Quantifying uncertainty in the contribution of mesopelagic fishes to the biological carbon pump in the Northeast Atlantic Ocean

Helena McMonagle1,2\*, Joel K. Llopiz2, Amy E. Maas3, Deborah K. Steinberg4, Annette F. Govindarajan2, and Timothy E. Essington1

1 University of Washington, School of Aquatic and Fishery Sciences, 1122 NE Boat St, Seattle, WA, USA

2 Woods Hole Oceanographic Institution, Biology Department, 266 Woods Hole Rd, Woods Hole, MA, USA

3 Bermuda Institute of Ocean Sciences, School of Ocean Futures, Arizona State University, 17 Biological Station, St. Georges, Bermuda

4 Virginia Institute of Marine Science, William & Mary, Gloucester Point, VA, USA

\* Corresponding author. Email: hmcmonag@uw.edu

**Abstract**

Mesopelagic fishes may contribute substantially to marine carbon export and sequestration. However, uncertainty in this contribution due to limited precision of mesopelagic biomass and bioenergetic rate estimates has not been thoroughly quantified for any study site. Datasets that can confront these challenges are rare, particularly for comparing fish-mediated carbon flux to other biological carbon pump pathways. Using data from a unique three-ship expedition in spring 2021 in the subarctic Northeast Atlantic Ocean, we compare carbon transported by adult fish, zooplankton, and sinking particles, and calculate uncertainty in the relative contribution of fishes. Results indicate biomass- and bioenergetic-based uncertainty contributed roughly equally to variance in estimated carbon transport. The plausible range of mesopelagic fish carbon flux spans an order of magnitude: 1.6-21 mg C m-2 d-1 to 200 m depth, and 0.52-9.6 mg C m-2 d-1 to 500 m. Fishes contributed ~0.52%-18% at 200 m to the total biological carbon pump, and ~0.43%-13% at 500 m. Of the fish-mediated carbon transport to 200 m, ~8%-30% is sequestered on climate-relevant time scales (>100 years). This reinforces that carbon transport should not be conflated with carbon sequestration. These findings have implications for prioritizing future empirical measurements, evaluating trade-offs in fisheries management, and understanding the role of fishes in the biological carbon pump.

**Keywords**Active carbon transport, diel vertical migration, biological carbon pump, carbon export, mesopelagic fish

**Introduction**

Mesopelagic fishes dominate the global biomass of fishes, with recent estimates placing mesopelagic fish biomass at 2-16 Gt (Proud *et al*., 2019, Irigoien *et al*., 2014). If this biomass range is correct, and if the biomass of other fishes is approximately 1 Gt (Jennings *et al.*, 2008, Wilson *et al.*, 2009, Bianchi et al., 2021), then mesopelagic fishes comprise roughly 67% to 94% of global fish biomass. This updated mesopelagic fish estimate, which was 2-10 times higher than previously thought (Gjøsaeter and Kawaguchi, 1980), led to increasing interest in mesopelagic fishes as potentially important components of the ocean carbon cycle (St. John *et al*., 2016, Hidalgo and Browman 2019, Saba *et al*., 2021). Mesopelagic fishes may play a key role in carbon export due to their extensive diel vertical migrations. A large proportion of these fishes consume organic carbon near the sea surface at night and then migrate down to the mesopelagic zone during the day, where they transport this carbon through their egestion of fecal matter and carbonates, respiration of carbon dioxide, and mortality such as via predation or dead fall (Klevjer *et al*., 2016, Saba *et al*., 2021).

The recent elevated estimates of mesopelagic fish biomass also led to questions about whether these fishes could sustain large commercial fisheries, which could have competing policy implications if fishing were to impact fish-mediated carbon transport (Hidalgo and Browman, 2019). Improved understanding of the role that mesopelagic fishes play is needed to evaluate the consequences of harvesting on carbon transport and sequestration. For instance, the magnitude of fish carbon transport is highly uncertain, and its contribution to climate-relevant carbon sequestration (i.e., storage from the atmosphere for at least 100 years, a typical planning time horizon for climate policy) is not yet known (Siegel *et al*., 2021).

Currently, the relative contribution of mesopelagic fishes to the consumption, vertical transport, and sequestration of carbon by the biological carbon pump is highly variable among ocean regions, and estimates are imprecise (Saba *et al*., 2021). We focus on mesopelagic fishes in part because they represent the majority of global fish biomass. Furthermore, many perform daily vertical migrations that can span from near the sea surface to 300-800 m deep (Klevjer *et al*., 2016). Thus, mesopelagic fishes have been found to contribute more than large pelagic fishes or epipelagic forage fishes to carbon transport out of the euphotic zone (Pinti *et al.,* 2023), though carbon transport associated with fishes in general is poorly constrained (Saba *et al.*, 2021). Among different regions, estimates of fish-mediated carbon transport vary from less than 1% to over 30% of biological carbon transport, where biological carbon transport includes both gravitational carbon flux of passively sinking particles and active transport by vertical migrators (Ariza *et al*., 2015; Belcher *et al*., 2019; Davison et al., 2013; Hernández-León *et al*., 2019; Hidaka *et al.*, 2001; Hudson *et al*., 2014). Within a region, estimated transport rates can vary by a factor of five (Belcher *et al*., 2019, Hudson *et al*., 2014). Globally, estimates of mean fish-mediated carbon transport out of the euphotic zone (defined as ~0-100 m) have been found to vary from 0.3 to 2.7 Gt C yr-1 (Saba *et al*., 2021), which is comparable to estimates of 0.2 to 2.0 Gt C yr-1 from a more recent, mechanistic model of global fish carbon transport (Pinti *et al.,* 2023). This variation stems not only from true temporal or regional variation in nature but also from imprecise estimates of mesopelagic fish biomass (Davison *et al*., 2015; Proud *et al*., 2019), and limited knowledge of bioenergetic and movement parameters (McMonagle *et al*., 2023). Further, differences in sampling method and study design contribute to this variation, including how fish carbon is quantified and compared to other carbon transport mechanisms besides fish transport. Studies can also differ in whether only respiratory flux or also egestion (fecal waste), excretion of carbonates (non-fecal waste) and mortality flux pathways are considered (Belcher *et al*., 2019; Davison *et al*., 2013; Hidaka *et al.*, 2001; Hudson *et al*., 2014; Saba *et al*., 2021). Studies estimating global fish-mediated carbon transport (hereafter also referred to as fish carbon transport or fish carbon flux) recommend more empirical research to validate and further constrain these estimates (Pinti *et al.,* 2023).

