## Thermal habitat shifts, but does not always widen, between embryonic and larval stages of fish

Running title: Thermal range differs for embryos vs. larvae

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*Statement of Authorship:* SJW, DR, and JAN designed the study. DR provided and helped analyze archival data. SJW collected samples. BJB, MKK, KC, HK, SLH, MR, OB, JM, SH, and AAVTS isolated individual ichthyoplankton and processed DNA barcoding results. SJW performed modeling work and analyzed results. SJW drafted the manuscript, to which all authors contributed feedback and substantial edits.

*Data Availability Statement:* EcoMon data are publicly available via NOAA’s National Centers for Environmental Information ([gov.noaa.nodc:0187513](https://www.ncei.noaa.gov/access/metadata/landing-page/bin/iso?id=gov.noaa.nodc:0187513)). DNA barcoding results from Lewis et al. (2016) are publicly available on BOLD under the project name [NIFEB](https://portal.boldsystems.org/result?query=NIFEB%5Brecordsetcode%5D), and those from the NYOS monitoring program are available under the dataset name NYOS ([dx.doi.org/10.5883/DS-NYOS](http://dx.doi.org/10.5883/DS-NYOS)), with additional data at [doi.org/10.6084/m9.figshare.27984443.v1](https://doi.org/10.6084/m9.figshare.27984443.v1). Code and auxiliary files to reproduce all analyses are available at [github.com/SarahJWeisberg/Ichthyoplankton-thermal-habitat](http://github.com/SarahJWeisberg/Ichthyoplankton_thermal_habitat).

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## Abstract

Temperature tolerance can vary greatly between ontogenetic stages of the same species and quantifying stage-specific thermal niches is critical for projecting climate impacts. For fish, ‘thermal bottleneck’ theory posits that temperature ranges are narrower for embryos than for larvae. However, this theory has not been fully validated with *in situ* evidence, in part due to lack of data on fish embryos, whose morphological similarities belie visual identification. Here, we used DNA barcoding to estimate thermal ranges of embryos and compare with those of larvae, for five species. None of the species we studied align with the predictions of thermal bottleneck theory. Instead, some species of embryos showed wider tolerances than their respective larvae, and all embryos had lower *in situ* thermal minima than conspecific larvae. Together, our results suggest that fish move through sliding windows, rather than bottlenecks, of thermal habitat as they progress from embryonic to larval stages.

## Introduction

Temperature is invoked to explain a wide range of global biological and ecological patterns, from mutation rates to body size to species diversity (Pianka et al., 1966; Angilletta et al., 2004; Allen et al., 2006). In our era of warming, examining and testing assumptions about temperature-driven processes and patterns is ever more urgent. This is especially true for ectotherms, whose survival, growth, and metabolic rates are more directly impacted by temperature changes than those of their endothermic counterparts (Deutsch et al., 2008; Buckley et al., 2012). Research on diverse taxa of ectotherms has shown that temperature impacts can vary meaningfully between life stages of the same species (Cupp, 1980; Floyd, 1983; Turriago et al., 2015; Pandori & Sorte, 2019; Enriquez-Urzelai et al., 2019; Ruthsatz et al., 2022).

Animals in their early life stages are thought to tolerate narrower ranges of temperatures than their adult counterparts (Keshavmurthy et al., 2014; Schiffer et al., 2014; Wang et al., 2017; Pandori & Sorte, 2019; Collin et al., 2021). One proposed mechanism posits that embryos in particular are limited in their oxygen transport functionality and thus more readily become metabolically limited at unfavorable temperatures (Pörtner et al., 2006; Melzner et al., 2009). However, the evidence is not entirely conclusive, as some results suggest that changes in thermal tolerance across ontogeny are species-specific and belie a coherent or generalizable explanation (Pörtner & Peck, 2010; Miller et al., 2013; Tangwancharoen & Burton, 2014; Hall & Warner; 2019; Cowan et al., 2023). Determining whether or not ectotherms display consistent ontogenetic patterns in their thermal biology is important for modeling climate change impacts; failing to properly account for life stage-specific temperature tolerances can strongly influence projections about species viability under future conditions (Radchuk et al., 2013; Levy et al., 2015; Hodgson et al., 2016; Morell et al., 2024).

For fish, the question of life cycle variation in thermal tolerance was examined in a recent meta-analysis of thermal tolerance of 694 fish species (Dahlke et al., 2020). The authors found that fish embryos and spawners tend to have narrower ranges of liveable temperatures than do larvae, with adults having the widest ranges. They propose that the full life cycle of fish is characterized by ‘thermal bottlenecks,’ and that survival hinges on the availability of suitable thermal habitat at the most sensitive life stage. If so, thermal bottlenecks likely dictate the susceptibility of a species to climate warming; relatedly, failure to study and understand thermal bottlenecks will almost inevitably lead to underestimations of climate change vulnerability.

The idea of thermal bottlenecks in the fish life cycle, while intriguing, has not yet been fully validated. Dahlke et al. (2020) relied on a good deal of phylogenetic data imputation for their analyses. Pottier et al. (2022) further pointed out that the metrics used by Dahlke et al. (2020) to estimate thermal habitats differed for embryos and larvae: the latter were largely derived from laboratory thermal tolerance studies, while the former were based on occurrence temperatures. This discrepancy may have confounded results by exaggerating the thermal habitat breadth of larvae (Pottier et al., 2022). Few studies have directly compared observed thermal habitats of the same fish species across all life stages, or of different co-occurring species across the same non-adult stages (McKenzie et al., 2020), and existing studies reveal species-specific, rather than universal, patterns (Pörtner & Peck, 2010).

Our understanding of how thermal habitat varies across life stages has been hindered in part because visual identification of fish embryos (i.e., unhatched eggs) to the species level is challenging, if not impossible, for many taxa. In contrast to the difficulty of visual identification, DNA barcoding of fish embryos provides a useful and reliable method for species-level classification (Hebert et al., 2003; Stoeckle, 2003; Kawakami et al., 2010; Harada et al., 2015; Lira et al., 2023). With DNA barcoding, PCR amplification and sequencing of a single gene – most commonly, the mitochondrial gene cytochrome c oxidase I (COI) – is used to identify samples. COI is well-conserved, such that it can be readily amplified with generic primers while still differing meaningfully between related species.

Here, we utilize DNA barcoding of fish embryos to test thermal bottleneck theory using *in situ* data collected across the Northeast US continental shelf. For several reasons, we focus on comparing embryos and larvae of the same species. First, existing knowledge about climate impacts on early life stages of fish is sparse, especially compared with our understanding of adults – even though survival through early life stages can determine population dynamics (Hjort, 1914; Houde, 2008; Hare, 2014). Second, thermal bottleneck theory, as articulated by Dahlke et al. (2020), describes a dramatic jump in thermal ranges between embryonic and larval stages: from 8.4 ± 0.4°C for embryos to 22.3 ± 0.7°C for larvae. Third, embryo and larval samples are collected in the same fashion (i.e., via plankton tows), allowing us to readily apply the same analytical approach to both stages and thus alleviate concerns about methodological disparities.

Based on thermal bottleneck theory as proposed by Dahlke et al. (2020), we generated three hypotheses:

1. For the same species, the lower bound of thermal habitat (Tmin) is lower for larvae than for embryos;
2. For the same species, the upper bound of thermal habitat (Tmax) is higher for larvae than for embryos;
3. For the same species, the range of thermal habitat (Tmax - Tmin = Trange) is wider for larvae than for embryos.

## Methods

### Data Collection & Collation

To compare the thermal habitats of fish residing in the Northeast US continental shelf across early life stages, we collated all available species-level data. The Northeast Fisheries Science Center conducts an extensive shelf-wide plankton survey every other month (Figure 1). These Ecosystem Monitoring (EcoMon) surveys use a stratified random sampling design and collect plankton using a 61cm diameter bongo frame with a 333μm mesh, which is towed obliquely at roughly 1.5 knots for at least 5 minutes per station. The net is towed within 5 m of the seafloor where possible, but tow depths do not exceed 200m. The total volume of water filtered is measured by mechanical flowmeters mounted on the net (NOAA Fisheries Northeast Fisheries Science Center, 2019). An earlier plankton survey called MARMAP ran from 1977-1987 and utilized a fixed station design but was otherwise similar to current EcoMon practices. In total, the EcoMon-MARMAP (hereafter, ‘EcoMon’) dataset includes 25,509 stations. Fish larvae are identified visually to the lowest taxonomic level possible, primarily by the Sea Fisheries Institute Sorting and Identification Center in Poland. Data are standardized and reported in units of individuals per 10m2 for each tow (Ejsymont & Sherman, 2000; Richardson et al., 2010). Sea surface temperature (SST) measurements from CTD sensors are included in the EcoMon dataset (NOAA Fisheries Northeast Fisheries Science Center, 2019). Recorded SST values range from -0.53 to 29.21°C, with a mean and median of 12.64 and 12.06°C, respectively (Figure S1A).

