less targeted feeding strategy of anchovies during the day would likely increase their relative microplastic exposure [13]. Overall, the highest microplastic exposure across all locations and sampling periods likely occurred for small, juvenile anchovy foraging during the day.

We could not account for anchovy food preferences or avoidance behavior when considering the potential relative microplastic exposure of anchovies. However, even if anchovy preferentially consumed all microplastic particles available, at a maximum, they would ingest one microplastic particle for every 20+ zooplankton individuals as microplastic particles constituted fewer than 5% of the available prey-sized items. The core habitat of Northern Anchovy is within the southern CCE, where our study was focused [67]. However, future investigations may expand into microplastic exposure throughout the wider habitat of Northern Anchovy across the central and northern CCE.

The potential physiological and ecological impacts of microplastic consumption on anchovy physiology and behavior are not well known. Ingested microplastic particles may cause intestinal damage, be retained indefinitely in the stomach, translocate to vital organs, or transfer chemical additives to fish [15, 68, 69]. Alternatively, microplastic particles may be eliminated or regurgitated by anchovies with minimal organismal impacts since microplastics are estimated to be retained in anchovy guts for approximately one



**Figure 4.** Conceptual schenatic detailing now projected changes in climate may affect the exposure of longe fishes to microplastics relative to their natural prey in offshore, open ocean regions. (a)–(c) Shading represents projected shifts from the current open ocean ecosystem state (blue) to the future state (orange). (a) & (b) CMIP5 model simulation and projections for (a) increases in stratification, defined as the density difference from 0 to 200 m depth, and (b) declines in net primary, productivity since 1990 [74]. Declines in net primary productivity may reduce the abundance and size of zooplankton individuals available for foraging anchovy. (c) Increase in microplastic exposure for Northern Anchovy with decreasing body size and gill raker spacing. Estimated based on the morphological constraint of anchovy's gill raker spacing on the smallest particle that can be consistently ingested [12]. (d) Current, offshore ecosystem state. Foraging fishes are exposed to zooplankton and positively buoyant microplastics (colored polygons) transported to depth via vertical mixing. (e) Expected future ecosystem state in offshore, open ocean regions. Increased stratification of the global ocean will limit the maximum depth positively buoyant microplastics can be transported to. Declines in productivity will likely decrease the size and abundance of available zooplankton prey. Declines in offshore primary productivity may also decrease both the body size and school size of forage fishes. Plastic pollution will likely increase microplastic availability to forage fishes. Illustration elements by K Lance.

day [68]. The peak of Northern Anchovy spawning is in the late winter—early spring (February—April) [67], corresponding with the peak in relative microplastic exposure for juvenile anchovy. As such, any negative consequences of microplastic ingestion by anchovy may be relevant for future assessments of anchovy recruitment.

Microplastic consumption by anchovies may also have consequences for the predators of anchovy, perhaps through the trophic transfer of microplastics. Anchovies are known to transfer pollutants to their predators, such as sea lions [70]. The potential for bioaccumulation of microplastic pollutants and trophic transfer of microplastic particles to anchovy predators depends on the rate of predation on anchovies and the number and sizes of ingested microplastics [71, 72]. Further investigation into the physiological and behavioral consequences of microplastic consumption on anchovy is necessary before we can fully understand how important anchovies are as a trophic pathway for microplastic cycling within marine food webs.

Other forage fish species may be more susceptible to microplastic ingestion than the Northern Anchovy. For example, Pacific Sardine (*Sardinops sagax*) can consume smaller particles on average than anchovies, preferentially filter feed [66], and are more abundant offshore in the CCE where zooplankton prey are less abundant [56, 64]. The large filtration area of sardines may also increase the number of particles they consume compared to anchovies [15]. Thus, sardines likely have a higher overall microplastic exposure than anchovies in the CCE. While sampling constraints prevented us from investigating sardine microplastic exposure, future studies may benefit from this examination.