The ideal scenario for empirically estimating the contribution of fishes to the biological carbon pump, and for identifying which parameters contribute most to uncertainty, is to have a data-rich sampling effort in which multiple carbon flux pathways are measured at the same time and place, along with detailed, depth-stratified sampling of the fish community. Such an effort was conducted in May 2021, comprising a three-ship operation led by two large projects: NASA EXport Processes in the Ocean from RemoTe Sensing (EXPORTS) (Siegel *et al*., 2016) and the Woods Hole Oceanographic Institution’s Ocean Twilight Zone Program. Teams collected sediment trap and Thorium-234 data for estimating passively sinking particle flux, and taxon- and depth-specific fish and zooplankton abundance for estimating fish- and zooplankton-mediated carbon flux.

Here, we estimate fish carbon flux at depth boundaries of 200 m and 500 m and compare to other biological carbon pump pathways, and examine how fish biomass and bioenergetic parameter uncertainties contribute to overall uncertainty in fish carbon flux estimates. We consider fish carbon flux in the form of egestion of particulate organic carbon, respiration and excretion of dissolved inorganic carbon, and mortality pathways. We further examine the extent to which fishes contribute to carbon sequestration. Since not all carbon transported to our chosen flux boundaries of 200 m and 500 m is also sequestered from the sea surface (and in turn, the atmosphere) on climate-relevant times scales, we also calculate fish carbon sequestration using results from a model of global ocean circulation (Siegel *et al*., 2021).

**Methods**

*Study region*

To obtain fish biomass data needed for the fish carbon flux model, we sampled fish during the field campaigns led by the EXPORTS and Ocean Twilight Zone projects in spring (May 4-26) 2021. Sampling took place ~170 km east of the Porcupine Abyssal Plain Observatory (Hartman *et al*., 2021) (Fig. 1) on the *R/V Sarmiento de Gamboa* (Ocean Twilight Zone project), *RRS James Cook*,and *RRS Discovery* (EXPORTS). Fishes were sampled in and around an anticyclonic mesoscale eddy during what appeared to be the end of a large phytoplankton bloom dominated by diatoms (Johnson *et al*., 2024). During this sampling period in May, the mixed layer depth varied from about 30-60 m, in part due to anomalous storms during the sampling period, though the mixed layer depth was potentially as deep as 250 m in April prior to our sampling based on glider data (Johnson *et al*., 2024).

*Sample collection*

We collected depth-stratified fish abundance and size distribution data between 0-1000 m using the Multiple Opening and Closing Net and Environmental Sensing System (MOCNESS) (Wiebe *et al*., 1985). The collection protocol was approved by the Institution Animal Care and Use Committee at Woods Hole Oceanographic Institution (WHOI ID Number 24708.01)*.* As is typical for MOCNESS tows, samples were collected while the net was being retrieved from its deepest to shallowest sampling depth. Depth-stratified samples from day and night tows were used to quantify the taxon-specific biomass of both the non-migratory fishes that are residents of the mesopelagic zone and the diel vertical migrators. Day tows are defined as those that occurred after the downward migration of the deep scattering layer was complete (i.e., after this layer as shown on an echogram reached its deepest depth), and before the upward migration began in the evening. Night tows are those that occurred after the upward migration was complete and before the downward migration began in the morning.   
  
Catch data from night tows were used to quantify fish density, vertical distribution, and diversity. The MOCNESS-10 on the R/V *Sarmiento de Gamboa* had a 10-m2 mouth and was outfitted with five, 333-µm mesh nets that sampled at depth intervals of 0-100, 100-300, 300-500 and 500-1000 m. The MOCNESS-1 on the RRS *James Cook* had a 1-m2 mouth fit with nine 200-µm mesh nets sampling from 0-50, 50-100, 100-150, 150-200, 200-300, 300-400, 400-500, 500-750, and 750-1000 m. The volume filtered per net ranged between 7194 and 33382 m3 (MOCNESS-10) and 443 and 1907 m3 (MOCNESS-1). Catch data were used to estimate the areal biomass density (g m-2)of fishes using volume swept, depths sampled, and estimated capture efficiency. Areal biomass (as opposed to volumetric biomass in g m-3) was used for easier comparison with literature values of fish and zooplankton biomass and carbon flux. Fishes were either flash frozen in liquid nitrogen immediately upon collection or preserved in 95% ethanol. All frozen specimens were stored at -80°C until further analysis.

*Fish identification, size distribution and biomass*

Fishes were measured and identified down to the lowest taxonomic level possible (often to species level) using morphological and genetic approaches. These data were used to obtain taxon-specific size distributions needed to calculate biomass. Fishes collected from the MOCNESS-10 were thawed and standard length and wet mass were measured using a Mettler Toledo AB204-S analytical balance. Fishes were morphologically identified using dichotomous keys (Carpenter, 2002, Sutton *et al*., 2020), and a small sample of muscle tissue was removed for DNA barcoding to confirm the morphological identifications (Appendix A). A representative subsample of the catch was genetically identified to the lowest (most specific) taxonomic level possible. Briefly, DNA analysis entailed extracting genomic DNA from the dissected muscle tissue and amplifying and sequencing the mitochondrial COI barcode marker (Ward et al., 2005; Govindarajan et al., 2023). Barcode sequences were compared to sequences on GenBank using BLAST. Species identifications were made when there was at least >98% identity over at least 90% of the sequence length (Appendix A). Fishes collected with the smaller MOCNESS-1 were photographed at sea with a ruler and lengths were measured using ImageJ. Smaller (<30 mm) fishes from both net systems that were not sorted at sea were later sorted from ethanol- or formalin-preserved samples. The standard lengths of those individual fishes were measured using ImageJ from photographs taken with a ruler (MOCNESS-10 samples) or from images taken by Zooscan (Appendix G) that were uploaded to the Ecotaxa website (following the methods in: Gorsky *et al*., 2010; Picheral *et al*., 2017; Maas *et al*., 2021) and then measured with ImageJ using the scale bar provided by the Ecotaxa (Appendix G) software (MOCNESS-1 samples). These smaller fishes measured from photographs were identified to the family level. We assumed that ethanol-preserved specimens (from the MOCNESS-10) were 5% larger prior to preservation (Moku *et al*., 2004), and that formalin-preserved specimens (from the MOCNESS-1) were 2% larger prior to preservation (Moku *et al*., 2004), thereby correcting for shrinkage.

For individuals measured for length but not weight, we estimated weight using empirical length-weight regressions further described in Appendix B. In summary, regression models were fit to data from the lowest taxonomic level possible given the sample size of length-weight data available for each taxon. If there were not enough individuals with both a length and weight measurement for a given species to fit a reliable species-specific regression, weight was estimated from a regression fit to genus-level or family-level data.