Due to difficulties in visual differentiation, fish embryos collected in EcoMon surveys are not routinely identified. However, DNA barcoding has recently been advanced within the Northeast Fisheries Science Center as a method for identifying embryos to the species level. In a pilot experiment, DNA barcoding analysis was conducted on samples collected at 480 EcoMon stations during surveys from 2002-2012 (Figure 1, inset). SST measurements were made as described above and ranged from 1.8 to 27.02°C, with a mean and median of 13.21°C and 12.39°C, respectively (Figure S1B). Of the 1603 isolated embryos in this pilot study, 1495 (93%) were identified to the species level (Lewis et al., 2016). This DNA barcoding dataset was included in our analyses.

Due to the limited size of this pre-existing embryo dataset, we supplemented archival EcoMon data with a second fish embryo dataset, compiled with samples we collected during the ongoing New York Offshore (NYOS) monitoring study. The area surveyed in NYOS cruises extends from the mouth of the Hudson River to Montauk Point and spans the continental shelf (Figure 2). NYOS sampling occurs seasonally and uses a fixed sampling design, although in 2023, winter sampling was eliminated and the number of transects and stations were reduced to more efficiently use available ship time. Plankton samples are collected using a 60cm diameter, 333μm mesh net lowered vertically to a depth of 25m or the maximum allowable water depth. These samples are stored in 95% ethanol and CTD casts occur at the same stations as plankton sampling. Animal collections are permitted by the NY State Department of Environmental Conservation (scientific permit #1145). Our analyses include data from 132 stations sampled from May 2021 to October 2023, with SST values ranging from 4.99 to 26.51°C (mean = 16.61°C, median = 14.80°C; Figure S1B).

Following each cruise, plankton samples were stored at 4°C and then sorted in their entirety to select individual ichthyoplankton for DNA barcoding. During sorting, plankton samples were diluted to a 2:1 ethanol:biovolume concentration and observed using a Nikon SMZ1270 stereo zoom microscope. Individual ichthyoplankton were isolated and micrograph images were recorded for each animal. We prepared 96-well plates with one animal and 30μL of 95% ethanol per well, filling 95 wells and leaving one empty as a negative control. All lab work – dilution, isolation, imaging, and sample storage – was conducted by a team of paid high school and undergraduate researchers. For this study, we isolated 1,229 fish embryos.

Filled 96-well plates were shipped to the University of Guelph’s Canadian Centre for DNA Barcoding. Standard DNA barcoding protocols were followed for DNA extraction, PCR, and DNA sequencing (see Lewis et al., 2016 for full details). PCR primers C\_FishF1t1 + C\_FishR1t1 (Ivanova et al., 2007) were used to amplify the ~658 base pair fish COI gene. Codon Code Aligner software (CodonCode Corp., Centerville, MA) allowed for trace files to yield contiguous sequences of DNA. Sequences and trace files were uploaded to the NYOS project on the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007).

COI sequences were queried against the BOLD database of published sequences, and matches above 99% were considered valid. Of the 1,229 fish embryo samples submitted to CCDB, 1,144 (93%) yielded sequences that met the BOLD standard for species identification. Of the remaining 85, we were able to salvage sequence information, which we cleaned using the *DNA Subway* toolkit and identified via a BLAST search, as in Marizzi et al. (2018). This resulted in the successful identification of 23 individuals, bringing our total number of identified embryos to 1,167 (95% success rate). We are preparing a separate manuscript describing the entire dataset (Hadjiargyrou et al., in prep). Upon its publication, all barcoding data will be available on BOLD.

### Thermal Habitat Analysis

Once we assembled all available species-level data on fish embryos and larvae in the Northeast US continental shelf, we chose five species for further thermal habitat analysis (Table 1). These species had a minimum of 100 embryos identified with DNA barcoding across both embryo datasets and larvae identified to the species level in the EcoMon survey. These constraints selected for the following species:

1. Silver Hake (*Merluccius bilinearis*)
2. Windowpane Flounder (*Scophthalmus aquosus*)
3. Gulf Stream Flounder (*Citharichthys arctifrons*)
4. Fourspot Flounder (*Hippoglossina oblonga*)
5. Butterfish (*Peprilus triacanthus*)

These species come from 4 different families, spawn in different seasons, and occupy a diversity of habitats as adults, including 3 benthic, 1 demersal, and 1 pelagic species (Table 1). Finally, we ensured a mix of geographic ranges for adults. All five species have ranges that span from Florida to Canada, but two of the species – Silver Hake and Windowpane Flounder – are concentrated north of Cape Hatteras (FishBase; Froese & Pauly, 2000). We quantified the biomass-weighted mean latitude and longitude values for adults in the NEUS using the approach of Mills et al. (2024). We recognize that ranges have shifted over time for many of these species (Nye et al., 2009; Mills et al., 2024), and therefore also report standard deviations of mean latitude and longitude across the available time series (1970-2019; Table 1).

We used single parameter quotient (SPQ) analysis to determine the thermal habitat of our five species of interest, in both embryo and larval stages. This approach has been used to evaluate fish habitat preferences, especially for spawning (e.g., Twatwa Mhlongo et al., 2005; Bernal et al., 2007; Overholtz et al., 2011; Henderson, 2019; Richardson et al., 2020). For quotient analysis, we binned the data into 0.5°C bins and calculated the SPQ for each temperature bin as:

[1]

where is the proportion of fish abundance in bin and is the proportion of samples collected in bin . Values greater than 1 indicate that the species prefers a given temperature bin, while values below 1 indicate that the species is avoiding that temperature. SPQ values approximating 1 are evidence that a temperature bin is tolerated, but neither preferred nor avoided.

Egg abundance was estimated differently in each of the two embryo datasets (EcoMon and NYOS), and therefore we had to calculate SPQ from each dataset separately. In the EcoMon embryo dataset, the number of embryos found in each station was multiplied by an ichthyoplankton haul factor to estimate effort-corrected abundance. Further, in isolating samples for DNA barcoding, Lewis et al. (2016) subsampled embryos based on diameter for many stations. We accounted for this subsampling by calculating the proportion of embryos of each species in each size bin and applying this proportion to the number of un-barcoded embryos in that same size bin from the same station. Visual inspection showed that estimates from the NYOS and EcoMon data were generally in agreement, with disparities that can primarily be attributed to differences in sampling effort (e.g., lack of NYOS sampling in the 16.5-17.5°C bins).

We developed two approaches to estimate the thermal habitats of the embryo and larval stages of the five species of interest using the SPQ metric. First, we used a bootstrapping routine to generate confidence intervals for each SPQ value. For each dataset (i.e., EcoMon larvae, EcoMon embryos, and NYOS embryos), we tallied the number of stations sampled (nstation). For each species, we resampled from its abundance dataset with replacement, nstation times, and calculated SPQ for each temperature bin. We repeated this process 1000x, and we determined the 5th and 95th quantiles of each SPQ value from the respective bootstrapped dataset.

We examined the output to determine how to define threshold preferences from our confidence intervals. Confidence intervals for embryo data were much wider compared with those of larvae, as is to be expected given the relative sizes of datasets (25,509 stations for larvae vs. 612 total stations for embryos; see, for example, Figure S2A vs. Figure S2B). For larvae, we estimated the lower thermal habitat limit as the lowest temperature bin in which the confidence interval includes SPQ = 1 for that and all higher temperatures. Similarly, we estimated the upper limit as that which the confidence interval includes SPQ = 1 for that bin but *not* all higher temperatures. However, we noticed that for three of the species examined – Gulf Stream Flounder, Fourspot Flounder, and Butterfish, the confidence interval crossed the 1 line for the second to highest temperature bin (28.5°C; Figures S4B, S5B, S6B). EcoMon sampling was very sparse at the highest temperatures, with only 10, 7, and 2 samples collected in the 28, 28.5, and 29°C bins, respectively. Therefore, we do not believe we can conservatively estimate a maximum temperature for these larvae. For embryos, we took advantage of the two datasets and determined the minimum and maximum temperature bins as the extreme values for which SPQ estimates from both surveys include 1. We made an exception for Windowpane Flounder, the species with the lowest data availability. For this species, we found it appropriate to estimate the thermal range as the minimum and maximum where the SPQ = 1 line was crossed in either survey. As with larvae, we found it difficult to confidently estimate a maximum value for Gulf Stream Flounder, Fourspot Flounder, and Butterfish.

