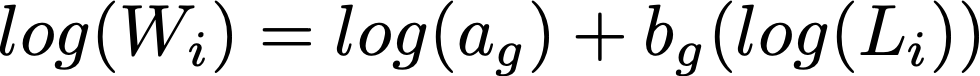
**Appendix A. DNA barcoding**

A subset of fish was barcoded to confirm the morphological species identifications. For these, a small piece of muscle tissue was dissected from the specimens, being careful to avoid external fish surfaces and gut areas. The tissues were placed in sterile 1.5 ml microcentrifuge tubes and stored in a -80 freezer until the DNA could be extracted. Genomic DNA was extracted with DNEasy Extraction Kits (Qiagen, Germantown, MD, USA) and a portion of the COI gene was amplified with PCR (Govindarajan *et al.*, 2023; Quigley *et al.*, 2023). Primer sets included Fish F1/R1 or Fish F2/R2 (Ward *et al.*, 2005) or HCO/LCO (Folmer et al., 1994) (Table S2). The PCR consisted of 95°C for 3 minutes, 35 cycles of 95°C for 30 seconds, 48°C for 30 seconds, 72°C for 1 minute, and 72°C for 5 minutes. Amplicons were visualized on 1.2% agarose gels stained with GelRed (Biotium, Hayward, CA, USA) and purified with Qiaquick PCR purification kits (Qiagen, Germantown, MD, USA). Purified DNA concentration was determined with a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Amplicons were sequenced in both directions at Eurofins Genomics. Raw sequence data was analyzed and consensus sequences were generated in the Geneious v.9.0.5 software platform (Biomatters, Inc). Sequences were compared to reference sequences in GenBank using BLAST. Matches with >98% identity over at least 90% of the sequence length were considered species identifications. DNA barcodes were deposited in GenBank (Table S2).

**Appendix B. Calculating missing weights using lengths and a hierarchical length-weight regression**

For some individuals, length was measured but not mass, yet the carbon transport model requires fish mass. Moreover, the taxonomic resolution among these samples varied—some were identified to species, others to genus or family. Thus data on length and mass across different taxa was used to estimate length-mass conversation parameters at a range of taxonomic levels.

For this we used a hierarchical random effects model that estimates traits *a* and *b* in the equation

  *(Eq. 3)*

where *a* and *b* are traits (the intercept and slope of the equation for log weight, respectively) that vary depending on the taxonomic group *g* of the individual *i,* while *Wi* and *Li* are mass and length for observation *i.* This model is described in detail by Thorson et al. (2017) as a multivariate model for trait evolution based on taxonomic trees. Traits are assumed to be random effects each with probability based on mean values of the parent taxonomic group (Thorson *et al*., 2017). Thus species-level traits are random effects within their genus, genus-level average traits are random effects within their family, and family-level average traits are random effects within their order. We then applied the length-weight conversion using parameters estimated at the lowest known taxonomic group for all observations where length but not mass was measured.

**Appendix C. Application of data filtering criteria**

The first data filtering criterion was that we only used tows that successfully fished the intended depth intervals for calculating areal biomass density of migrating versus non-migrating fish taxa, and for identifying migration depths and which taxa undergo diel vertical migration. From the MOCNESS-10, day tows 1 (May 6), 5 (May 13) and 7 (May 17) and night tows 2 (May 6), 4 (May 13), 6 (May 14), 8 (May 17), and 9 (May 18) were used (Fig. 4). For the MOCNESS-1, day tows 10 (May 6), 47 (May 17) and 89 (May 26) and night tows 11 (May 7), 48 (May 17) and 90 (May 26) were used (Fig. 4). These MOCNESS-1 tows were used in building the vertical distribution plot (Fig. 6), because these tows represent three, paired day and night tows that occurred in a similar time and place and thus are appropriate for making day versus night comparisons of biomass and distribution. These tows from the MOCNESS-1 were also more appropriate than the MOCNESS-10 tows to visualize vertical distribution (Fig. 6) because the depth strata were higher resolution (8 nets sampling instead of just 4 nets sampling from 0 - 1000 m), and there were more usable day tows (three instead of two). Thus, both day and night catch data were used to identify migration depths and whether fish taxa would be considered migrators or non-migrators in the fish carbon flux model.

The second data filtering criterion was to compare day and night tows between net types (MOCNESS-1 and MOCNESS-10) to determine whether there were differences in net capture efficiency. If catches were similar, net types would be pooled to increase sample size and generate a more representative sample. Based on catch results by tow (Fig. 4), only the night tows were chosen for further consideration because the MOCNESS-10 had consistently lower catches during the day. The relatively low MOCNESS-10 catches may be due to high net avoidance of this larger net during the day by mesopelagic fishes when there are potentially slightly higher light levels, even at their mesopelagic daytime depths. The MOCNESS-1 catches at night were still higher than the MOCNESS-10 catches at night, after standardization by volume filtered and depth interval sampled (grams of fish wet mass per square meter sampled). Night catches were more similar between the two nets. Therefore, only night tows were used for subsequent biomass estimation used in carbon flux calculations.