*Data filtering*

To increase our sample size and evaluate the impact of sampling gear on fish biomass and carbon flux estimation, we pooled data from the MOCNESS-1 and MOCNESS-10 and filtered the data for analysis according to predetermined data criteria. Further details on applying these criteria for data filtering are described in Appendix C. In summary, these criteria aimed to maximize the percentage of the total catch used in the analysis without sacrificing the accuracy of the fish biomass data. Carbon flux estimation required fish biomass standardized by sampling effort and vertical distribution, which could only be reliably determined for the more abundant taxa. As a result, rare taxa (n=1) were excluded, including fishes from two rare families (Alepocephalidae and Melamphaidae). The more abundant taxa (Myctophidae, Gonostomatidae and Sternoptychidae) were used for subsequent analyses. Of the subsample of 410 individuals that were both morphologically and genetically identified from MOCNESS-10 tows, this decision to focus on more abundant taxa resulted in removal of 10 individuals. We excluded fishes <10 mm in standard length, as mesopelagic fishes like myctophids and bristlemouths that are smaller than this are generally preflexion larvae (Richards, 2005), and the bioenergetics and migratory behaviour of such early-stage mesopelagic fishes are not well understood. Finally, we selected which tows to use to estimate fish biomass on the basis of how net size versus time of day appeared to impact catch (Appendix C), because differences in capture efficiency between the two net systems is unknown.

Where possible, we incorporated cruise-specific empirical measurements into the fish carbon flux model, rather than relying on parameter values from the literature. Shipboard acoustic data (18kHz and 38 kHz) from the *R/V Sarmiento de Gamboa* and *RRS James Cook* were used as independent checks of the minimum and maximum depth of the fish vertical migration compared to net-derived migration depths. We assume that the dominant scattering layer visible in the echograms is representative of general patterns in mesopelagic fish migration behaviour. Further details related to these calculations are available in our public repository for this study (<https://github.com/hmcmonagle/Fish-carbon-flux-N-Atlantic>/data/Swim\_speed\_from\_acoustics.xlsx). For vertically migrating fishes, acoustic data were also used to estimate the average migration swimming speed in the carbon transport model and time spent in various assumed activity states (foraging near the sea surface, migrating, or swimming at depth). Temperature probes (Sea-Bird 911; Sea-Bird Electronics, Inc., Bellevue, United States) on the MOCNESS-10 collected water temperature data that were used in the fish carbon transport model.

*Carbon flux estimation and uncertainty analysis*

Carbon flux per areal biomass density of fish per day (mg C m-2 d-1) was estimated from the length composition of the catch, carbon flux associated with each length of fish, catch biomass, and assumed capture efficiencies of the net systems. This was done separately for each family—Myctophidae, Gonostomatidae, and Sternoptychidae—and then summed across all taxa. Carbon flux was based on fish areal biomass densities (wet mass of fish per unit area swept), calculated from the catch from eight night tows (n=5 from the MOCNESS-10 and n=3 from the MOCNESS-1, Fig. 1) as

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Description automatically generated with medium confidence (Eq. 1)*

where *F*is the total fish carbon flux in *mg C m-2 d-1* associated with all three taxonomic groups *g*, *Pg,l* is the proportion of fish within each of 100 length bins *l*, *Fg,l* is the carbon flux associated with each taxonomic group *g* within length bin *l*, *Bg* is the mean catch biomass of taxonomic group *g* in grams of wet mass per square meter sampled, and *q* is the assumed length-independent capture efficiency of the net systems. For example, if we assume that (due to net avoidance) we catch only 10% of the fish that would normally be distributed in that area sampled, then *q* = 0.10. We allow *q* to vary in our sensitivity analysis from 0.05 to 0.50 (Belcher et al., 2019; Williams and Koslow 1997; Pakhomov et al., 2019) (Table S1). Uncertainty in the mean catch biomass of each taxonomic group was found by averaging across eight simulated tows, where each tow’s areal biomass density (*Bg*) was drawn from a normal distribution

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Description automatically generated with medium confidence (Eq. 2)*

with mean μ and standard deviation σ, where the mean and standard deviation represent the sample mean and standard deviation of the eight empirical tows for that taxon. We chose a normal distribution because its limitations (e.g., that normal distributions tend to have too few observations in the tails) are well known, and we lack sufficient data to draw conclusions about the underlying probability distribution of tow catches. Simulated tows are used here to represent uncertainty in subsequent sensitivity analyses because although a different set of tows would not have had the same catch biomass and composition, the catches obtained from the true tows were presumably sampled from a true distribution of fish biomass at the study site. In summary, empirical tow data was used to estimate the mean and standard deviation of the true probability distribution of fish biomass.

We found the range of plausible fish carbon flux estimates using a Monte Carlo sensitivity analysis. This Monte Carlo analysis propagated uncertainty in fish biomass as well as bioenergetic parameter uncertainty. To calculate the range of feasible carbon flux estimates given parameter uncertainties, we identified feasible ranges for each parameter using cruise data when available or literature data when appropriate cruise data were unavailable (nominal values and ranges are provided in Appendix D). Biomass was standardized by sampling effort in grams of wet mass per unit area swept (hereafter referred to as areal biomass density) and calculated from the catch of both the MOCNESS-1 and MOCNESS-10, where the mean and standard deviation of areal biomass density at the study site was based on fish densities and fish length distributions from as many tows as possible (Appendix C). The capture efficiency of these net systems was allowed to vary between 5% and 50% in the sensitivity analysis based on previous studies using nets of comparable mouth opening sizes (Belcher et al., 2019; Kaartvedt et al., 2012; Pakhomov et al., 2019; Williams and Koslow, 1997). Probability distribution functions were defined for each parameter (Appendix D). We then used a Monte Carlo analysis to generate 1,000 simulations that randomly sampled parameter space and calculated carbon flux for each simulated vector of parameters. Where fish carbon flux calculations for migrating and non-migrating fishes shared a parameter, the same parameter value was used for both types of fish within that simulation. To compare uncertainty associated with bioenergetic parameters versus biomass estimation, this Monte Carlo analysis was repeated under three scenarios: one where only bioenergetic parameters (Appendix D) varied, one where only estimated or assumed biomass parameters (e.g., catch and net capture efficiency) varied, and one where both bioenergetic and biomass parameters varied.

We calculated carbon transport for the fish community at this study site using a model that incorporates bioenergetics and movement parameters of mesopelagic fishes and determines the fate of the carbon in their bioenergetic output past a chosen flux boundary (Fig. 2). The structure and parameterization of this model is described in detail by McMonagle *et al*., (2023). In summary, this model estimates the amount of carbon transported daily by the fish community. Biomass information, migration behaviour, and bioenergetic accounting is used to estimate the transport of carbon via specific dynamic action (respiration associated with the digestion of food), non-digestion related respiration (based on routine metabolic rate according to Ikeda 2016), mortality, egestion (fecal waste) and excretion (non-fecal waste) past a chosen flux boundary. We use published rates of dissolved inorganic carbon (carbonate) production by marine fishes for calculating excretion flux (Wilson *et al.*, 2009); to our knowledge, substantial rates of dissolved organic carbon production that are independent from egestion (e.g., digestible carbon that is shed from skin as mucus or released as metabolic waste products from gills) have not yet been quantified for marine fishes (Saba *et al.,* 2021). We consider some respiration to be associated with specific dynamic action because the regression used to estimate fish respiration rates (Ikeda, 2016) is based on empirical measurements of routine respiration rates from typically food-deprived fish. In contrast, the *in situ* fish represented in our calculations had been recently feeding based on morphological gut content analysis of a subset of the catch (Appendix F), so they presumably were releasing additional respired carbon dioxide associated with digestion, i.e., specific dynamic action. Though specific dynamic action and respiration not related to digestion are distinguished in our calculations, total carbon dioxide production via respiration would be the sum of specific dynamic action (i.e., heat increment) and other processes related to metabolism such as maintenance, heat loss, and activity (Brett and Groves, 1979).