To increase confidence in our inferences, we employed a second approach to estimating thermal habitat. As in Henderson (2019), we normalized SPQ values for each species-dataset combination and then calculated a cumulative distribution function. Then, we fit a generalized linear model to the cumulative distribution function using a quasibinomial link (*stats* package in R, version 4.4.0). The quasibinomial is similar to the binomial but includes a dispersion parameter 𝜏, which allows for better fits to under-dispersed data like ours here. Quasibinomial models can fit proportional response data rather than the binary data required by binomial models. Finally, the generalized linear models can estimate response values for unsampled predictor variables, such as temperatures higher than 27°C. For the embryo data, we treated both survey estimates as inputs. Quasibinomial fits were statistically significant for all parameters in all species and across all stages (Table S1). From these models, we estimated the 5th and 95th percentiles of the cumulative distribution function for each species in each early life history stage and used these as estimates of the lower and upper bounds of thermal habitat.

## Results

We estimated the thermal habitat of larval and embryonic stages of five fish species (Table 2, Figure 3, Figures S2-S6) and compared our results with the predictions of thermal bottleneck theory (Dahlke et al., 2020). Contrary to our first hypothesis, Tmin was higher for larvae than embryos for all five species. Predictions of the second hypothesis, that larvae would have higher Tmax than embryos, were met for two of the species (Silver Hake and Windowpane Flounder). For the other three species, it was challenging to identify Tmax since both larvae and embryos were found at or near the maximum temperatures surveyed. However, our quasibinomial models predict similar Tmax values for the embryos and larvae of these three species (Gulf Stream Flounder, Fourspot Flounder, and Butterfish). Lastly, our results align with the hypothesis that Trange is wider for larvae than for embryos in two species (again, Silver Hake and Windowpane Flounder). For the other three species, model results indicate that Trange is wider for embryos than for larvae.

Additionally, the notion proposed by Dahlke et al. (2020) that Trange for larvae is ~22°C, was firmly rejected by our analyses. Instead, we found that larval Trange has a mean of 9.6°C with a range of 9-10.5°C. Our Trange values for embryos were more aligned with their predictions of ~8°C. We estimated Trange of Windowpane Flounder embryos as 8-8.5°C. Trange for Silver Hake was lower (6.5-7°C), while for the other three species, Trange was several degrees higher. For these three – Gulf Stream Flounder, Fourspot Flounder, and Butterfish – we can conclude that Trange is *at least* 13, 15, and 13.5°C, respectively.

Based on these results, we identified two clusters of species. The first consists of our two more northern species, Silver Hake and Windowpane Flounder (Table 2, Figures S2-S3). For these two species, we find that Tmin, Tmax, and Trange values are all higher for larvae than for embryos, and the Trange values for embryos are 6.5-8.5°C. The second cluster includes three species whose geographic centers are further south: Gulf Stream Flounder, Fourspot Flounder, and Butterfish (Table 2, Figures S4-S6). For these three species, we observed Tmax values at or near the maximum surveyed temperatures. In this cluster, we observed particularly large differences in Tmin between embryos and larvae. The most extreme example is Fourspot Flounder, whose embryos have a Tmin that is ~6°C lower than the larvae. For the southern cluster, our model predictions indicate that Trange is wider for embryos than larvae.

Several aspects of our approach make us highly confident in the results we present here. Due to the low availability of embryo data, we supplemented the EcoMon dataset with newly collected and barcoded embryos from 132 stations of NYOS surveys. This allowed us to nearly double the embryo sample size included in our analyses of five selected species, from 666 to 1,142. The EcoMon and NYOS embryo samples were collected in two different time periods – 2002-2012 and 2021-2023, respectively – as well as across two different geographic ranges (Figures 1 and 2). Nevertheless, we saw a high degree of agreement between the estimates derived from each survey, suggesting that our results are robust. Unsurprisingly, we found wider confidence intervals for embryos than for larvae, which we ascribe to lower data availability. To account for this, we added a more stringent interpretation threshold for the embryo data, requiring agreement between both EcoMon and NYOS surveys to determine Tmin and Tmax. To further increase confidence in our inferences, we employed two different approaches to estimate thermal habitats from single parameter quotients: a bootstrapping-based confidence interval estimation and generalized linear modeling. Our results from these two approaches were highly consistent: across the 14 parameters estimated by both models, differences ranged from 0-1°C with an average of 0.46°C (Table 2).

## Discussion

None of the five species we tested fully conformed with the predictions of thermal bottleneck theory as articulated by Dahlke et al. (2020). Rather than bottlenecks, our evidence suggests ‘sliding windows’ of thermal habitat as fish progress between embryonic and larval stages. For all species, embryos occupied colder temperatures compared with conspecific larvae, indicating that their thermal habitat window opens at a lower threshold. This window stays open past the minimum value for larvae, and so there is some overlap between embryonic and larval habitats in all species tested. Depending on the species, however, the thermal habitat windows of embryos shut at the same or lower temperatures as that of larvae. As a result, we found no singular pattern as to whether embryos or larvae have wider temperature ranges.

We estimated the realized, rather than fundamental, thermal niches of fish embryos and larvae, and the difference between these niche types might explain why our results diverge from the findings of Dahlke et al. (2020) and others. In the controlled laboratory studies often used to estimate critical or lethal temperature values, stage-correlated aerobic limits might indeed be the key factor determining survival. In real ecological settings, however, temperature is linked with development time, predation rates, abundances of both predators and prey, pH and CO2 concentrations, as well as local hydrography and transport to suitable juvenile habitat (Llopiz et al., 2014; Morell et al., 2024). We presume that the stage-specific realized thermal niches observed here reflect a combination of these and perhaps additional factors.

Ecological, rather than mechanistic, thinking can help explain our finding that embryos occupy colder thermal habitats than their conspecific larvae. This was especially true for the southern cluster of species, whose lower thermal habitat bounds are 2.5-6°C colder for the embryos than the larvae. While water may warm between spawning and hatching, hatching time for most species included in our study ranges from 2-3 days at relevant temperatures (Kendall & Naplin, 1981; Miller et al., 1991; Able & Fahay, 1998; Morse et al., 1999). Even accounting for a longer lag until the larvae are collected, it is unlikely that water temperature would rise by several degrees in such a short period. Rather, there may be survival advantages conferred by colder habitats that are specific to the embryonic stage. Indeed, Pepin (1991) found lower cumulative mortality for embryos at lower temperatures – a trend that reversed for yolk-sac larvae. We speculate that survival advantages for embryos at low temperatures could include decreased exposure to multiple stressors, which can impact embryos more acutely than larvae (Schwemmer et al., 2020), reduced metabolism and thus starvation rates (Suneetha et al., 1999; Houde, 1974), or reduced consumption rates by predators and thus reduced predation risk (Elliott & Leggett, 1996). Additionally, larvae are sensitive to extrinsic food availability, which is likely temperature-dependent (Morell et al., 2024). Differences between the northern and southern species clusters may also reflect differing transport dynamics associated with geographically distinct source communities (Grothues et al., 2002). These and other possible explanations should be further explored – especially since as waters warm, embryos may lose access to colder temperatures, which could shrink their thermal habitat. If this happens, we may see replacement of cold water species by the higher survival of the embryos of warm water species, contributing to ongoing tropicalization of the Northeast shelf region (Friedland et al., 2023; Fenwick et al., 2024).

As alluded to above, our results clustered into two geographically distinct groups: one more northern and cold-biased, the second southerly and warmer. In our northern cluster, we did indeed find a narrower thermal range at the embryo stage relative to larvae, by ~1°C for Windowpane Flounder and ~2.5°C for Silver Hake. Although much slimmer than the differences proposed by Dahlke et al. (2020), this finding implies that climate vulnerability for these northern species might be determined at the embryonic stage. In contrast, our southern cluster showed larger temperature ranges for embryos than for larvae, indicating that the larval period may be the bottleneck. Further research should extend this to additional species and determine if these clusters are robust, as well as how rising temperatures might impact population dynamics for each cluster.