The third criterion for data filtering was to include as many fish taxa and as high a percentage of the total catch as possible within the bounds of our data limitations to focus the analysis on the most abundant taxa. The highest taxonomic resolution available for most fish was family level. The exceptions were the fish sorted and flash frozen at sea aboard the R/V *Sarmiento de Gamboa* from the MOCNESS-10 catch, as these were processed individually with morphological identification and, in many cases, genetic barcoding. Based on these results, fish of the families Myctophidae, Gonostomatidae, and Sternoptychidae made up ~90% of the catch. We focused the rest of the analysis on fish of these three families for the following reasons: First, the weights of many individuals were estimated using empirical length-weight regression models fit to cruise data for each family, which could only be done reliably on the more abundant families. Second, some of the rarer taxa (e.g., Alepocephalidae and Melamphaidae) tended to include larger individuals, which may be more likely to be piscivorous than zooplanktivorous. We restricted the scope of this analysis to fishes that are mainly zooplanktivorous because these were the vast majority of fishes in our catches, and because this avoided double-counting fish-mediated carbon flux that would appear in the mortality flux of prey individuals as well as the egestion, specific dynamic action, excretion and respiration fluxes of fish predators at higher trophic levels. Finally, determining which taxa were vertical migrators versus non-migrators from the cruise data (Fig. 6) was most reliable for abundant taxa with higher sample sizes.

**Appendix D. Table of fish-mediated carbon flux parameter values used in the carbon flux model**

Parameters used in the carbon flux model are provided in Table S1 with their nominal value, allowed range, units, and citation where applicable. Parameters without mean values were calculated rather than entered as inputs. Parameters without ranges were entered as a constant rather than allowing the value to range in the sensitivity analysis. Parameters without units are unitless. Parameters apply to both vertically migrating (VM) fishes like myctophids and hatchetfish and to non-migrating (NM) fishes like bristlemouths, unless otherwise noted. The abbreviation W stands for wet weight, DW for wet weight, and SDA for specific dynamic action.

Table S1. Parameters used for calculating fish-mediated carbon flux. Parameters are shown with their mean (nominal) value, range, units, and associated reference. Blank nominal (often the mean) values indicate that this parameter was calculated based on another parameter. Blank ranges indicate that the value entered is a constant and does not vary in the sensitivity analyses. Blank units indicate unitless parameters. The abbreviation W is used for wet weight, DW for wet weight, and SDA for specific dynamic action. d