The model of fish carbon flux defines the daily activity pattern of the taxa described. For vertically migrating taxa, we incorporate the minimum and maximum depth of migration, and the amount of time spent migrating versus foraging or at a potentially lower metabolic state during the day. We also consider the contribution of non-migrating fishes; although they do not actively transport carbon across the flux boundary, we assume they ingest some zooplankton that may have otherwise migrated and released carbon above the flux boundary. To estimate carbon transported by each fish carbon flux pathway, we estimate the daily ingestion of energy needed to meet the sum of energy requirements. These assumed energy requirements include energy lost to respiration, specific dynamic action, mortality, egestion and excretion according to a set of equations that are not species-specific, also known as a standard base energy budget for fishes (Kitchell *et al*., 1977, 1974). Respiration is defined as including activity, maintenance and heat loss. Growth is assumed to equal mortality, which is more intuitive at the population level than individual level for a population assumed to be at steady state at the time of sampling. Units of energy are converted to units of carbon, and carbon transported past the flux boundary by each flux pathway is summed for both migrating and non-migrating fishes.

*Comparing fish carbon flux to other biological carbon flux pathways*

After calculating fish carbon flux for this study site, we placed fish carbon flux into the context of total carbon flux via the biological carbon pump by comparing our findings to other EXPORTS results. Total biological carbon flux is defined as the sum of carbon transported by fish, zooplankton, and sinking particles. Carbon transported by fecal pellets are considered part of fish- or zooplankton-mediated carbon flux, estimated using fish and zooplankton bioenergetic models. Zooplankton-mediated carbon flux is typically referred to as a component of active flux, along with fish flux (Boyd *et al*., 2019). The last biological carbon flux considered was that associated with sinking particles, sometimes referred to as gravitational or passive flux (the non fish- or zooplankton-mediated flux).

Sinking particle flux at this study site was estimated at 200 and 500 m using two independent methods: sediment traps and the Thorium-234 disequilibrium approach. Sediment traps estimated passive flux by sampling the accumulation of sinking particles over discrete depths and time intervals into a 0.0226 m2 collection area (Estapa *et al.,* 2023). Neutrally buoyant sediment traps and surface-tethered sediment traps were used as one data source for estimating sinking particle flux, with sampling and analysis details further described elsewhere (Estapa *et al*., 2023 and references therein). The Thorium-234 approach was used as a second data source for estimating passive sinking particle flux based on the disequilibrium between the naturally-occurring Uranium-234 and the particle-reactive radionuclide Thorium-234 in seawater samples collected at various depths, including 200 and 500 m, from CTD rosette casts (Clevenger *et al*., 2024). For the purposes of presenting the relative contribution of fishes to the total biological carbon pump at 200 m and 500 m, we primarily use the sinking particle flux results derived from sediment traps, with the exception of plotting sinking particle flux results derived from both sediment traps and the Thorium-234 method.

Zooplankton flux was estimated to 200 and 500 m depth using MOCNESS-1 samples from the *RRS James Cook* and bioenergetic modelling (e.g., Steinberg *et al*., 2023). We found the marginal (additional) carbon transported by zooplankton, beyond that which is transported by their fish predators, by subtracting the zooplankton mortality flux from the sum of zooplankton carbon flux. In describing fish carbon flux, we focus on fish carbon transported by small, mainly zooplanktivorous (Appendix F) mesopelagic fishes, which constitute the majority of the catch. Thus, we avoid double counting carbon transported by fish predators and their zooplankton prey. We defined zooplankton flux as the sum of carbon transported via respiration (as carbon dioxide), egestion (as particulate organic carbon), or excretion (as dissolved organic carbon) at this study site (Appendix G). Our choice to remove zooplankton mortality flux from total zooplankton flux favours a higher relative contribution of fish compared to zooplankton. Embedded in this choice is the assumption that all mortality flux from zooplankton is due to predation by mesopelagic fishes and that in the absence of the fish, this zooplankton mortality flux would not occur. Although this is not entirely realistic–zooplankton are consumed by other secondary consumers that are not fish such as squid or macrozooplankton–we make this choice to obtain a conservative estimate of zooplankton carbon flux relative to fish carbon flux. Future studies examining gut contents of secondary consumers other than fishes could quantitatively assess how much zooplankton mortality flux is unrelated to predation by fishes.

*Comparing carbon transport to sequestration*

To estimate carbon sequestration times of carbon transported by fishes, we calculate how much carbon is transported to 200 m and 500 m in various forms (e.g., respired inorganic carbon or egested particulate organic carbon), and consider storage times for each carbon pool. We use results from an inverse ocean circulation model (Siegel *et al*., 2021) to find the proportion of carbon that is sequestered for each carbon flux pathway and flux boundary at our study site in the eastern North Atlantic. The ocean circulation model (Siegel *et al*., 2021) estimates the proportion of carbon sequestered as a function of the depth to which that carbon is transported (Siegel *et al*., 2021, Supplemental figure S2A). Based on these model results at our specific study site above the Porcupine Abyssal Plain, 4.8% of carbon transported to 200 m and 5.3% of carbon transported to 500 m is sequestered from the sea surface for a climate-relevant time scale of 100 years (Siegel *et al*., 2021). We assumed that carbon transported via respiration pathways (including specific dynamic action) was sequestered at these proportions. Carbon transported via egestion of fecal matter (particulate organic carbon), excretion of carbonates (particulate inorganic carbon), and mortality pathways could potentially sink deeper than the depth of release by each fish. To account for these contributions to deeper flux, we assumed that 10% to 50% of carbon released via egestion, excretion, and mortality pathways sinks deep enough to be fully sequestered for 100 years, which at this study site could require that this material sinks to ~3000 m (Siegel *et al.*, 2021). Compared to the North Pacific, carbon in the North Atlantic must generally sink deeper to be fully sequestered due to differences in the global thermohaline circulation; this overturning circulation brings deep water to the sea surface faster in the North Atlantic. The assumption that roughly 10% to 50% of particulates sink from the carbon transport flux boundary to sequestration depths before being remineralized has not been quantified in the literature to our knowledge for fish waste and carcasses. However, there is no empirical evidence that 100% of fish wastes or carcasses transported to 200 or 500 m depths would sink as deep as ~3000 m before being remineralized, disaggregated, or consumed, so we allow this proportion to vary from ~10-50%. To calculate the minimum carbon sequestered, we chose a lower range estimate (5th percentile of estimates from Monte Carlo simulations) of carbon transported by each pathway and assume that 10% of carbon flux from egestion, excretion, or mortality pathways sinks to a depth of long-term sequestration. To find the maximum carbon sequestered, we chose a higher range estimate (95th percentile from simulations) of carbon transported by each pathway and assume that 50% of carbon flux from egestion, excretion, or mortality pathways is sequestered.