# 3.5. Forage fishes exposure to microplastics with a changing climate

Given our findings and the clear linkages between climate change and marine plastic pollution [73], climate change in the coming century will likely alter the exposure of forage fishes to microplastic particles. Microplastic exposure in anchovies is constrained by (1) near-surface mixing (which transports microplastic particles to depth), (2) primary productivity (which limits zooplankton abundance), (3) anchovy body size (which controls the smallest particle that can be ingested), and (4) microplastic abundance in the surface ocean. Projected changes in global climate will likely alter each of these factors in offshore, open ocean environments, subsequently increasing the relative exposure of forage fishes to microplastics offshore. Although anchovies are typically a nearshore species, other forage fishes such as the Pacific Sardine are found offshore, and may be more affected by relative increases in microplastic exposure [64]. Surface waters in the open ocean (0–200 m) are projected to increase in stratification by up to 30% in the year 2100 [74] (figure 4(a)). Increased stratification will decrease the maximum depth of transport for positively buoyant microplastics, resulting in higher potential microplastic exposure for forage fishes that feed shallowly. Increased stratification of the ocean will also limit the transport of deep, nutrient-rich waters to the surface, reducing primary production on average. Although the impacts of climate change on coastal upwelling systems are not well constrained [75, 76], in the global ocean, net primary productivity is expected to decrease by up to 16% over the next century [74] (figure 4(b)). Lower net primary productivity may decrease the size and abundance of zooplankton prey [56], consequently increasing relative microplastic exposure for zooplanktivorous forage fishes. Lower productivity and prey availability may also decrease the overall body size and abundance of forage fishes in the future [77]. The smaller gill raker spacing associated with smaller anchovies and other forage fishes that filter feed would increase exposure to smaller, more abundant microplastics (figure 4(c)). Finally, marine plastic pollution has increased exponentially, and plastic inputs to the ocean are projected to continue increasing [78, 79] (figure 4(c)). Together, future shifts in the physical environment, prey field, fish body size, and plastic concentration will likely increase microplastic exposure for

forage fishes that filter feed in the open ocean (figures 4(d) and (e)).

## 4. Conclusions

Despite a growing literature of marine species ingesting microplastic particles across the global ocean, Northern Anchovy in the CCE had relatively low exposure to microplastics during the sampling period. Microplastics constituted fewer than 5% of all prey-sized items. The highest potential exposure to microplastics was for small, juvenile anchovy ( $\sim$ 1 year old) foraging offshore, during the day, in the winter. Due to continued increases in microplastic pollution, small forage fishes that filter feed in the open ocean are likely to experience higher levels of microplastic exposure in future global climate scenarios.

## Data availability statement

The data that support the findings of this study are openly available at the following URL: https:// bitbucket.org/anelachoy/microplastic\_exposure\_cha varry/src/master/.

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## Author contributions

C A C, K L L, and N M B conceptualized research; C A C supervised research and acquired funding; M D O generated data; J M C curated data with assistance from N M B; J M C performed data analysis and visualization with substantial assistance from C A C, K L L, and A D B; J M C wrote the paper; C A C, K L L, A D B, N M B and M D O reviewed the paper.

# Appendix A. Figures and tables

**Table A1.** Parameters used in the depth decay model to account for wind driven vertical mixing of positively buoyant microplastic particles. Parameters come from Kukulka *et al* [34], Kooi *et al* [35], Thorpe *et al* [80], and Taylor [81].

Variable	Name	Value	Units	Source
Pair	Air density	Varies	$\mathrm{kg}\mathrm{m}^{-3}$	Cruises 0604, 0607 and 0610: CalCOFI Underway Data reported air temperature and relative humidity, converted to air density by sea-mat in MATLAB. Cruise 0701: Hydrographic data from a CTD cast at the same station recorded dry and wet temperature. Converted to relative humidity using sea-mat in MATLAB, then converted again to air density.
$\kappa$	von Karman constant	0.4	N/A	Kukulka <i>et al</i> [34]
g	Gravity	$9.81 \text{ m s}^{-2}$	Kukulka <i>et al</i> [34]	
$\sigma$	Wave age	35	N/A	Kukulka et al [34], originally from
				Komen et al 1996
$w_b$	Plastic rise speed	0.009	${ m m~s^{-1}}$	Kooi <i>et al</i> [35]
d	Net immersion depth	0.155	m	CalCOFI Report
$U_{10}$	Wind speed at 10 m above sea surface	Varies	m s <sup>-1</sup>	Cruises 0604, 0607 and 0610: CalCOFI Underway Data wind speed and reported anemometer height converted to $U_{10}$ using <i>cdnlp</i> from sea-mat in MATLAB. Cruise 0701: Wind speed recorded during a CTD cast. Anemometer height inferred from a sketch. Converted to $U_{10}$ using <i>cdnlp</i> from sea-mat in MATLAB.
$u_{*}$	Frictional air velocity	$\sqrt{C_d U_{10}^2}$	${ m m~s^{-1}}$	Thorpe <i>et al</i> [80]
$H_s$	Significant wave height	$0.96 g^{-1} \sigma^{\frac{3}{2}} u_{*_a}^2$	m	Kukulka et al [34]
$C_d$	Drag coefficient	for $U_{10} < 4 = N/A$ for $U_{10} > 4 =$ MATLAB Toolbox Result	N/A	Cruises 0604, 0607 and 0610: CalCOFI Underway Data wind speed and reported anemometer height converted to Cd using <i>cdnlp</i> from sea-mat in MATLAB. Cruise 0701: Wind speed recorded during a CTD cast and anemometer height (inferred from a sketch of the ship) were converted to Cd using <i>cdnlp</i> from sea-mat in MATLAB.
au	Wind stress	$C_{40}$ in $U_{10}^2$	$\mathrm{N}\mathrm{m}^{-2}$	Taylor [81]
$\rho_{water}$	Water density	Varies	$kg m^{-3}$	Hydrographic data recorded surface seawater density during a CTD cast.
${\mathcal U_*}_w$	Frictional velocity of water	$\sqrt{rac{ au}{p_{water}}}$	${\rm m~s^{-1}}$	Kukulka et al [34]
$A_o$	Near-surface mixing due to breaking waves	1.5 $u_{*_w}\kappa H_s$	$m^2 s^{-1}$	Kukulka et al [34]
Ν	Total number of depth-integrated, buoyant plastic particles	$\frac{N_{PlasticCollected}}{1 - e^{-dw_{p}A_{o}^{-1}}}$	#	Kukulka <i>et al</i> [34]