|  | **Description** | **Nominal** | **Range** | **Units** | **Reference, data source, notes, or equation used to calculate parameter** |
| --- | --- | --- | --- | --- | --- |
|  | Intercept in respiration rate regression | 4.325 | ±2 SE |  | SE = 0.236, modified from Ikeda 2016 in McMonagle et al., 2023 |
|  | Coefficient for individual fish wet mass | 0.731 | ±2 SE |  | SE = 0.059, modified from Ikeda 2016 in McMonagle et al., 2023 |
|  | Coefficient for temperature | -6.899 | ±2 SE |  | SE = 1.56, modified from Ikeda 2016 in McMonagle et al., 2023 |
|  | Coefficient for migrating behaviour | 0.814 | ±2 SE |  | SE = 0.20, modified from Ikeda 2016 in McMonagle et al., 2023 |
|  | Indicator variable (1 for VM or 0 for NM) | 0 or 1 |  |  | Categorical habitat depth variable, modified from Ikeda 2016 in in McMonagle et al., 2023 |
|  | Mean temperature in epipelagic | 14 | (13.5, 14.5) | °C | Temperature measured using sensor on MOCNESS-10 |
|  | Mean temperature in mesopelagic | 11 | (10.5, 11.5) | °C | Temperature measured using sensor on MOCNESS-10 |
|  | Mean temperature across migration depth interval | 12.5 | (12, 13) | °C | Temperature measured using sensor on MOCNESS-10 |
|  | Activity factor while foraging | 2.5 | (1, 4) |  | Brett and Groves 1979; Smith and Laver 1981 |
|  | Activity factor while resting | 0.75 | (0.5, 1) |  | Brett and Groves 1979 |
|  | Activity factor while migrating (VM fish) | 2.5 | (1, 4) |  | Brett and Groves 1979; Smith and Laver 1981 |
|  | Respiration rate in units of oxygen utilization |  |  | μl O2/h | Calculated in carbon flux model (see Appendix B, Eq. B.1 in McMonagle et al., 2023) |
|  | Proportion of day resting | 0.455 | (0.44, 0.47) |  | Derived from R/V *James Cook* acoustic data at 38 kHz |
|  | Proportion of day migrating (VM fish) |  |  |  | Calculated in carbon flux model (see Appendix B, Eq. B.4b in McMonagle et al., 2023) |
|  | Proportion of day foraging |  |  |  | Calculated in carbon flux model (see Appendix B, Eq. B.4c in McMonagle et al., 2023) |
|  | Energy ingested |  |  | kJ/d | Calculated in carbon flux model (see Appendix B, Eq. B.5 in McMonagle et al., 2023) |
|  | Energy lost to metabolism (non-SDA) |  |  | kJ/d | Calculated in carbon flux model (see Appendix B, Eq. B.3 in McMonagle et al., 2023) |
|  | Energy lost to growth (VM fish) | 0.018 | ± 50% | kJ/d | Calculated from data in Childress et al., 1980 |
|  | Energy lost to growth (NM fish) | 0.007 | ± 50% | kJ/d | Calculated from data in Childress et al., 1980 |
|  | Proportion of ingestion lost to SDA | 0.14 | ± 20% |  | Brett and Groves 1979 |
|  | Proportion of ingestion lost to fecal waste | 0.2 | ± 20% |  | Brett and Groves 1979 |
|  | Proportion of ingestion lost to excretion | 0.07 | ± 20% |  | Brett and Groves 1979 |
|  | Proportion of oxygen utilization not due to SDA | 0.77 | (0.54, 1) |  | Brett and Groves 1979 (min =1-(0.14/0.30) = 0.54) |
|  | NM fish prey (zooplankton) of non-detrital origin | 0.6 | ± 50% |  | Steinberg et al., 2008 |
|  | Wet mass of individual fish |  |  | g |  |
|  | Ratio of respiration exported > flux boundary |  |  | kJ/d | Calculated in carbon flux model (see Eq. 1b in McMonagle et al., 2023) |
|  | VM fish non-SDA respiratory export flux |  |  | mg C/d | Calculated in carbon flux model (see Eq. 1a in McMonagle et al., 2023) |
|  | NM fish non-SDA respiratory export flux |  |  | mg C/d | Calculated in carbon flux model (see Eq. 1c in McMonagle et al., 2023) |
|  | Mean of gamma distribution for SDA flux | 3 | ± 80% | hr | Used in carbon flux model (see Appendix C, which informs Eq. C.5 in McMonagle et al., 2023) |
|  | Time to 90% SDA | 12 | (10, 14) | hr | Used in carbon flux model (see Appendix C, which informs Eq. C.5 in McMonagle et al., 2023) |
|  | Ratio SDA exported > flux boundary |  |  |  | Calculated in carbon flux model (see Appendix C, Eq. C.5 in McMonagle et al., 2023) |
|  | SDA export flux (VM fish) |  |  | mg C/d | Calculated in Eq. 2a in McMonagle et al., 2023 |
|  | SDA export flux (NM fish) |  |  | mg C/d | Calculated in Eq. 2b in McMonagle et al., 2023 |
|  | Ratio of fecal egestion exported > flux boundary | 0.7 | (0.5, 0.9) |  | Robison and Bailey 1981; Saba and Steinberg 2012 |
|  | Fecal pellet export flux (VM fish) |  |  | mg C/d | Calculated in Eq. 3a in McMonagle et al., 2023 |
|  | Fecal pellet export flux (NM fish) |  |  | mg C/d | Calculated in Eq. 3b in McMonagle et al., 2023 |
|  | Ratio of excreted carbon exported > flux boundary | 0.75 | (0.5, 1) |  | Assumed to be similar to export ratio of fecal egestion due to association with dense calcium carbonate |
|  | Carbon excretion rate | 0.008 | ± 80% | mg C/d | Wilson et al., 2009 and references therein |
|  | Excretion export flux (VM fish) |  |  | mg C/d | Calculated in Eq. 4a in McMonagle et al., 2023 |
|  | Excretion export flux (NM fish) |  |  | mg C/d | Calculated in Eq. 4b in McMonagle et al., 2023 |
|  | Asymptotic length of mesopelagic fish | 21 |  | cm | Gjøsaeter 1973; Childress et al., 1980; Wörner 1975 |
|  | Growth coefficient | 0.3 |  |  | Gjøsaeter 1973; Childress et al., 1980; Wörner 1975 |
|  | Intercept in mortality rate equation | -0.0066 |  |  | Pauly 1980 |
|  | Coefficient for asymptotic length | -0.279 |  |  | Pauly 1980 |
|  | Coefficient for growth coefficient | 0.6543 |  |  | Pauly 1980 |
|  | Coefficient for mean water temperature | 0.4634 |  |  | Pauly 1980 |
|  | Annual instantaneous mortality rate (VM and NM fish) | 0.606 | ± 50% |  | Calculated in Eq. 5a in McMonagle et al., 2023 |
|  | Mortality export flux (VM fish) |  |  | mg C/d | Calculated in Eq. 5b in McMonagle et al., 2023 |
|  | Mortality export flux (NM fish) |  |  | mg C/d | Calculated in Eq. 5c in McMonagle et al., 2023 |
|  | Oxycalorific coefficient | 0.0134 | ± 10% | kJ/mg O2 | Jobling 1994 |
|  | Respiratory quotient | 1.35 | (0.7, 2) |  | Brett and Groves 1979; Jobling 1994 |
|  | Maximum depth of migration of VM fish | 500 | (300, 700) | m | Based on results of vertical distribution (Fig. 6) |
|  | Minimum depth of migration for VM fish | 50 | (10, 100) | m | Based on results of vertical distribution (Fig. 6) |
|  | Flux boundary | 200 | (199, 201) | m | Either 200 m (commonly used fish carbon flux boundary) or 500 m. The 500 m flux boundary is only allowed to vary from 499 to 501 m. |
|  | Migration swimming speed (VM fish) | 0.04 | (0.02, 0.059) | m/s | Derived from R/V *James Cook* acoustic data at 38 kHz |
|  | Energy density of fecal pellets | 2 | (0.6, 3.5) | kcal/g DW | Bailey and Robertson 1982 |
|  | Ratio of carbon to dry weight of fecal pellets | 0.075 | (0.05, 0.1) |  | Saba and Steinberg 2012 |
|  | Ratio of carbon to wet mass of a VM or NM fish | 143 | (38, 250) | mg C/g W | Childress and Nygaard 1973 |
|  | Ratio of mortality flux > flux boundary | 0.65 | (0.40, 0.90) |  | Sutton and Hopkins 1996; Ochoa et al., 2013; Olson et al., 2014; O’Dwyer et al., 2015; Watanuki and Thiebot 2018; Stewart et al., 2018; Goetsch et al., 2018; Braun et al., 2019; McBride et al., 2022 |
| *q* | Capture efficiency | 0.275 | (0.05, 0.50) |  | Belcher et al., 2019; Williams and Koslow 1997; Pakhomov et al., 2019 |
| *cal* | Calibration factor correction | 1 | (1, 1.5) |  | Peter Wiebe, pers. comm. 26 January 2024. Allows for uncertainty in calibration factors used (4.63 for the MOCNESS-10 and 4.136 for the MOCNESS-1). Calibration factors can vary from about 4 to 6 for these flow meters. The maximum calibration factor correction of 1.45 accounts for the maximum potential flow meter calibration factor of 6. |