**Results**

*Fish diversity, biomass, and vertical distribution*

The dataset included a total of 1,415 individuals, including 1,170 fishes sampled from the MOCNESS-10 and 245 fishes from the MOCNESS-1. A subsample of the MOCNESS-10 catch (n = 410) was identified to species level (n = 405), for which 19 fish species were identified (Fig. 3). Fish identifications, tow information, and other metadata are available in a Github repository (<https://github.com/hmcmonagle/Fish-carbon-flux-N-Atlantic>).

Although larger nets are generally assumed to catch more fish per unit sampling effort and a larger size distribution due to lower net avoidance rates, we calculated a higher areal biomass density (g m-2) and catch per unit volume (g m-3) from the MOCNESS-1 compared to the MOCNESS-10 (Fig. 4). There is inevitably some uncertainty in the calibration factors used to convert from flow meter counts to volume filtered (Appendix H). The smaller net also unexpectedly had higher catch rates for some of the larger size classes, though the larger net did catch the largest several fishes in the dataset (Fig. 5). The mean areal (depth integrated, 0-1000 m) biomass density, calculated only from night tows, was 1.2 grams of wet mass per square meter (g m-2). However, there was about a two-fold difference in areal biomass density among night tows alone, with 0.79 g m-2 caught in the smallest MOCNESS-10 tow at night and the 1.6 gm-2 caught in the largest MOCNESS-1 tow at night (Fig. 4). Sampling procedures did not change among tows, suggesting high patchiness in fish distribution.

Night tows had more similar catches between the two nets than day tows, but day/night patterns of catch biomass were not consistent between the two nets (Fig. 4). For the MOCNESS-10, catch densities from night tows were higher than day tows, potentially due to greater net avoidance during the day. This difference was not evident for the smaller MOCNESS-1.

Across the entire fish catch from both net systems, three fish families—Myctophidae, Gonostomatidae, and Sternoptychidae—composed ~90% of the total catch biomass and number of individuals. The size distribution (Fig. 5) was not significantly different when we included only these three fish families versus when we included all fish families (p > 0.05; Kolmogorov-Smirnov test, which tests for differences between groups). Further analysis focuses on fishes of these families, which had sufficient data available for fitting reliable, empirical length-weight regressions for individuals without an empirical weight measurement.

The vertical distribution of mesopelagic fishes by fish family indicates that fishes of the families Myctophidae (myctophids) and Sternoptychidae (hatchetfish) generally performed extensive diel vertical migration, while fishes of the family Gonostomatidae (bristlemouths) generally did not (Fig. 6). Myctophids typically migrated from 300-500 m during the day to 0-50 m at night, while hatchetfish migrated from 300-500 m during the day to 100-200 m at night. Bristlemouths primarily resided in the mesopelagic zone between 500 m and 1000 m during both the day and night. A small proportion of myctophids did not migrate during the day, and a small proportion of bristlemouths were found near the sea surface during day and night.

Although not a focus of the study, morphological gut content analysis revealed that the myctophids, hatchetfish and bristlemouths caught were generally zooplanktivorous. The identifiable myctophid gut contents primarily consisted of calanoid copepods and hyperiid amphipods, with rarer occurrence of ostracods, mysids, pteropods, and decapods (Appendix F). Hatchetfish gut contents generally consisted of calanoid copepods with some ostracods. Bristlemouth guts contained calanoid copepods and some ostracods.

*Carbon flux estimation*

Based on a carbon flux model for mesopelagic fishes (McMonagle *et al*., 2023), and Monte Carlo simulation to propagate parameter uncertainty related to bioenergetic and biomass parameters, fish transported 1.6 to 21 mg C m-2 d-1 past 200 m depth and 0.52 to 9.6 mg C m-2 d-1 past 500 m (Fig. 7). These ranges represent the inner 90th percentile range of model estimates from the Monte Carlo simulations (n=1000). Fish carbon flux past 500 m was lower in part because migrating myctophids and hatchetfish generally did not migrate below 500 m (Fig. 6). Therefore, carbon released as dissolved carbon dioxide via respiration was not transported below 500 m. The parameter ranges used in the carbon flux model, including the proportion of carbon transported via egestion and mortality below the chosen flux boundary, are provided in Appendix D.

Comparison of fish carbon flux with other export pathways, such as active zooplankton carbon flux and passively sinking particles, shows that fishes at this study site contributed 0.52%-18% to total carbon export at a flux boundary of 200 m (Fig. 7). This range is based on the inner 90th percentile range of model estimates for fish flux, the range for passive sinking particle flux from sediment traps during three different time periods, and the range for zooplankton flux based on biomass calculated from three different tows. At a flux boundary of 500 m, fishes contributed 0.43%-13% to total carbon export, and this range was calculated the same way as for 200 m.

*Uncertainty analysis*

In addition to finding the plausible range of carbon flux estimates, the Monte Carlo analysis was also used to propagate parameter uncertainty related to specific sources of uncertainty, i.e., bioenergetic versus biomass parameters. We repeated the Monte Carlo analysis under three scenarios: one where only bioenergetic parameters were allowed to vary (Appendix D), one where only biomass estimation parameters (e.g., areal biomass density and net capture efficiency) were allowed to vary, and one where both types of parameters were allowed to vary. Biomass-related uncertainties contributed at least as much uncertainty as bioenergetic parameter uncertainty (Fig. 8). There was over an order of magnitude difference between the lower and upper bound (5th and 95th quartile) estimates of fish carbon flux when both bioenergetic and biomass-related parameter uncertainties were considered (Fig. 8). The standard deviation of estimates when bioenergetics, biomass, or both were allowed to vary was 3.10, 5.38, and 6.99, respectively. Even with multiple parameter ranges based on cruise data (Appendix D), with lower parameter ranges than would otherwise be required if relying only on literature data (McMonagle *et al*., 2023, Table B.1), there was a ~12-fold difference between the minimum and maximum fish carbon flux estimate at a 200 m flux boundary. Results of the sensitivity analysis for all bioenergetic and movement parameters for vertically migrating fishes for a 200 m flux boundary can be found in Appendix E.