While adult geography may explain some of our results, we found that larval habitat spans roughly 9.5°C, regardless of adult habitat, adult geographic range, or spawning seasonality. This indicates the range may be a conserved trait advantageous for overall species persistence. As waters warm, available thermal habitat might constrict for many of these species, and they may no longer be able to span a 9.5°C range during their larval stages. This could alter survival, dispersal and/or phenology at the population level.

Our results may have been influenced by factors we were not able to consider due to data limitations. First, three of the five species analyzed here were found at the maximum observed temperatures in our region. For these three, we had to rely on model-derived estimates, which may be biased. Additional research is needed to analyze the embryos and larvae of these species in the lower latitude portions of their ranges to confirm their maximum temperatures. We binned our data into 0.5°C bins, as has been done in prior studies (e.g., Bernal et al., 2007; Asch & Erisman, 2017), but therefore our results are only accurate to the nearest 0.5°C. Additionally, we did not factor larval age, stage, or length into our analyses. The larval samples identified by the EcoMon program may be biased in some fashion, perhaps toward higher age, which could in turn bias our results. Such a bias might occur if the earlier yolk-sac stages are more difficult to identify to the species level, or if they are excluded from the 333μm mesh used for EcoMon sampling (Schobernd et al., 2018). However, even accounting for possible bias, it is unlikely that the true larval temperature minima are lower than those of conspecific embryos, or that their thermal habitat ranges are anywhere close to the 22°C proposed by Dahlke et al. (2020). Finally, we did not factor possible vertical migration into our analyses since we lacked data on species-specific depth ranges. If larvae are not in the surface mixed layer, SST might not reflect their experienced temperatures.

Our species choices may have introduced biases as well. We selected species with high abundances of embryos across our two embryo datasets to ensure robust inferences. These species may have particularly long spawning seasons, which could explain why their embryos are adapted to a wider temperature range (though the same would likely apply to larvae). This might not be true of other, rarer species. Finally, the species we chose all spawn pelagic embryos – a trait that characterizes most, but not all, fish species that occur along the Northeast US continental shelf. It remains unclear how our findings might apply to fish with other reproductive strategies; this would be a very interesting avenue for further study.

Future research should expand to include not only additional species and life history strategies but also the life cycle stages excluded here. Thermal habitats of adults and spawners must be analyzed to test the full suite of predictions of thermal bottleneck theory. It is often difficult to find metrics for spawners comparable to those of non-spawning adults (Pottier et al., 2022). However, embryo occurrence and abundance data like those from our NYOS dataset can be used to estimate spawning locations (see, for example, Harada et al., 2015) and, therefore, spawning temperatures. Such analyses could help illuminate the thermal habitats of fish across their entire life cycle, yielding information essential for determining species-specific climate vulnerabilities. This information can and should feed into formal vulnerability assessments used to prioritize management actions (Pecl et al., 2014; Morrison et al., 2015; Hare et al., 2016; Hodgson et al., 2016; Saba et al., 2023).

A full picture of climate vulnerability across fish life cycles will be greatly aided by continued monitoring. We have demonstrated that the simple SPQ metric can be used to compare across life stages, but inferences would be greatly strengthened by additional data. Further study could also shed light on the degree to which Tmax, Tmin, and Trange values are plastic and adaptable in the context of changing temperatures. EcoMon and NYOS datasets appear to yield very similar results despite significant warming in the decade between the last EcoMon sample collection and the first of NYOS, suggesting a certain level of fixity in the thermal habitat of NEUS fish embryos.

Here, we have shown that the tenets of thermal bottleneck theory, as proposed by Dahlke et al. (2020), do not universally apply. However, we have identified two possible elements of life stage-specific vulnerability, both of which could lead to fish population declines: (1) if embryos lose access to the cold portions of their thermal habitats, they may lose a survival advantage, and (2) if larvae are no longer able to occupy a ~9.5°C thermal range, populations might decrease. These and future ontogenetic observations can illuminate the full suite of risks and opportunities that animals will encounter in a warming ocean.

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We are grateful for the funding support that made this work possible. Funding for NYOS monitoring cruises and related DNA barcoding work was provided by the New York State Department of Environmental Conservation (MOU #900286-02), and SJW was supported by the NOAA-Sea Grant Population and Ecosystem Dynamics Fellowship. We thank several partner organizations for their funding support via student stipends: BioBus, Inc., NY Sea Grant, NY State Department of Environmental Conservation, the American Fisheries Society Hutton Scholars program, East Side Community School (part of NYC Public Schools), and the National Science Foundation’s GEOPaths program. We are deeply grateful to those responsible for decades-long work of collecting and curating the EcoMon dataset, and to the scientists and ship crews involved in the NYOS monitoring program. Finally, we appreciate the support from Stony Brook University’s Simons STEM Scholars and WISE programs, who facilitated student participation in this project.

## Conflict of Interest Statement

We declare no conflict of interest.

## Tables

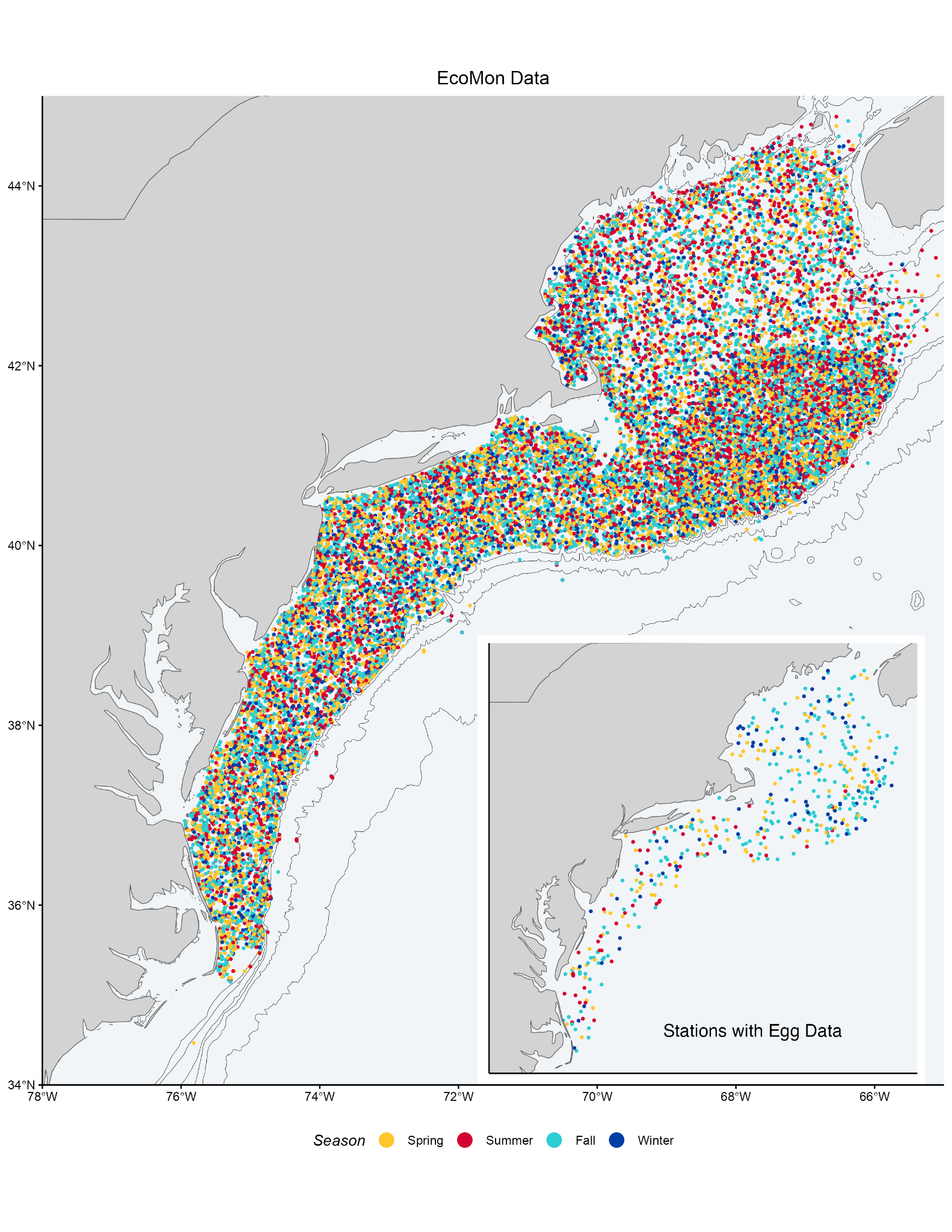
Table 1. Information on species selected for thermal habitat analysis. Species were selected if they had a minimum of 100 embryos identified by DNA barcoding across both EcoMon and NYOS datasets. Latitude (in °N) and longitude (in °W) values reflect biomass-weighted means in the Northeast US continental shelf, calculated across all available time periods (see Mills et al., 2024). SD = standard deviation.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species** | **# Eggs**  **(Ecomon, NYOS)** | **Seasons Eggs Found** | **Mean Latitude (Adults, ± SD)** | **Mean Longitude (Adults, ± SD)** | **Adult Habitat** |
| Silver Hake | 304  (196, 108) | Spring, Summer, Fall | 41.731  ± 0.581 | -69.163  ± 0.639 | Demersal |
| Windowpane Flounder | 104  (81, 23) | Spring, Summer, Fall | 40.913  ± 0.199 | -69.186  ± 0.658 | Benthic |
| Gulfstream Flounder | 378  (153, 225) | Spring, Summer, Fall | 40.141  ± 0.276 | -70.874  ± 0.598 | Benthic |
| Fourspot Flounder | 236  (184, 52) | Spring, Summer, Fall | 40.106  ± 0.145 | -70.620  ± 0.424 | Benthic |
| Butterfish | 120  (52, 68) | Spring, Summer | 39.119  ± 0.700 | -72.038  ± 0.873 | Pelagic |