**Appendix E. Monte Carlo simulation results showing parameter sensitivities**

The figure below shows results of a Monte Carlo simulation (n = 1000) and the sensitivity of carbon flux estimates to 200 m by vertically migrating fishes (Fig. S1) and non-migrating fishes (Fig. S2) to both bioenergetic and biomass-related parameter uncertainty. Only parameters allowed to vary in the Monte Carlo simulation are plotted. On the x-axis, parameter values are converted into z-score units. For each parameter value draw, the resulting carbon flux estimate is provided on the y-axis. Descriptions of each parameter are provided in the table in Appendix D. Parameters and colour-coded and grouped according to the component of the carbon flux model in which the parameter appears. The inner 80th percentile is shaded in colour.Capture efficiency (*q*) and catch (areal biomass density in grams per square meters sampled) were the parameters that fish carbon flux estimates were most sensitive too, but some bioenergetic and movement parameters were also influential such as those relating to the respiration rates of these fishes (e.g., a0, a3, RQ, and AM).

A chart of different values

Description automatically generated with medium confidence

Fig. S1. Results of a Monte Carlo simulation (n = 1000) for vertically migrating mesopelagic fishes. The values of all parameters are converted to unitless z-scores on the x-axis. Carbon flux estimates to 200 m for each randomly assigned vector of parameter values are shown on the y-axis. Parameters and associated symbols are described in Table S1. Parameters are color-coded according to how they are used in carbon flux calculations. The inner 80th percentile range is shaded.

A chart of different values

Description automatically generated

Fig. S2. Results of a Monte Carlo simulation (n = 1000) for non-migrating mesopelagic fishes. The values of all parameters are converted to unitless z-scores on the x-axis. Carbon flux estimates to 200 m for each randomly assigned vector of parameter values are shown on the y-axis. Parameters and associated symbols are described in Table S1. Parameters are color-coded according to how they are used in carbon flux calculations. The inner 80th percentile range is shaded.