*Converting from carbon transport to carbon sequestration*

The proportion of carbon transport that was also sequestered from the sea surface on climate-relevant time scales was estimated from an inverse ocean circulation model that estimates the proportion of carbon sequestered at the depth to which that carbon is transported (Siegel *et al*., 2021, Supplemental figure S2A). Our calculations result in a range of carbon sequestration of 0.13-6.3 mg C m-2 d-1 at 200 m, with 8%-30% of the total fish-mediated carbon transported to 200 m also sequestered on climate-relevant time scales (100 years of storage from the sea surface). At 500 m, we calculate 0.13-6.4 mg C m-2 d-1 in fish-mediated carbon sequestration. Calculations are further described in Methods, and in our public repository (Appendix I).

**Discussion**

*Biomass and bioenergetic uncertainty*

Biomass remains the leading contributor to uncertainty in estimates of fish-mediated carbon transport, though the combined total of bioenergetic uncertainties (encompassing uncertainty associated with about 30 bioenergetic parameters) nearly equals biomass uncertainties. Sensitivity analyses that show specifically which bioenergetics parameters contribute most to uncertainty are available (McMonagle et al., 2023) and can be used to prioritize bioenergetics measurements aimed at constraining fish carbon transport estimates. Here, we find that net capture efficiency (*q*) was the single most influential parameter in the fish carbon flux model according to the Monte Carlo results for both vertically migrating and non-migrating fishes (Appendix E). While a higher number of tows could provide a more representative sample of fish densities and increase precision, unknown capture efficiency remains a major contributor of uncertainty in converting from catch rates to areal (or volumetric) biomass density.

Variation in catch among tows, presumably from patchiness in vertical and horizontal fish distribution, was also a major contributor of uncertainty in fish carbon flux estimates (Appendix E). This patchiness, which is largely a precision issue, implies that more extensive fish sampling approaches (e.g., more frequent sampling., integrated tow-net and acoustic sampling) is important for more accurate biomass estimates at a given study site. Biomass precision could be improved with a combined acoustic and net-based sampling effort, which could resolve variation in spatial structure of these fishes using acoustic information while using nets for species identification and relating backscatter to biomass. Further research might also examine how mesopelagic fish biomass varies across oceanographic features. For example, anticyclonic mesoscale eddies such as the one sampled during this sampling campaign are thought to accumulate zooplankton (Goldthwait and Steinberg, 2008, Eden *et al*., 2009, Yebra *et al*., 2005), fish larvae (Lobel and Robinson, 1986), and to attract predators of mesopelagic fishes, potentially because they can serve as a thermal refuge to reach mesopelagic depths (Braun *et al*., 2019). However, it is not well known how adult mesopelagic fishes are distributed across these features. Future investigations of patchiness in mesopelagic fish biomass could be relevant in the context of future mesopelagic fisheries management, such as how to interpret fishery-independent or -dependent surveys, if certain oceanographic features create biomass hotspots of these fishes.

We note that there are other well-established and emerging approaches for fish biomass estimation and taxonomic identification besides sampling with nets and processing each specimen in the catch. Acoustic methods can be used for estimating mesopelagic fish biomass across large geographic areas without further laboratory collection or processing of the catch (Irigoien *et al*., 2014; Klevjer *et al*., 2016), but these methods are also associated with high biomass uncertainties in part due to unknown target strengths and lack of taxonomic identification of the organisms that produce the backscatter (Proud *et al*., 2019). Furthermore, while eDNA approaches may detect the presence of fish species missed by net systems like the MOCNESS (Govindarajan *et al*., 2023), inferring biomass from eDNA data is considerably more challenging (Rourke *et al*., 2022).

This was a rare opportunity to compare fish catch from two independent, depth-stratified net systems towed in the same place and time, yet there was still high uncertainty in biomass estimates. Unexpectedly, there was little difference between the size distribution and estimated areal biomass density between the two net systems, even though we expected higher capture efficiency (lower net avoidance) with the larger net. Even if the fish densities between the two nets had been extremely different and could point to some consistent difference in relative capture efficiency, absolute net capture efficiency is still unknown and remains a perennial challenge in estimating fish abundance from towed nets (Gunderson, 1993). Therefore, while our net-based approach to biomass estimation is arguably as good as any other single method available at this time, we are still left with high biomass uncertainty. Best practices in fish biomass estimation and identification could, however, evolve as other techniques and technologies for biomass estimation advance, which could further constrain estimates of fish carbon export.

*Relative contribution of fish to the biological carbon pump*

We find a relatively modest contribution of fish-mediated export to the biological carbon pump at this study site. However, the time of year that the data were collected might affect the estimated proportion of carbon transport attributable to diel migrating fishes, given that seasonality affects sinking particle flux (Henson *et al.*, 2015., de Melo Viríssimo *et al*., 2022). Data were collected during a declining North Atlantic spring bloom (Clevenger *et al*., 2024; Johnson *et al*., 2024), and late spring or early summer is generally when passive sinking particle flux is highest in the region (Henson *et al.*, 2015., de Melo Viríssimo *et al*., 2024). Consequently, the relative contribution of fish during this time period may be lower than at other times of the year when rates of passive sinking particle flux are lower. Small, zooplanktivorous (Appendix F) mesopelagic fishes have lifespans of ~2-8 years (Caiger *et al*., 2021), thus are likely transporting a more consistent amount of carbon throughout the year compared to passive sinking particles. No studies exist yet on the seasonality of fish carbon transport in absolute or relative terms; our estimates of relative fish carbon flux should, however, be considered in the context of the season in which they were generated.

We focused this modelling effort on the more abundant, smaller fish species at this study site in part because our nets do not effectively sample larger organisms that have greater net avoidance capability. However, other larger taxa also contribute to carbon flux. To roughly compare our carbon flux estimates to those based on larger secondary consumers, we drew from a study estimating carbon flux associated with baleen whales in the Southern Ocean (Durfort et al., 2022). This comparison revealed that Southern Ocean whales contribute roughly 0.04% to 3% as much carbon flux as the small fishes in our North Atlantic study site (Appendix J). This percentage depends on whether we use the lower or upper bound of our fish flux estimates and depending on whether we use whale carbon flux estimates from before or after large-scale industrial exploitation of Southern Ocean whales. These calculations (available in our public repository) are intended only to roughly put fish carbon flux at our study site into context with carbon flux associated with one higher trophic level taxon, cetaceans, albeit in an entirely different region. We expect that our estimate of fish carbon flux (and zooplankton carbon flux) is so much higher than the estimate of whale carbon flux calculated by Durfort et al. (2022) because of the considerably lower relative abundance of large-bodied species in ecosystems in general. Further research may refine and expand on this comparison by examining carbon flux associated with larger zooplankton, larger pelagic fishes, and other higher trophic level predators, ideally enabling comparison at the same time and study site.