Table 2. Results of thermal habitat estimation for all species and life stages analyzed. Model and bootstrap estimates reflect the two approaches used for estimation, see text for details. All values given in °C. ‘Max’ indicates that the upper limit was not estimable from the bootstrapped dataset, ‘range’ is the difference between maximum and minimum values. Ranges estimated from models are highlighted for easy comparison.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Egg Range** Model Estimate | **Larval Range** Model Estimate | **Egg Range** Bootstrap Estimate | **Larval Range** Bootstrap Estimate |
| Silver Hake  *range* | 12 - 18.5 | 13.5 - 22.5 | 12 - 19 | 13.5 - 23 |
| *6.5* | *9* | *7* | *9.5* |
| Windowpane Flounder  *range* | 9.5 - 17.5 | 10.5 - 20 | 9 - 17.5 | 10 - 19 |
| *8* | *9.5* | *8.5* | *9* |
| Gulfstream Flounder  *range* | 14.5 - 27.5 | 17 - 27.5 | 14 - max | 16.5 - max |
| *13* | *10.5* | – | – |
| Fourspot Flounder  *range* | 11.5 - 26.5 | 18 - 27.5 | 12 - max | 17 - max |
| *15* | *9.5* | *–* | – |
| Butterfish  *range* | 14 - 27.5 | 17.5 - 27.5 | 14.5 - max | 17 - max |
| *13.5* | *10* | *–* | – |

## Figures

Figure 1. Maps of sample collection locations and seasons for EcoMon samples included in analyses. Larger map shows all samples that have species-level larval data; inset shows stations with species-level embryo data from samples that were analyzed using DNA barcoding from 2002-2012 (Lewis et al., 2016). Color represents seasonal breakdown. Winter = Dec-Feb, Spring = March-May, Summer = June-Aug, Fall = Sept-Nov. Contour lines show the 50, 100, 500, 1000, and 2000m isobaths.

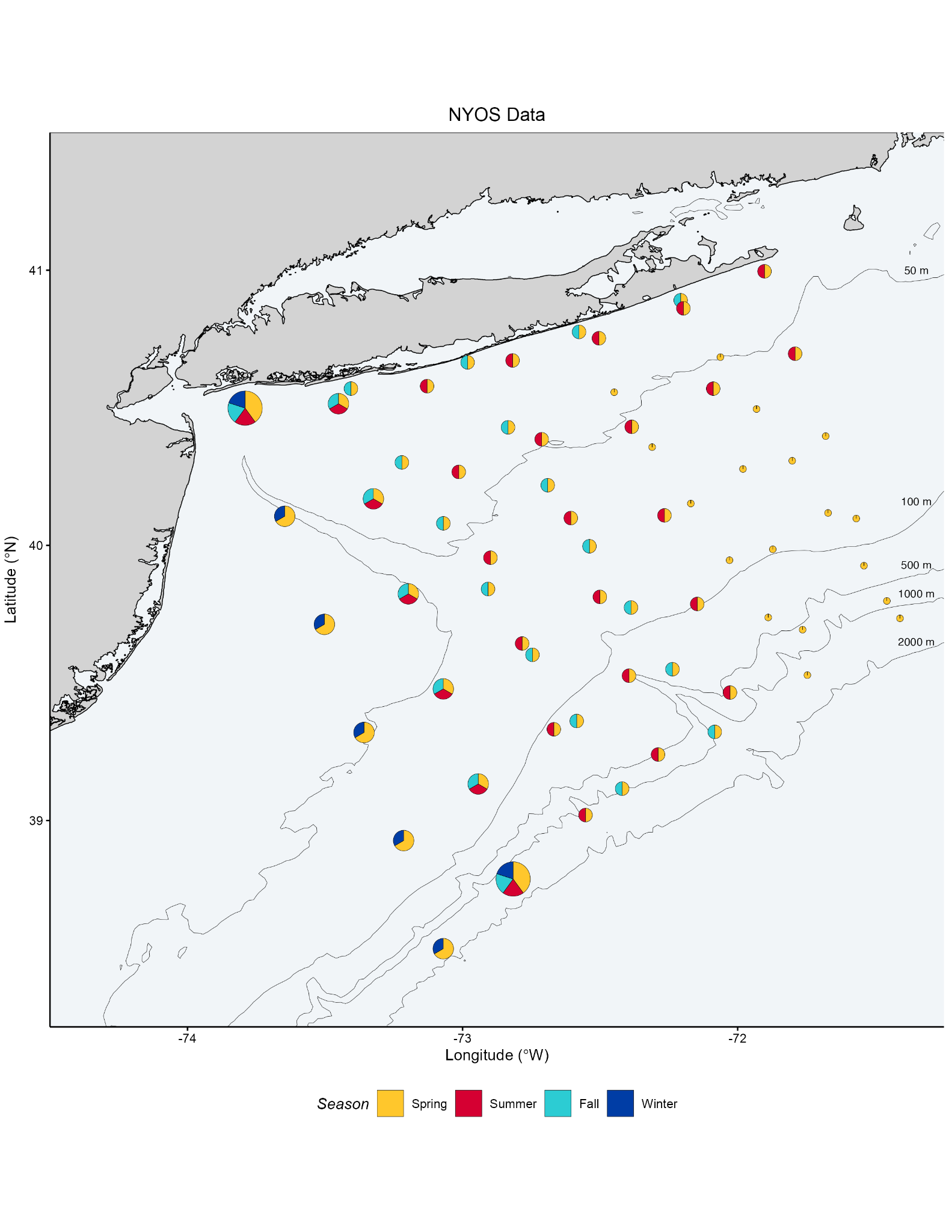
**

Figure 2. Map of sample collection locations and seasons for New York Offshore (NYOS) samples included in analyses. The size of each pie chart corresponds to the number of samples collected at a given location; color represents seasonal breakdown. Winter = Dec-Feb, Spring = March-May, Summer = June-Aug, Fall = Sept-Nov. Contour lines show the 50, 100, 500, 1000, and 2000m isobaths.

## 

Figure 3. Comparisons of thermal habitats of embryos (green) and larvae (purple) of the five study species, based on the cumulative fraction of single parameter quotient (SPQ) values. Lines reflect model-based estimates, shading indicates 95% confidence intervals.