**Appendix F. Gut content analysis of mesopelagic fishes**

Gut content analysis data for a subset of mesopelagic fishes sampled during this cruise are available in the Github repository for this manuscript: <https://github.com/hmcmonagle/Fish-carbon-flux-N-Atlantic/blob/8244af58b4d30d63a7998208083409b78cae0c92/data/Gut_content_Llopiz_Lab_fish/OTZ_MorphID_PreyData_Entry_SG2105.xlsx>

**Appendix G. Zooplankton-mediated carbon flux results**

*Sampling*

Zooplankton metabolic contributions to active flux were calculated using abundance and dry mass analysis of the MOCNESS-1 on the RRS *James Cook*. Each net tow sample was split using a Folsom plankton splitter and processed using protocols described in Steinberg *et al*., (2008). Half of the sample was size-fractionated using nested sieves (200, 500, 1000, 2000, and 5000 µm), rinsed onto pre-weighed 0.2 mm Nitex mesh filters, and frozen at -20oC for biomass analysis. The other half sample was further split for additional analyses, with a portion preserved in sodium borate-buffered 4% formaldehyde for ZooScan image analysis. For the May 17th day and night tows the formaldehyde sample was size-fractionated using the same nested sieves prior to preservation. There were abundant large pteropods (>1.5 cm) in the MOCNESS sampling. These individuals were removed from the whole net sample, individuals enumerated, photographed, and preserved as a separate size fraction for dry mass analysis.

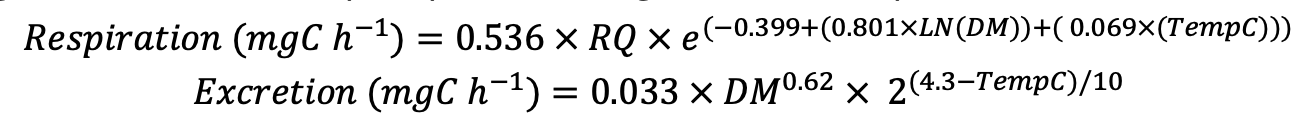
*Dry Mass*

Dry mass for zooplankton were measured on a Sartorius BP211D or Mettler AE 160 balance. First samples were thawed on paper towels to remove excess water (~ 20 minutes), then dried for at least 24 h at 60oC and re-weighed. Dry biomass of each size fraction within a net (mg m-3) was determined by dividing the biomass by the seawater volume filtered through the net during sampling. The average dry mass of the pteropods was 105.3 mg.

*ZooScan of abundance*

To estimate mesozooplankton abundance and average size, the formalin preserved samples from May 17 were imaged with a ZooSCAN version 3 at 2400 dpi as described in Maas et al. (2021). Briefly, at least 1500 particles per size fraction were scanned after subsampling using a Motoda splitter (Motoda, 1959). Raw images were processed in ZooProcess (Gorsky et al., 2010, Vandromme et al., 2012), then uploaded to EcoTaxa (https://ecotaxa.obs-vlfr.fr/; Picheral et al., 2017) for machine assisted identification. The entirety of the May 17 scan was manually validated for taxonomy. This dataset provides the abundance of zooplankton from each size fraction from each depth interval after correcting for fraction imaged and volume filtered. This average dry mass, which varied day vs. night and between depths (Table G1), was used to calculate the average metabolic contribution of a representative zooplankter within each size fraction using the allometric equations detailed below.

Physiological rates for both the pteropods and the general mesozooplankton were calculated as:

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where DM is the estimated average dry mass (mg) of an individual within a size fraction, RQ is 0.87 for copepods (Mayzaud et al., 2005), and 0.536 allows conversion from µL CO2 to µg C h-1. Temperature (TempC, °C) is the average temperature measured by the MOCNESS in the 200-1000 m interval and corrects the rates the average temperature below the flux boundaries (11.1°C). The remaining coefficients for the respiration equation are from Ikeda et al., (2001) and were chosen as the best predictive equation based on measured metabolic rates, while the equation for DOC comes directly from measured regressions to zooplankton size (Maas et al., 2021). POC was estimated as 31% of respiratory CO2 (Schnetzer and Steinberg, 2002; Steinberg *et al.*, 2012).

Once the rate for a representative organism for a particular net and fraction was computed, this was scaled to the community by multiplying by the estimated average size (Table G1) divided by the measured dry mass of that net (which equates to the abundance of individuals in a net for that tow) to calculate hourly production of waste for the zooplankton community (mg C m-3 h-1). This protocol was required because the abundance of individuals was only fully enumerated for the May 17th tows.

To calculate total active flux below a specific boundary, all of the night time respiration of mesozooplankton and pteropods above that boundary were summed and then subtracted from the daytime respiration of zooplankton above the boundary. This assumes that the physiological processes of these absent organisms were performed below the flux boundary during the day. This hourly rate of waste production was multiplied by the day length (15 h during the cruise) to estimate the total daily active flux of the mesozooplankton community at each flux boundary.

