



Fundamental Niche Narrows through Larval Stages of a Filter-Feeding Marine Invertebrate

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Abstract

*Ontogenetic niche theory predicts that resource use should change across complex life histories. To date, studies of ontogenetic shifts in food niches have mainly focused on a few systems (e.g., fish), with less attention on organisms with filter-feeding larval stages (e.g., marine invertebrates). Recent studies suggest that filter-feeding organisms can select specific particles, but our understanding of whether niche theory applies to this group is limited. We characterized the fundamental niche (i.e., feeding proficiency) by examining how niche breadth changes across the larval stages of the filter-feeding marine polychaete *Galeolaria caespitosa*. Using a no-choice experimental design, we measured feeding rates of trochophore, intermediate-stage, and metatrochophore larvae on the prey phytoplankton species *Nannochloropsis oculata*, *Tisochrysis lutea*, *Dunaliella tertiolecta*, and *Rhodomonas salina*, which vary 10-fold in size, from the smallest to the largest. We formally estimated Levins's niche breadth index to determine the relative proportions of each species in the diet of the three larval stages and also tested how feeding rates vary with algal species and stage. We found that early stages eat all four algal species in roughly equal proportions, but niche breadth narrows during ontogeny, such that metatrochophores are feeding specialists relative to early stages. We also found that feeding rates differed across phytoplankton species: the medium-sized cells (*Tisochrysis* and *Dunaliella*) were eaten most, and the smallest species (*Nannochloropsis*) was eaten the least. Our results demonstrate that ontogenetic niche theory describes changes in fundamental niche in filter feeders. An important next step is to test whether the realized niche (i.e., preference) changes during the larval phase as well.*

Introduction

According to ontogenetic niche theory, species with complex life cycles that change body form and habitat during their life history can also change trophic niche; classic examples include caterpillars that eat leaves but feed on nectar as butterflies (Altermatt and Pearse, 2011) and tadpoles that consume algae (Jenssen, 1967; Schriever and Williams, 2013) but switch to insects after metamorphosis (Lima and Moreira, 1993; Hirai, 2002). To date, ontogenetic niche theory in complex life histories has focused primarily on optimal resource size in predator-prey systems based on how the feeding rate of the predator scales with body size (MacArthur and Pianka, 1966; Pastorok, 1981; Wirtz, 2012); as an individual grows, proficiency of feeding on a resource changes, so diet changes to optimize growth (Mittelbach, 1981; Werner and Gilliam, 1984; Lundberg

and Persson, 1993; Persson *et al.*, 1998). The diet can broaden or narrow to include more or fewer food items, collectively making up the fundamental niche (Mittelbach, 1981; Werner and Gilliam, 1984; Lundberg and Persson, 1993; Persson *et al.*, 1998). The theory has primarily been tested when comparing the niche of juveniles to that of adults (MacArthur and Pianka, 1966; Pastorok, 1981; Wirtz, 2012), so tests of how fundamental niche changes in other stages of the life history, such as within the larval phase, remain relatively rare.

The mechanisms that drive changes in trophic niche in juveniles and adults may also apply to larvae. Species can increase in size by several orders of magnitude during the larval phase; for example, lepidopterans grow by $\sim 10^3$ in mass before pupation (Kivela *et al.*, 2020), so proficiency may change across instars. A few studies have demonstrated

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how diet changes during the larval phase. For example, pre- and postflexion larvae of the fish *Lampanyctus pusillus* feed on different zooplankton (Bernal *et al.*, 2013), and *Lithobates sylvaticus* tadpoles switch from herbivory in the beginning of development to carnivory close to metamorphosis (Schriever and Williams, 2013). Diet can also broaden or narrow to include more or fewer food items, which may yield specialist and generalist stages within the larval phase. For example, the first- and second-instar larvae of the beetle *Cybister japonicus* specialize on insects, and as third instars, their diet broadens to include tadpoles and fish (Ohba, 2009). Changes in diet within the larval phase suggest that fundamental niche is changing; however widespread, direct tests of feeding proficiency are needed to understand whether shifts in diet during the larval phase fit under the ontogenetic niche theory framework.