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# Supplemental Tables and Figures

Table S1. Scaled deviance and p-values for all generalized linear models fit to data. P-values are calculated from the distribution.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Scaled deviance**  Eggs | **p-value**  Eggs | **Scaled deviance**  Larvae | **p-value**  Larvae |
| Silver Hake | 2079.163 | 0 | 50226.64 | 0 |
| Windowpane Flounder | 1741.69 | 0 | 11069.13 | 0 |
| Gulf Stream Flounder | 1474.251 | 1.53E-322 | 7578.301 | 0 |
| Fourspot Flounder | 838.2736 | 2.58E-184 | 11285.22 | 0 |
| Butterfish | 633.3645 | 9.27E-140 | 8887.725 | 0 |

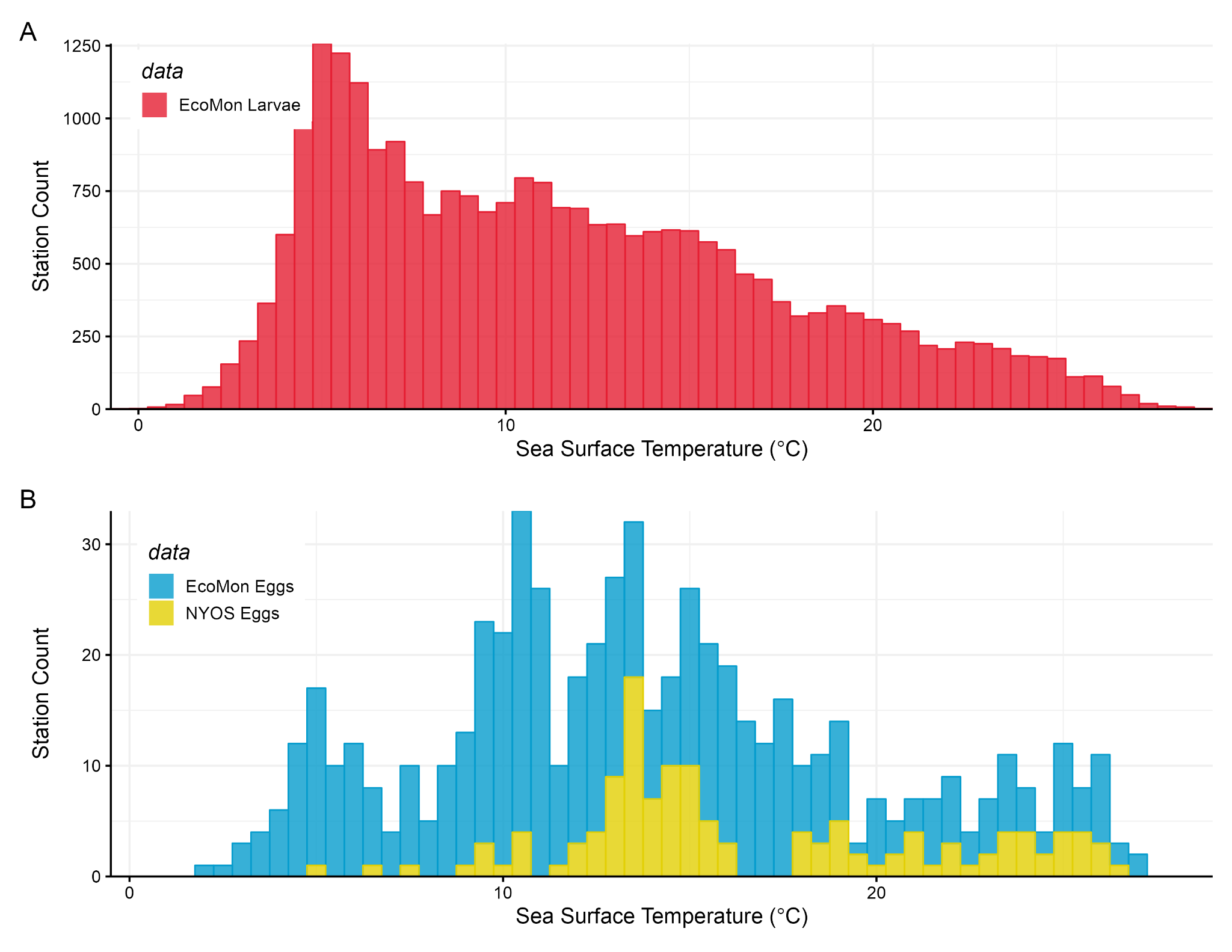


Figure S1. Distribution of sea surface temperatures at sample collection sites for samples included in analyses. Note differences in y-axis scale. Temperature is binned to 0.5°C bins.

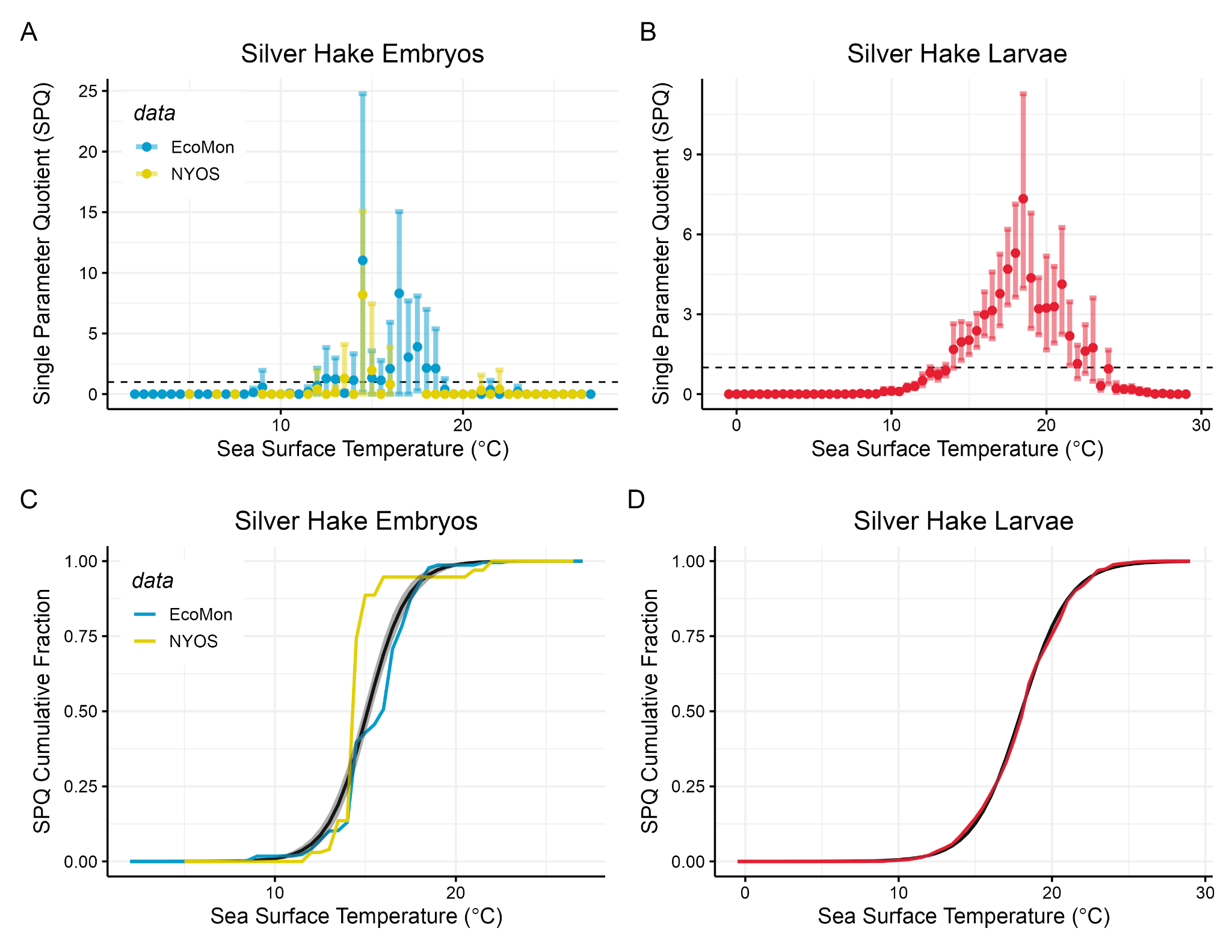


Figure S2. Thermal habitat of Silver Hake embryos and larvae estimated by single parameter quotient (SPQ) analysis. A-B: dots represent estimates from original dataset, 90% confidence intervals are derived from bootstrapping. Dashed line at SPQ = 1, indicating temperature is neither preferred nor avoided. C-D: results from generalized linear modeling, black lines represent model predictions, colored lines show estimates from data, shading indicates 95% confidence intervals from model-based estimation.