Table SG1: Dry mass (mg) of an average mesozooplankton individual from each size fraction of the daytime or nighttime tow from May 17th, 2021. The large pteropod average mass was estimated to be 105.3 mg at all depths and times.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| D/N | depth | 200 (mg) | 500 (mg) | 1000 (mg) | 2000 (mg) | 5000 (mg) |
| Day | 1000 - 750 | 0.0015 | 0.0142 | 0.0561 | 0.5323 | 1.0815 |
| Day | 750 - 500 | 0.0032 | 0.0075 | 0.0384 | 0.1451 | 0.1939 |
| Day | 500 - 400 | 0.0014 | 0.0067 | 0.0555 | 0.1252 | 0.4306 |
| Day | 400 - 300 | 0.0016 | 0.0069 | 0.0404 | 0.0584 | 0.2641 |
| Day | 300 - 200 | 0.0015 | 0.0091 | 0.0723 | 0.2104 | 0.1885 |
| Day | 200 - 150 | 0.0016 | 0.0074 | 0.0309 | 0.1184 | 0.1868 |
| Day | 150 - 100 | 0.0017 | 0.0081 | 0.0243 | 0.2397 | 0.0717 |
| Day | 100 - 50 | 0.0011 | 0.0021 | 0.0034 | 0.0118 | 0.4692 |
| Day | 50 - 0 | 0.0015 | 0.0031 | 0.0135 | 0.1075 | 0.4995 |
| Night | 1000 - 750 | 0.0013 | 0.0109 | 0.0471 | 0.1298 | 1.2558 |
| Night | 750 - 500 | 0.0018 | 0.0072 | 0.0384 | 0.2897 | 0.4097 |
| Night | 500 - 400 | 0.0013 | 0.0056 | 0.0374 | 0.1973 | 0.1358 |
| Night | 400 - 300 | 0.0014 | 0.0047 | 0.0286 | 0.0771 | 0.1351 |
| Night | 300 - 200 | 0.0018 | 0.0052 | 0.0123 | 0.0834 | 0.4262 |
| Night | 200 - 150 | 0.0015 | 0.0053 | 0.0268 | 0.0853 | 0.4215 |
| Night | 150 - 100 | 0.0014 | 0.0043 | 0.0182 | 0.0816 | 0.2749 |
| Night | 100 - 50 | 0.0012 | 0.0037 | 0.0280 | 0.1042 | 0.3633 |
| Night | 50 - 0 | 0.0014 | 0.0031 | 0.0049 | 0.0190 | 0.1863 |

Table SG2: Flux contributions by the large pteropod and mesozooplankton community to active flux (mg C m-2 d-1) below specified flux boundaries during the three MOCNESS tow periods.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Flux boundary | Date | Respiration  (CO2) | Excretion (DOC) | Egestion  (POC) | Total C |
| 500 | May 11 | 13.66 | 14.50 | 4.24 | 32.40 |
|  | May 17 | 20.04 | 23.10 | 6.21 | 49.35 |
|  | May 26 | 10.56 | 16.80 | 3.27 | 30.64 |
| 200 | May 11 | 23.59 | 20.30 | 7.31 | 51.21 |
|  | May 17 | 31.71 | 27.87 | 9.83 | 69.40 |
|  | May 26 | 33.18 | 28.75 | 10.28 | 72.21 |

**Appendix H. MOCNESS-1 and MOCNESS-10 flow meter calibration information**

For the larger MOCNESS-10 on the *R/V Sarmiento de Gamboa*, the original MOCNESS software and flow meter was used (Biological Environmental Sampling Systems, BESS, Falmouth, MA). The flow meter calibration factor, which is used by the BESS software to calculate volume filtered by each net on the MOCNESS-10, was determined as follows at the start of this cruise (before the first tow used to estimate fish biomass). The MOCNESS-10 was towed near the sea surface in a straight line with Net 1 open for 1 nautical mile (nm) and the number of flow meter counts was recorded. To account for the effect of any water currents on the resulting flow meter counts per meter travelled, this protocol was then repeated after the ship turned 180 degrees. During the turn, Net 1 was closed and Net 2 was opened. After the turn was complete, Net 2 was closed and Net 3 was opened to obtain a second flow meter count per meter travelled in the opposite direction for 1 nm. The final flow meter calibration factor of 4.63 meters per flow meter count was calculated as 1852/400, where 1852 converted from nautical miles to meters and 400 was the average flow meter count (363 counts per nautical mile in the first direction and 437 counts per nautical mile in the opposite direction).

For the smaller MOCNESS-1 on the *RRS James Cook*, the Scripps Institution of Oceanography’s MOCNESS software (either version 1.00g or 1.00e) was used with a pre-calibrated flow meter (Hydrobios 3) with a calibration factor of 4.136. Due to uncertainty in this calibration factor, and that flow meter calibration factors can vary from ~4 to 6 (P. Wiebe, *pers. comm.,* 2024), we re-calculated Fig. 4 with a flow meter calibration factor of 6 instead of 4.136 (Fig. S3). Even with this higher calibration factor, which leads to higher volume filtered and thus lower biomass, the smaller MOCNESS-1 net still had significantly higher biomass than the larger MOCNESS-10 net (Fig. S3) (Two-way ANOVA, p < 0.05). In the Monte Carlo sensitivity analysis, a calibration factor correction is used to determine the influence of a calibration factor increasing by 50% on carbon flux estimates, which would cause calibration factors considered to vary within the approximate known range of calibration factors for these flow meters (P. Wiebe, *pers. comm.,* 2024).