While larvae remain poorly tested, another group that has largely been ignored in tests of ontogenetic niche theory is filter-feeding organisms. Filter feeders are a large group that include adults and larvae of marine invertebrates. The plankton was once considered a homogenous resource (Buss and Jackson, 1981), so some assumed that filter feeders consume all particles and that feeding niche is invariable through ontogeny (Werner and Gilliam, 1984). However, empirical work shows that feeding niches exist in communities of filter feeders (Rosa *et al.*, 2018; Comerford *et al.*, 2020), so different feeding niches probably exist within a species as well. Filter-feeding larvae increase several orders of magnitude in body size (*e.g.*, crustaceans increase 20-fold; Anger, 1991), so the optimal resource across stages may differ. Differential use of resources during the larval phase suggests that filter feeders may be under similar constraints as predators: evidence in four filter-feeding caddis fly species found that microhabitat use changes across instars (Muotka, 1990), and marine invertebrate larvae can select specific particles when given multiple choices (Romero *et al.*, 2010; Dadon-Piloso *et al.*, 2019; Rosa and Padilla, 2022). Thus, because filter-feeding larvae can feed on certain particles, it is reasonable to expect that fundamental niche might change within the plankton.

Here, using three larval stages of the sessile marine polychaete *Galeolaria caespitosa*, we tested the hypothesis that fundamental niche changes during the larval phase of a filter feeder. *Galeolaria caespitosa* has morphologically distinct larval stages that increase about sixfold in mass in five days (ELR, unpubl. data), and empirical work with marine invertebrates suggests that larvae can select particles (Rico-Villa *et al.*, 2006; Rosa and Padilla, 2020, 2022). We used a no-choice design to estimate consumption rates of larvae on four species of phytoplankton that vary 10-fold in size. Giving larvae a single algal species allowed us to test the central tenet of ontogenetic niche theory: feeding proficiency on a single resource underlies fundamental niche broadening or narrowing during development. Providing larvae a mix of algal species could confound proficiency with

resource preference. To compare fundamental niches across ontogeny, we estimated the relative proportion of each alga in the diet of each larval stage by using the Levins's standardized niche breadth index (Levins, 1968; Hurlbert, 1978). Niche breadth indexes are classically used to compare resource use across species; our study is unique in that we use an analytical community ecology framework to quantify changes in niche breadth across multiple stages within a single species. We were interested in testing (1) whether niche breadth changed across larval stages (either broadened or narrowed) and (2) whether feeding rates differed across stages and algal species, which allowed us to tease apart what underlies changes in niche breadth. We expected early-stage larvae to have a narrow niche breadth and feed on small algal species because larvae may be constrained by their gape/body size and for late-stage larvae to broaden their niche, meaning that they consume more algal species and in equal proportion.

Materials and Methods

In August 2019, we collected and transported aggregations of the sessile marine polychaete *Galeolaria caespitosa* Lamarck, 1818 from Chelsea Pier (Port Phillip Bay, Victoria, Australia) to Monash University for experiments. *Galeolaria* are fertile year-round, and adults release gametes when removed from their tube and placed in a dish with seawater (Chirgwin *et al.*, 2018). After extracting adults, we pooled eggs (~500,000) from 30 females immediately after spawning and placed them in 200-mL filtered (0.25 μm), sterilized seawater at 18 °C. We then collected sperm from 30 males and initiated fertilization by adding 1 mL of sperm (~30,000,000 sperm mL^{-1}) to the egg stock and gently mixed. We added sperm two more times in 5-min intervals to reduce risk of polyspermy. The final sperm concentration was ~450,000 sperm mL^{-1} , a concentration shown to optimize fertilization (Guillaume *et al.*, 2016). After 15 min, we rinsed the fertilized eggs over 20- μm mesh to remove sperm and gently bubbled embryos at a lower density in glass beakers (~200 embryos mL^{-1}). Fertilization success was ~70% after 2 h.