We made a simplifying assumption that allowed us to estimate a maximum contribution of mesopelagic fishes compared to zooplankton carbon flux. That is, we assumed that all zooplankton mortality is due to mesopelagic fish predation, a decision that could result in an overestimate of the marginal contribution of mesopelagic fishes to carbon transport compared to that of zooplankton. Our carbon flux estimates may also be an overestimate in the context of potential impacts of mesopelagic fish removal from harvesting; if mesopelagic fishes were removed from the system, another organism that contributes to active carbon transport could fill the niche of those fishes (such as via release from predation or reduced competition for prey resources) and transport some of that carbon by a different pathway. Such a scenario is evidenced by our data, as we find migrating zooplankton in the diets of these migrating mesopelagic fishes (Appendix F). We mention this to caution one-to-one applications of these fish carbon flux estimates to calculate the extent to which fish harvest might decrease overall carbon export by the biological carbon pump. Furthermore, in the absence of food web analyses that could indicate otherwise, we allow the percentage of mortality of mesopelagic fishes resulting in fish carbon transport past a given flux boundary to vary from 40% to 90% in our sensitivity analyses. Mesopelagic fishes are not only consumed by deep-dwelling predators (Iglesias *et al*., 2023; McBride *et al*., 2022; Sutton and Hopkins, 1996) but also by diving tunas, marine mammals, and seabirds (Braun *et al*., 2022; Iglesias *et al*., 2023; Watanuki and Thiebot, 2018). These predators presumably release some of the ingested carbon at depths shallower than our flux boundaries, or even into the atmosphere in the case of some seabird and marine mammal carbon transport pathways. Therefore, we avoid a previously common assumption in fish carbon flux studies that all mortality results in downward carbon transport.

*Application of results for biogeochemical modelling and fisheries management*

These results have potential applications both for biogeochemical modelling and fisheries management. Marine biogeochemistry models often find a deficit of carbon in the mesopelagic zone, with a carbon supply from surface waters that is inadequate to support metabolism of mesopelagic biota (Burd *et al*., 2010; Steinberg *et al*., 2008). This may be due in part to the exclusion of active transport by zooplankton (Steinberg *et al*., 2008) and fish from many of these models. If so, our sensitivity analysis shows a plausible range of the magnitude of carbon flux in this region that could come from fishes, which are generally not included in global biogeochemical models. Our analysis of carbon sequestration times is also relevant to fisheries management that seeks to preserve ecosystem services of carbon sequestration; concerns about the impacts of fishing to carbon sequestration are raised in various news articles about harvesting mesopelagic fishes (e.g., CNN, 2021; Guardian, 2022; Nature News; 2020; Vox, 2021), but these claims have not yet been quantitatively assessed. In terms of management, the marginal contribution of fishes to carbon flux beyond that of their zooplankton prey can be used to inform trade-off analysis between fish harvest and carbon transport, but the difference between carbon transport and carbon sequestration should be considered (Pinti *et al.,* 2022). This study site is near proposed experimental mesopelagic fisheries in the Northeast Atlantic (Standal & Grimaldo 2020, Schadeberg *et al*., 2021), making it a relatively high priority area to study the contribution of fishes to carbon transport and carbon sequestration.

Thus far, prior studies of fish carbon flux have generally used a flux boundary of ~150-200 m, although global carbon cycling studies often use deeper carbon flux boundaries to evaluate carbon storage on climate-relevant time scales (Bisson *et al*., 2020; Jin et al., 2020). The recent U.S. National Academies of Science and Engineering and Medicine (NASEM) report (2021), uses 1,000 m as the generally accepted depth of “long-term sequestration” (NASEM report Chapter 6, section on “Deadfall and Excreted Carbon”). Another appropriate flux boundary may be the maximum mixed layer depth, which generally occurs during winter months, as any depth shallower than this is mixed toward the atmosphere annually (Buesseler *et al*., 2020; de Boyer Montégut *et al*., 2004). Globally, the winter mixed layer depth varies greatly but can be > 300 m deep, particularly in the North Atlantic, North Pacific, and Southern Oceans (de Boyer Montégut *et al*., 2004). Globally-averaged, median sequestration times are ~ 20 years when carbon is released at a depth of 208 m (with a standard deviation of 58 years about the global mean) (Siegel *et al*., 2021). At the Bermuda Atlantic Time-series Study (BATS) station in the Sargasso Sea, carbon release as deep as 1022 m is stored for a median of 30 years (Siegel *et al.,* 2021). The relatively deep flux boundary depths used in biogeochemical analyses, and modest storage times at shallower depths, raises the question of whether it is accurate for fish-mediated carbon flux studies to claim that carbon flux past 150 or 200 m is stored long-term without providing storage time estimates at the chosen depths (Trueman *et al*., 2014). Carbon dioxide removal on the order of decades to centuries are considered policy-relevant times scales for carbon sequestration, so we attempt to put fish carbon into context of carbon sequestration timelines here and find that carbon sequestration could be only about 8% of carbon transport past 200 m. We caution not to conflate carbon transport and climate-relevant carbon sequestration.

*Conclusions*

We find that fishes may contribute 0.52%-18% of the total biological carbon pump flux past 200 m. Even with a uniquely comprehensive sampling effort, this relative contribution is still poorly constrained with over an order of magnitude of uncertainty in estimates of fish-mediated carbon transport when considering uncertainty from both biomass estimation and bioenergetic parameters. Empirical and assumed biomass parameters (catch, capture efficiency, and net calibration factor) alone contribute slightly more uncertainty than all bioenergetic parameter uncertainty combined. These results can be used to prioritize efforts to further constrain fish carbon flux estimates in the future, and in the meantime, to justify caution in the way these imprecise estimates are used. We also show that while there is over an order of magnitude of uncertainty in fish carbon transport estimates, there is even more uncertainty in fish carbon sequestration estimates, and the contribution of fishes to climate-relevant carbon sequestration could be relatively modest. Therefore, we recommend that claims about the magnitude of carbon transported and sequestered by marine fishes be considered in light of the substantial imprecision in existing estimates.

**Data Availability Statement**

All data and code underlying this article are available via*Github at* https://github.com/hmcmonagle/Fish-carbon-N-Atlantic,and can be accessed publicly. Sinking particle flux results from the Thorium-234 approach were found in Clevenger *et al.,* 2024, Table 4, at 195 (closest depth to 200 m) and 500 m and averaged over the three epochs (time periods during the cruise). Sinking particle flux results from the sediment trap approach are further described by Estapa *et al*., (2023) and are available at Estapa, M. L. 2022. EXPORTSNA. SeaWIFS Bio-optical Archive and Storage System (SeaBASS). NASA. doi:10.5067/SeaBASS/EXPORTS/DATA001. Raw data used for the calculation of all carbon fluxes are available in the SeaWIFS Bio-optical Archive and Storage System (SeaBASS) repository. See <https://seabass.gsfc.nasa.gov/experiment/EXPORTS> (DOI 10.5067/SeaBASS/EXPORTS/DATA001) for data from the RRS James Cook, and [https://seabass.gsfc.nasa.gov/experiment/OTZ\_WHOI](https://urldefense.com/v3/__https:/seabass.gsfc.nasa.gov/experiment/OTZ_WHOI__;!!K-Hz7m0Vt54!nPP6bYq0masyu7coit7D0UP83gc3JVtZUDeNNxy75VHr-0GGG95WrpI0bbZo8Th8_VT8LsuIubwUfOrjUTadkxye4qFe$) (DOI 10.5067/SeaBASS/OTZ\_WHOI/DATA001) for the data from the R/V Sarmiento de Gamboa. Alternatively, on the SeaBASS website, go to EXPORTSNA (EXPORTS) experiment or SG2105 (OTZ\_WHOI) and selection the main PI: Joel Llopiz. Genbank accession numbers and links to associated files in SeaBASS are available in Appendix A.