*A graph showing the number of days and months of the year

Description automatically generated with medium confidence*

Fig. S3. Fish biomass (0 - 1000 m) standardized by sampling effort, with the previous calibration factor of 4.136 for the MOCNESS-1 replaced with a potential maximum calibration factor of 6. Sampling effort is calculated as volume filtered multiplied by depth interval sampled for each net, summed by tow. Each bar indicates a distinct tow that began on the date in May indicated in x-axis labels. Day tows are shown on the left panel and night tows are shown on the right panel.

**Appendix I: Calculations of carbon sequestration for at least 100 years**

Carbon sequestration calculations, based on results from Siegel et al., 2021, can be found in the “code” folder of our public repository at:

https://github.com/hmcmonagle/Fish-carbon-flux-N-Atlantic/blob/main/code/09-C-sequestration-100-years.R

**Appendix J: Calculations comparing fish carbon flux in the eastern North Atlantic (this study) with whale carbon flux in the Southern Ocean (Durfort et al., 2022)**

Estimates of the contribution of whales to carbon flux in the Southern Ocean are taken from Durfort et al., 2022. These calculations comparing whale carbon flux to fish carbon flux from our study can be accessed in our public repository in the “code” folder at:

https://github.com/hmcmonagle/Fish-carbon-flux-N-Atlantic/blob/main/code/08-flux\_comparison\_bar\_plots.R

**References for supplementary material**

Bailey, T. G., and Robertson, D. R. 1982. Organic and caloric levels of fish feces relative to its consumption by coprophagous reef fishes. Marine Biology, 69: 45–50.

Braun, C. D., Gaube, P., Sinclair-Taylor, T. H., Skomal, G. B., and Thorrold, S. R. 2019. Mesoscale eddies release pelagic sharks from thermal constraints to foraging in the ocean twilight zone. Proceedings of the National Academy of Sciences, 116: 17187–17192.

Brett J. R. and Groves, T. D. D. 1979. Physiological energetics. *In* Bioenergetics and Growth, *Edited by* W. S. Hoar and D. J. Randall. Academic Press, New York. pp. 279-352.

Childress, J. J., and Nygaard, M. H. 1973. The chemical composition of midwater fishes as a function of depth of occurence off southern California. Deep Sea Research and Oceanographic Abstracts, 20: 1093–1109.

Childress, J. J., Taylor, S. M., Cailliet, G. M., and Price, M. H. 1980. Patterns of growth, energy utilization and reproduction in some meso- and bathypelagic fishes off Southern California. Marine Biology, 61: 27–40.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit i from diverse

metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3 (5), 294–299*.*

Gjøsæter, J. 1973. Age, growth, and mortality of the mygtophid fish, *Benthosema glaciale* (Reinhardt), from Western Norway. Sarsia, 52: 1–14.

Goetsch, C., Conners, M. G., Budge, S. M., Mitani, Y., Walker, W. A., Bromaghin, J. F., Simmons, S. E., *et al.,* 2018. Energy-Rich Mesopelagic Fishes Revealed as a Critical Prey Resource for a Deep-Diving Predator Using Quantitative Fatty Acid Signature Analysis. Frontiers in Marine Science, 5: 430.

Gorsky, G., Ohman, M. D., Picheral, M., Gasparini, S., Stemmann, L., Romagnan, J.-B., Cawood, A., Pesant, S. et al. 2010. Digital zooplankton image analysis using the ZooScan integrated system. J. Plankton Res., 32, 285–30.

Govindarajan, A. F., Llopiz, J. K., Caiger, P. E., Jech, J. M., Lavery, A. C., McMonagle, H., Wiebe, P. H., *et al.* 2023. Assessing mesopelagic fish diversity and diel vertical migration with environmental DNA. Frontiers in Marine Science, 10: 1219993.

Jobling, M., 1994. Fish Bioenergetics. Chapman & Hall, London, p. 95.

Maas, A. E., Gossner, H., Smith, M. J., and Blanco-Bercial, L. 2021. Use of optical imaging datasets to assess biogeochemical contributions of the mesozooplankton. Journal of Plankton Research, 43: 475–491.

McBride, L. E., Braid, H. E., Stevens, D. W., and Bolstad, K. S. R. 2022. Trophic ecology of the deep-sea squid *Moroteuthopsis ingens* (Cephalopoda: Onychoteuthidae) from the Chatham Rise, Aotearoa New Zealand. New Zealand Journal of Marine and Freshwater Research: 1–15.

McMonagle, H., Llopiz, J. K., Hilborn, R., and Essington, T. E. 2023. High uncertainty in fish bioenergetics impedes precision of fish-mediated carbon transport estimates into the ocean’s twilight zone. Progress in Oceanography, 217: 103078.