One day after fertilization, we set up 200-mL culture beakers ($n = 30$), each with 100 mL seawater and 2000 gastrula, and gently bubbled the cultures. Larvae reach competence to settlement in ~9 d. Every 2 d after fertilization we fed larvae 15,000 cells mL^{-1} of each of four phytoplankton species: *Nannochloropsis oculata*, *Tisochrysis lutea*, *Dunaliella tertiolecta*, and *Rhodomonas salina*. On days we fed larvae, we replaced 50% of culture seawater and cleaned beakers.

Measuring feeding rates and niche breadth

The goal of the feeding rate experiment was to estimate the consumption of four algal species (*Nannochloropsis*, *Tisochrysis*, *Dunaliella*, and *Rhodomonas*) across three ontogenetic larval stages: trochophore, intermediate, and metatrochophore. We chose these phytoplankton species because they

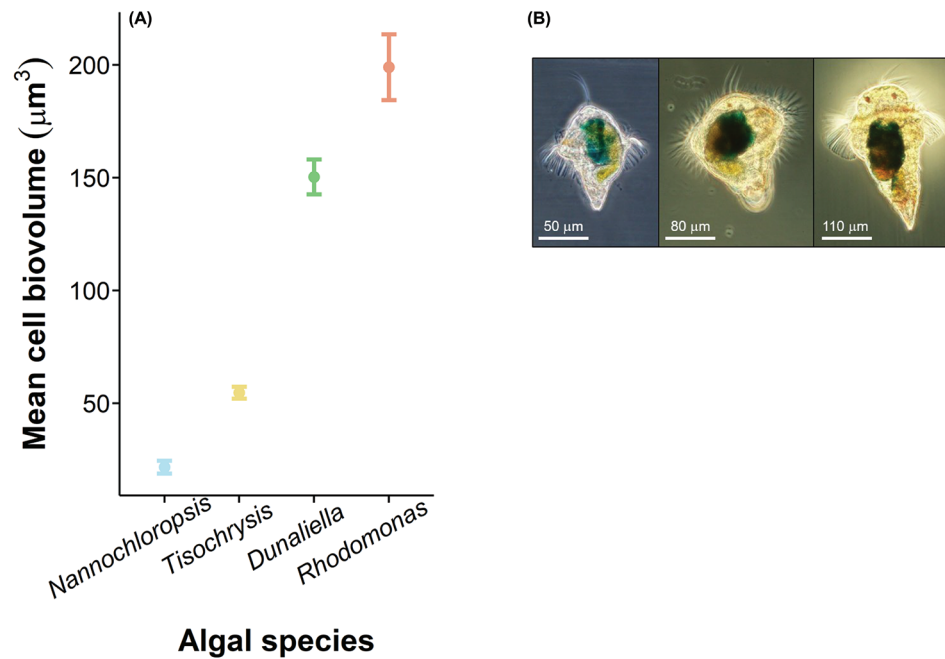


Figure 1. Algal species and larval stages used in experiment. (A) Mean (\pm SE) cell biovolume of the algal species *Nannochloropsis oculata*, *Tisochrysis lutea*, *Dunaliella tertiolecta*, and *Rhodomonas salina* used in feeding rate trials. (B) Three larval stages of *Galeolaria caespitosa* used in feeding rate trials: trochophore, intermediate, and metatrochophore. The intermediate stage has morphological traits of both the trochophore and metatrochophore stages (occurring between 5 and 6 d). Notice that the scale bars differ.

are commonly used to feed marine invertebrate larvae (Rosa and Padilla, 2020, 2022) and they vary 10-fold in volume from smallest to largest (Fig. 1A). The morphological traits for the trochophore and metatrochophore stages are well described in *Galeolaria* (Grant, 1981; Nelson *et al.*, 2017; Watson *et al.*, 2017). We define the intermediate stage as having both trochophore and metatrochophore features, and it typically occurs from day 5 to 6 (observed in pilot study; Fig. 1B).