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**Author contributions**

This study was conceptualized by H. McMonagle, J.K. Llopiz, A.E. Maas and T.E. Essington. J.K. Llopiz and H. McMonagle collected fish data aboard the R/V *Sarmiento de Gamboa* and A.E. Maas and D.K. Steinberg collected fish and zooplankton data aboard the RRS *James Cook*. DNA sequencing and identification of fish specimens was led by A.F. Govindarajan. Estimation of zooplankton carbon flux was led by A.E. Maas and D.K. Steinberg. Data visualization and other code was primarily written by H. McMonagle with some assistance for data filtering and data visualization code from Github’s Copilot for R. The original draft was written by H. McMonagle. T.E. Essington, A.E. Maas, D.K. Steinberg, A.F. Govindarajan, and J. K. Llopiz contributed to review and editing.

**Declaration of competing interests**

The authors have no competing interests to declare.

**Supplementary Data**

All appendices can be found in supplementary material, available at the ICESJMS online version of the manuscript. Appendices include:

Appendix A. DNA barcoding

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

Appendix C. Application of data filtering criteria

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

Appendix E. Monte Carlo simulation results showing parameter sensitivities

Appendix F. Gut content analysis of mesopelagic fishes

Appendix G. Zooplankton-mediated carbon flux results

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

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

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)

Appendix K: Responses to reviewers during the peer review process

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**Additional reading**

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Fig. 1. Map of study site over the Porcupine Abyssal Plain in the Northeast Atlantic Ocean. The black rectangle indicates the study site and has a vertical height of 50 kilometres, which is magnified in the inset bottom left. The inset (study site with tows) shows the five MOCNESS-10 tows deployed at night from the *R/V Sarmiento de Gamboa* (orange) and three MOCNESS-1 tows deployed at night from the *RRS Cook* (purple). These tows were used for fish biomass estimation. Circles and triangles represent the start and end of each tow, respectively, with connecting lines indicating ship track during each tow.

Fig. 2. Visualization of the fish carbon flux model and fish-mediated flux pathways considered, including respiration, mortality, and egestion (fecal waste) or excretion (non-fecal waste). Alongside the estimates from the fish carbon flux estimates for this study site, we also compare fish carbon flux to carbon flux associated with zooplankton and sinking particles.

Fig. 3. Stacked bar plot showing fish diversity from the subset of MOCNESS-10 samples (n=403) that were identified to the lowest taxonomic level possible with both morphological and genetic identification techniques. Tows are plotted by date in May, 2021 and labelled with time of day. All taxa are included that had more than one individual sampled. Taxa are colour-coded by family with Sternoptychidae in orange (Argyropelecus sp., Valenciennellus sp.), Gonostomatidae in purple (Cyclothone sp.), Melamphaidae in pink (Scopelogadus sp.), Alepocephalidae in yellow (Xenodermichthys sp.), and Myctophidae in green (Benthosema sp., Lampanyctus sp., Myctophum sp., Nannobrachium sp., Protomyctophum sp., and Symbolophorus sp.).

Fig. 4. Fish biomass (0-1000 m) standardized by sampling effort. 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, 2021 indicated in x-axis labels. Day tows are shown on the left panel and night tows are shown on the right panel.

Fig. 5. Mesopelagic fish catch in number of individuals per square meter sampled from the MOCNESS-1 (1 m2 mouth opening) in purple and the larger MOCNESS-10 (10 m2 mouth opening) in green. Data shown are from night tows (n= 3 MOCNESS-1; n=5 MOCNESS-10). The grey overlapping colour represents 1 sq. meter MOCNESS catch where the 10 sq. meter MOCNESS catch is higher, and vice versa.

Fig. 6. Vertical distribution of fish biomass by fish family. Biomass standardized by volume filtered. Bar widths align with the minimum and maximum depth sampled by each of the 8 nets on the MOCNESS-1. Colours indicate Myctophidae (myctophids), Sternoptychidae (hatchetfishes), or Gonostomatidae (bristlemouths). MOCNESS-1 data is used because this net had higher resolution depth strata (8 nets instead of 4 nets as on the MOCNESS-10), there were more day tows than available from the MOCNESS-10 (three tows instead of two tows), and because the MOCNESS-1 tows represent paired day and night tows in similar space and time.

Fig. 7. Comparison of carbon flux associated with fish compared to zooplankton and passive sinking particles, from sediment traps or the Thorium-234 disequilibrium method, past flux boundaries of a) 200 and b) 500 m. Measurements taken in May, 2021 are broken into time periods t1, t2 and t3 for passive sinking particle flux measurements. These time periods were defined by ~8 day periods of flux collection punctuated by intense storm events (see EXPORTS “epochs” in Johnson et al 2024), and were delineated as follows: t1 = May 4-10, t2 = May 11-20, and t3 = May 21-29. Dots represent the minimum, nominal, and upper bound estimates for each flux pathway described on the y-axis. The fish carbon flux range represents the inner 90th percentile range and the nominal value is the mean fish flux estimate from Monte Carlo simulations (n = 1000 simulations). The zooplankton range represents variability by tow used to calculate zooplankton biomass, and the nominal value is the mean estimate (n = 3 tows). The Thorium-234 range represents the standard deviation for each measurement and the nominal value represents the mean measurement for each time period (Clevenger *et al.,* 2024). The sediment trap range represents measurement error and the nominal value represents the mean estimate for each time period (Estapa *et al.,* 2023).

Fig. 8. Violin plot comparing overall uncertainty in fish carbon flux estimates at 200 m from bioenergetic parameter uncertainty versus from biomass estimation. The inner 90th percentile range of estimates is indicated by the lower and upper bounds along the y-axis of the violin plot shapes. The x-axis shows the number of simulations that led to each estimate. This 90th percentile range for bioenergetics or biomass uncertainty alone is similar--consideration of either bioenergetic or biomass uncertainty results in comparable minimum and maximum estimates--indicating that biomass estimation alone contributes approximately the same amount of uncertainty as all bioenergetic parameter uncertainties combined.