Ochoa, J., Maske, H., Sheinbaumc, J., and Candela, J. 2013. Diel and lunar cycles of vertical migration extending to below 1000 m in the ocean and the vertical connectivity of depth-tiered populations. Limnology and Oceanography, 58: 1207–1214.

O’Dwyer, P., Berrow, S., López-Suárez, P., and Oujo Lamao, C. 2015. Insights into the diet of a poorly known species: pygmy killer whale *Feresa attenuata* from Cape Verde, West Africa. African Journal of Marine Science, 37: 427–430.

Olson, R., Duffy, L., Kuhnert, P., Galván-Magaña, F., Bocanegra-Castillo, N., and Alatorre-Ramírez, V. 2014. Decadal diet shift in yellowfin tuna Thunnus albacares suggests broad-scale food web changes in the eastern tropical Pacific Ocean. Marine Ecology Progress Series, 497: 157–178.

Pauly, D. 1980. On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. ICES Journal of Marine Science, 39: 175–192.

Pakhomov, E. A., Podeswa, Y., Hunt, B. P. V., and Kwong, L. E. 2019. Vertical distribution and active carbon transport by pelagic decapods in the North Pacific Subtropical Gyre. ICES Journal of Marine Science, 76: 702–717.

Picheral, M., Colin, S. and Irisson, J.-O. (2017) EcoTaxa, a Tool for the Taxonomic Classification of Images. http://ecotaxa.obs-vlfr.fr.

Quigley, L. A., Caiger, P. E., Govindarajan, A. F., McMonagle, H., Jech, J. M., Lavery, A. C., Sosik, H. M., *et al.* 2023. Otolith characterization and integrative species identification of adult mesopelagic fishes from the western North Atlantic Ocean. Frontiers in Marine Science, 10: 1217779.

Robison, B. H., and Bailey, T. G. 1981. Sinking rates and dissolution of midwater fish fecal matter. Marine Biology, 65: 135–142.

Saba, G. K., and Steinberg, D. K. 2012. Abundance, Composition and Sinking Rates of Fish Fecal Pellets in the Santa Barbara Channel. Scientific Reports, 2: 716.

Schnetzer, A., and Steinberg, D. 2002. Natural diets of vertically migrating zooplankton in the Sargasso Sea. Marine Biology, 141: 89–99.

Steinberg, D. K., Lomas, M. W., and Cope, J. S. 2012. Long-term increase in mesozooplankton biomass in the Sargasso Sea: Linkage to climate and implications for food web dynamics and biogeochemical cycling: INCREASE IN MESOZOOPLANKTON AT BATS. Global Biogeochemical Cycles, 26.

Steinberg, D. K., Van Mooy, B. A. S., Buesseler, K. O., Boyd, P. W., Kobari, T., and Karl, D. M. 2008. Bacterial vs. zooplankton control of sinking particle flux in the ocean’s twilight zone. Limnology and Oceanography, 53: 1327–1338.

Stewart, J. D., Barroso, A., Butler, R. H., and Munns, R. J. 2018. Caught at the surface: myctophids make easy prey for dolphins and devil rays. Ecology, 99: 1894–1896.

Thorson, J. T., Munch, S. B., Cope, J. M., and Gao, J. 2017. Predicting life history parameters for all fishes worldwide. Ecological Applications, 27: 2262–2276.

Vandromme, P., Stemmann, L., Garc.a-Comas, C., Berline, L., Sun, X. and Gorsky, G. 2012. Assessing biases in computing size spectra of automatically classified zooplankton from imaging systems: a case study with the ZooScan integrated system. Methods in Oceanography, 1–2, 3–21.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., and Hebert, P. D. N. 2005. DNA barcoding Australia’s fish species. Philosophical Transactions of the Royal Society B: Biological Sciences, 360: 1847–1857.

Watanuki, Y., and Thiebot, J.-B. 2018. Factors affecting the importance of myctophids in the diet of the world’s seabirds. Mar Biol **165**(4): 79. doi:10.1007/s00227-018-3334-y.

Wiebe, P.W. 26 January, 2024. Discussed calibration factor variation of MOCNESS flowmeters.

Williams, A., and Koslow, J. A. 1997. Species composition, biomass and vertical distribution of micronekton over the mid-slope region off southern Tasmania, Australia. Marine Biology, 130: 259–276.

Wilson, R. W., Millero, F. J., Taylor, J. R., Walsh, P. J., Christensen, V., Jennings, S., and Grosell, M. 2009. Contribution of Fish to the Marine Inorganic Carbon Cycle. Science, 323: 359–362.

Wörner, F. G. 1975. Untersuchungen an drei Myctophidenarten Benthosema glaciale (Reinhardt, 1837), Ceratoscopelus maderensis (Lowe, 1839) und Myctophum punctatum (Rafinesque 1810) aus dem nordwestafrikanischen Auftriebsgebiet im Frühjahr 1972. Thesis, University of Kiel. 136 pp.