## Appendix B. Datasets

All data and analytical code are available in a public repository (see Data Availability statement). Datasets are as follows:

**CalCOFIEffort.csv**—Data for the general Cal-COFI sampling grid. *cruise*—month of sampling. *line*—CalCOFI line of sampling. *station*—CalCOFI station of sampling. *latitude* and *longitude*—sampling location in decimal degrees.

**monthly\_wind\_CCMP.csv**—Monthly-averaged wind speed at 10 m above the sea surface from Atlas *et al* (2011) [28]. *time*—modeled month. *latitude* and *longitude*—modeled location in decimal degrees. *wspd*—wind speed (m  $s^{-1}$ ) 10 m above the sea surface.

AnchovyGillSpacing.csv—Change in anchovy gill raker spacing from Rykaczewski (2009) [12]. anch\_standard\_length\_mm—anchovy standard length in mm. gill\_raker\_spacing\_mm—anchovy gill spacing in mm.

ProportionalAbundancebySize.csv—Change in the median abundance of microplastics and zooplankton based on particle and prey size. size\_mm—the minimum particle or prey size. *median zp abun m3*—the median abundance of zooplankton prey available to a foraging anchovy (# m<sup>-3</sup>) within the size bin (size\_mm + 0.04 mm). relative\_zp-the median proportion of zooplankton prey available to a foraging anchovy (%) within the size bin (size\_mm + 0.04 mm). median\_plas\_abun\_m3-the median depth-integrated, size-corrected abundance of microplastic particles available to a foraging anchovy  $(\# m^{-3})$  within the size bin (size mm + 0.04 mm). relative\_plas-the median proportion of microplastic particles available to a foraging anchovy (%) within the size bin (*size\_mm* + 0.04 mm).

RelExpData.csv—Primary data used in the manuscript. cruise-month of sampling. line-CalCOFI line of sampling. station—CalCOFI station of sampling. time\_sampled-sampling time, either day or night. latitude and longitude-sampling location in decimal degrees. *surf\_plas\_avail\_per\_m3*—the abundance of surface microplastic particles within the feeding size range of an anchovy  $(\# m^{-3})$ . *depth\_int\_plas\_avail\_per\_nm3*—the abundance of depth-integrated microplastic particles within the feeding size range of an anchovy (#  $m^{-3}$ ). zoop\_avail\_per\_m3-the abundance of depthintegrated zooplankton available within the feeding size range of an anchovy (#  $m^{-3}$ ). *u10\_ms*—the wind speed 10 m above the sea surface at the time of plastic collection (m s<sup>-1</sup>). *distance\_to\_shore\_m*—the distance from the sampling station to shore, where shore is approximated by the CalCOFI station closest to shore (m).

**RelExpChangeWithAnchovySize.csv**—Change in anchovy exposure with increased anchovy size and

gill raker spacing. gill\_raker\_spacing\_um—the mean inter-gillraker spacing ( $\mu$ m). max\_exposure\_plas\_zp —anchovy maximum exposure to microplastic relative to zooplankton prey.

**Fu2016SI.csv**—CMIP5 modeled projections for changes in ocean stratification from Fu *et al* (2016) [74]. *strat\_perc\_change*—the projected change change in stratification from 0 to 200 m depth in the global ocean since 1990 (%).

**Fu2016NPP.csv**—CMIP5 modeled projections for changes in ocean net primary productivity from Fu *et al* (2016) [74]. *npp\_perc\_change*—the projected change change in net primary productivity in the global ocean since 1990 (%).

## **ORCID** iDs

J M Chavarry lo https://orcid.org/0000-0001-9932-3632

K L Law <sup>(b)</sup> https://orcid.org/0000-0002-5298-6751 M D Ohman <sup>(b)</sup> https://orcid.org/0000-0001-8136-3695

C A Choy lo https://orcid.org/0000-0002-0305-1159

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