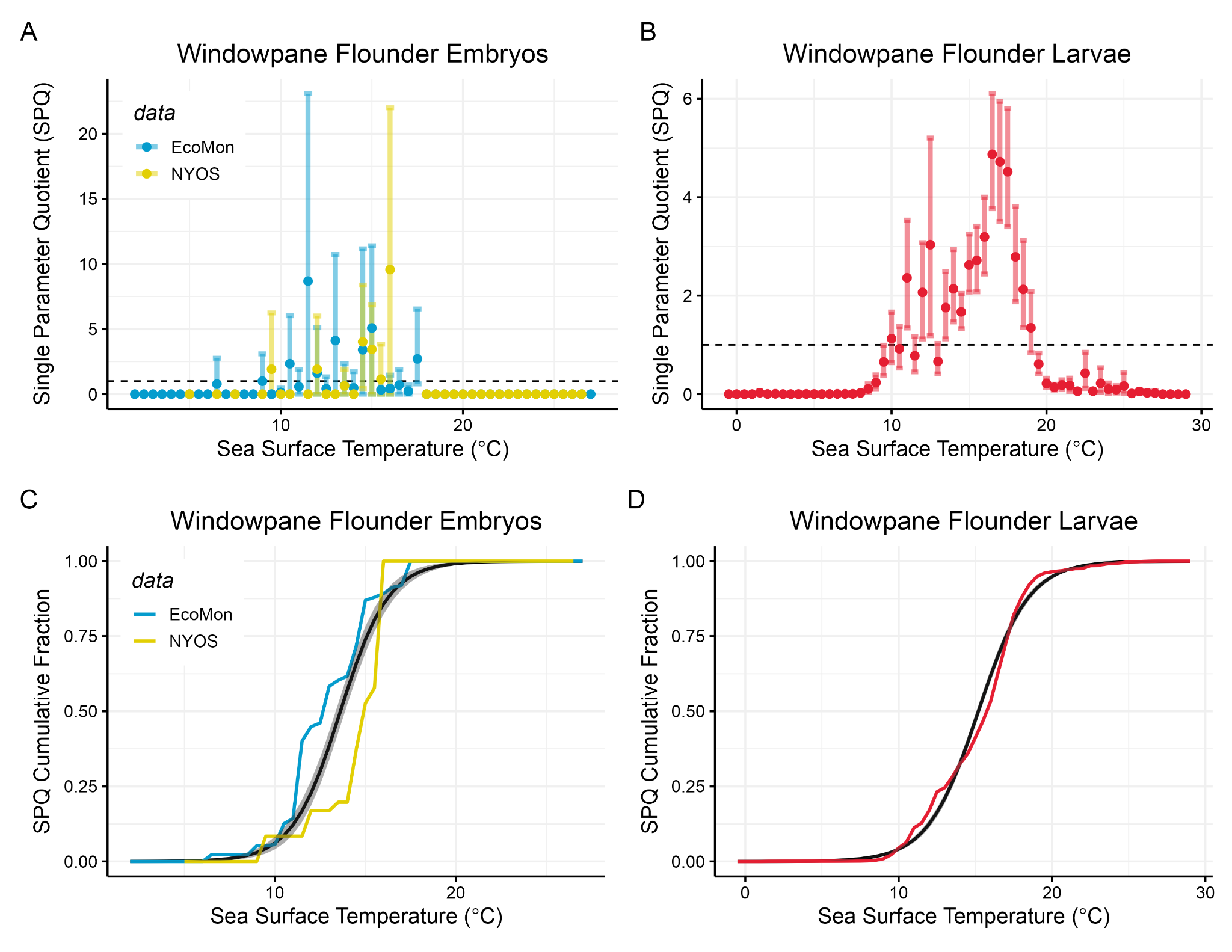


Figure S3. Thermal habitat of Windowpane Flounder embryos and larvae estimated by single parameter quotient (SPQ) analysis. A-B: dots represent estimates from original dataset, 90% confidence intervals are derived from bootstrapping. Dashed line at SPQ = 1, indicating temperature is neither preferred nor avoided. C-D: results from generalized linear modeling, black lines represent model predictions, colored lines show estimates from data, shading indicates 95% confidence intervals from model-based estimation.

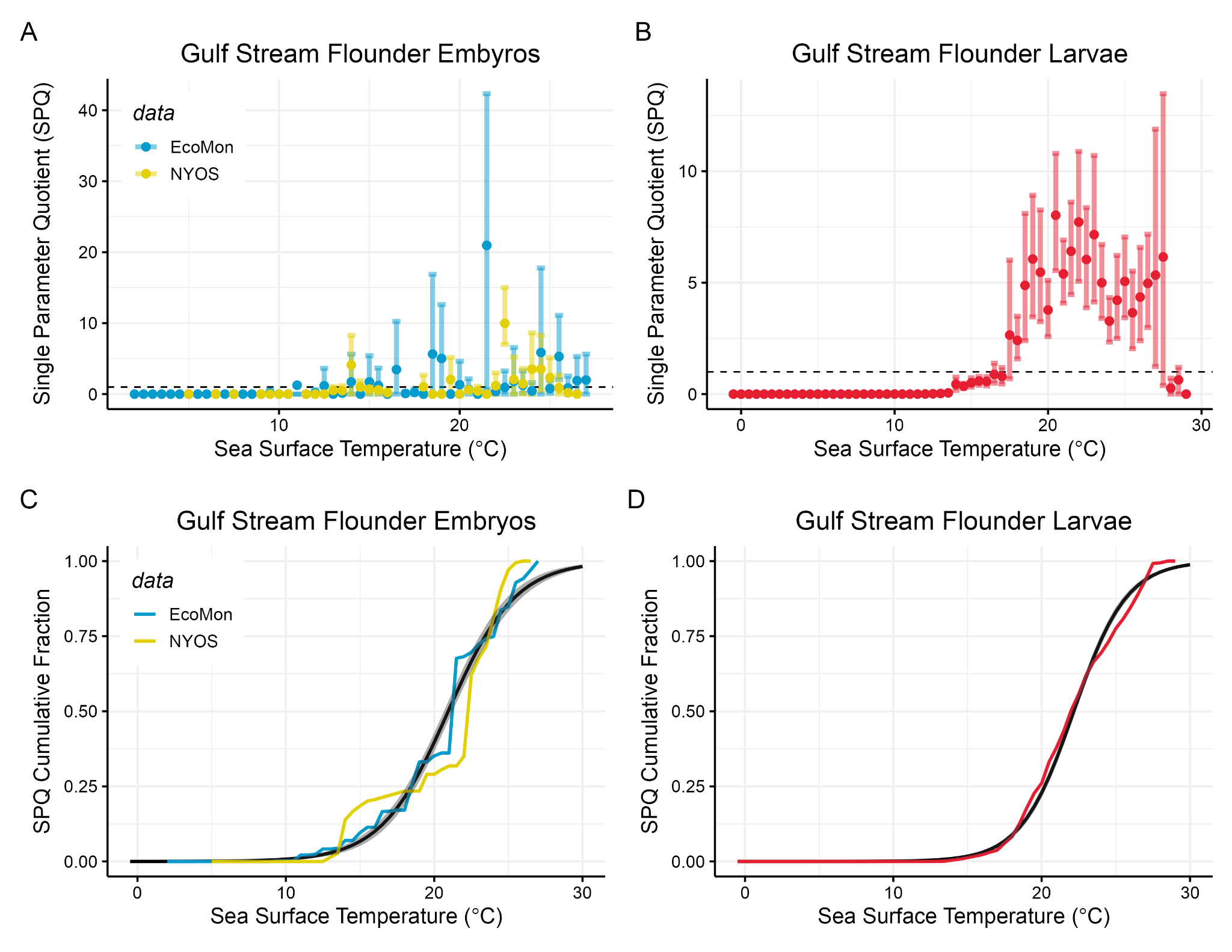


Figure S4. Thermal habitat of Gulf Stream Flounder embryos and larvae estimated by single parameter quotient (SPQ) analysis. A-B: dots represent estimates from original dataset, 90% confidence intervals are derived from bootstrapping. Dashed line at SPQ = 1, indicating temperature is neither preferred nor avoided. C-D: results from generalized linear modeling, black lines represent model predictions, colored lines show estimates from data, shading indicates 95% confidence intervals from model-based estimation.

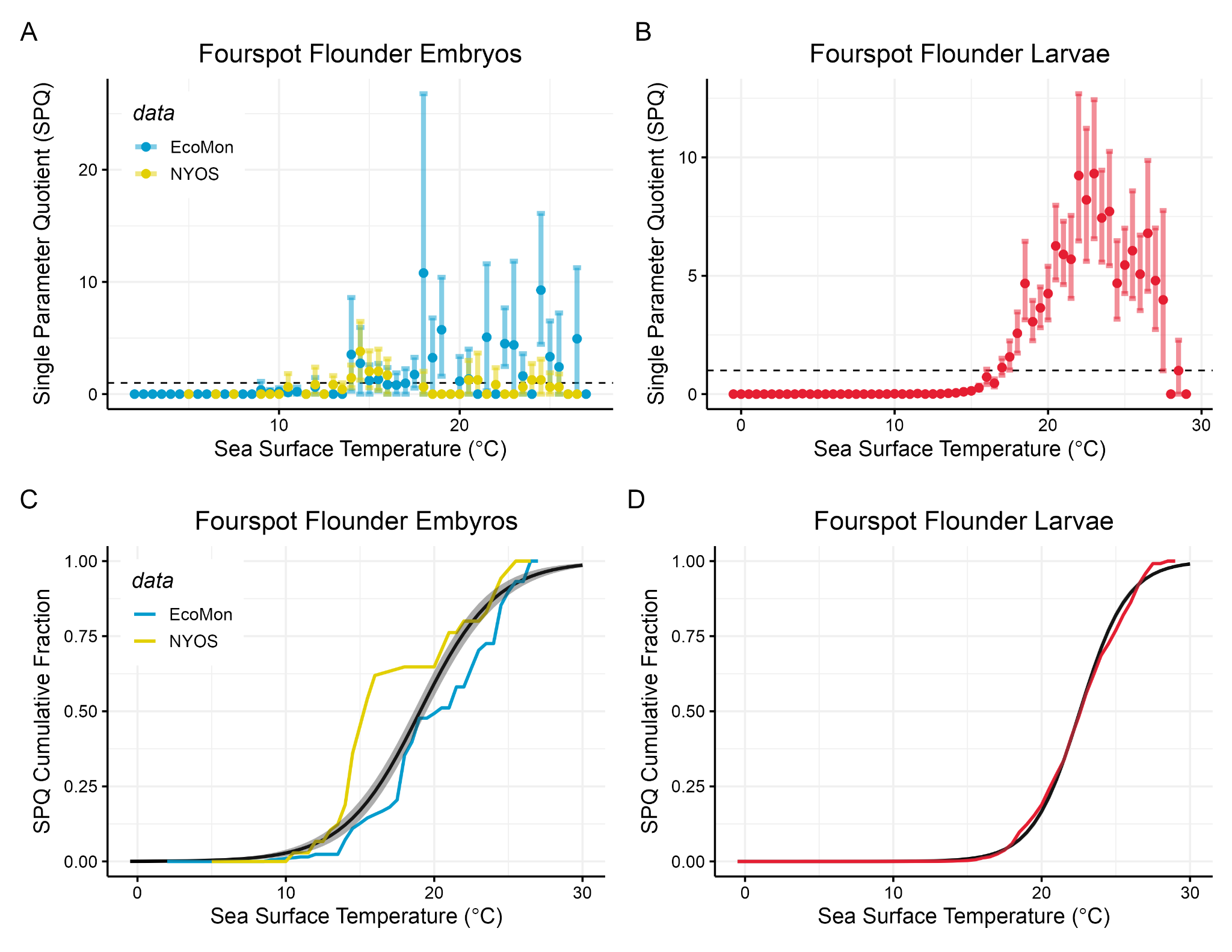


Figure S5. Thermal habitat of Fourspot Flounder embryos and larvae estimated by single parameter quotient (SPQ) analysis. A-B: dots represent estimates from original dataset, 90% confidence intervals are derived from bootstrapping. Dashed line at SPQ = 1, indicating temperature is neither preferred nor avoided. C-D: results from generalized linear modeling, black lines represent model predictions, colored lines show estimates from data, shading indicates 95% confidence intervals from model-based estimation.

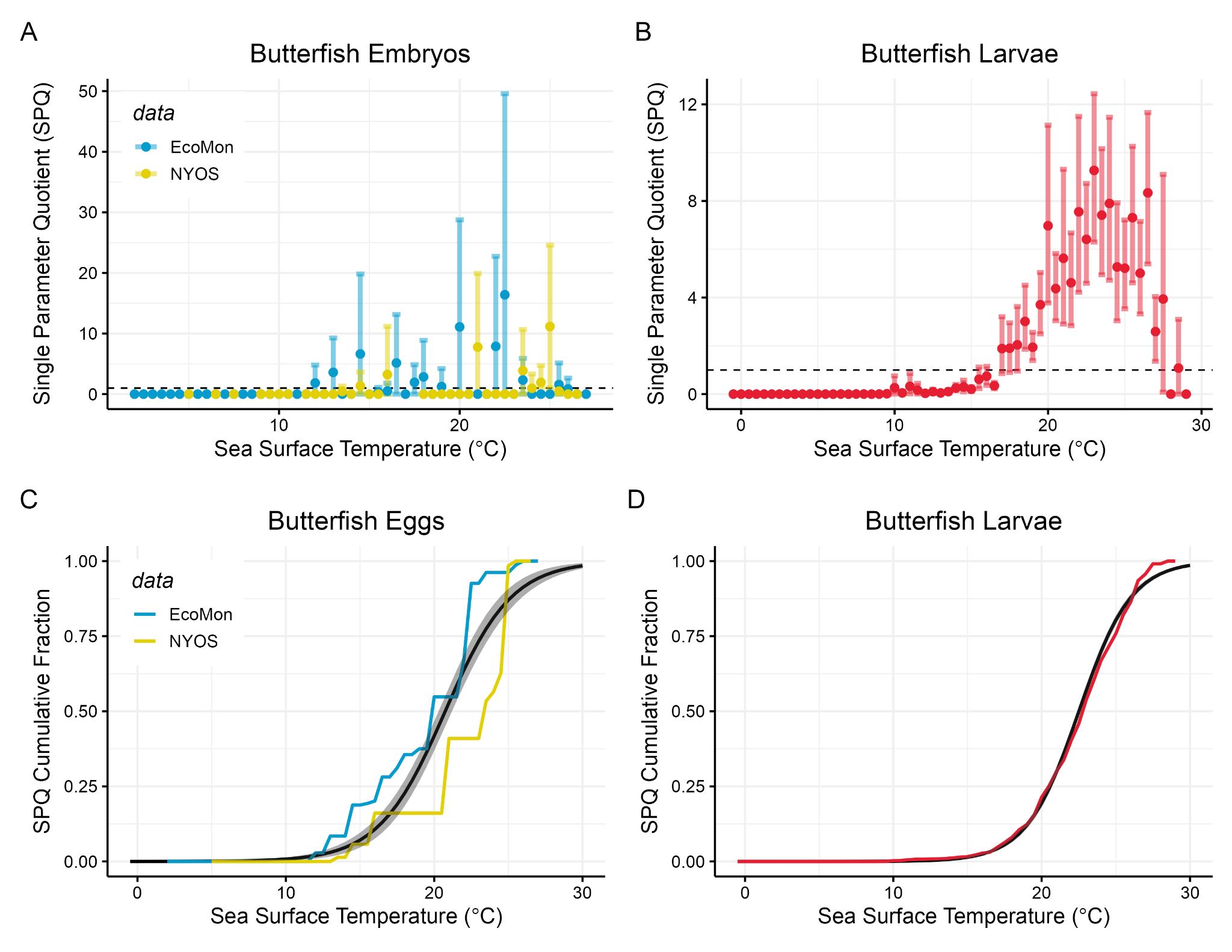


Figure S6. Thermal habitat of Butterfish embryos and larvae estimated by single parameter quotient (SPQ) analysis. A-B: dots represent estimates from original dataset, 90% confidence intervals are derived from bootstrapping. Dashed line at SPQ = 1, indicating temperature is neither preferred nor avoided. C-D: results from generalized linear modeling, black lines represent model predictions, colored lines show estimates from data, shading indicates 95% confidence intervals from model-based estimation.