Three, five, and seven days after fertilization (corresponding to each of the three morphological stages above), we measured larval feeding rates on the four algal species. We combined 300 larvae and 3 mL of algal solution in 5-mL plastic vials and allowed larvae to feed for 6 h (concentrations established in a pilot study to optimize experiment length and feeding signal). Because our algal species vary 10-fold in volume, we fed larvae equal biovolumes across the four algae, rather than equal cell counts. Each sampling day, we found the larval density of 10 cultures and then diluted them to a concentration of 150 larvae mL^{-1} (~15 mL). Then, for each culture, we set up four feeding trial vials, filled with 300 larvae in 2 mL of seawater, one vial for each of the four algal treatments (Fig. 2). For each species of algae, we then added 1 mL of algal stock solution at the standardized biovolume (final vial biovolume: $4.5 \times 10^7 \mu\text{m}^3$ alga). Algal stock concentrations were measured using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA). We then measured cell area for each species using ImageJ (v1.53e; Schneider *et al.*, 2012) and converted area to cell

biovolume using the most appropriate geometric shape (*Nannochloropsis* and *Tisochrysis* treated as spheres, *Dunaliella* and *Rhodomonas* as prolate spheroids, as per Sun and Liu, 2003). We then multiplied cell concentration by cell biovolume to obtain biovolume concentration ($\mu\text{m}^3 \text{mL}^{-1}$).

To estimate larval feeding rates, we took a 500- μL sample from each vial at the start of the experiment and again after 6 h. To count the total number of cells, 20 μL of each 500- μL sample was run through the flow cytometer, and then the number of cells in the sample was converted to total vial biovolume in the experimental vial for analyses. Every hour during the feeding trial, vials were gently vortexed to resuspend algae.

We then calculated niche breadth for each replicate across the three larval stages. The Levins's standardized niche breadth index (Levins, 1968; Colwell and Futuyma, 1971; Hurlbert, 1978) estimates the relative abundance of each algal species in the diet. We used the equations

$$B = \frac{1}{\sum p_i^2}, \quad (1)$$

$$B_s = \frac{B - 1}{n - 1}, \quad (2)$$

where B is the Levins's index, p_i is the relative proportion of algal species i in the diet, n is the number of algal species (in this study, 4), and B_s is the standardized B . The standardized index is bound between 0 and 1; niche breadth

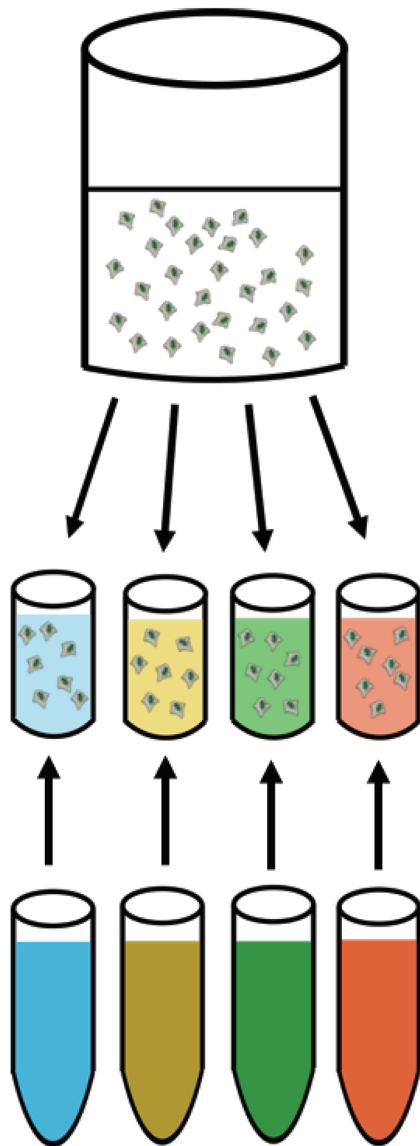


Figure 2. Experimental setup for measuring feeding rates of *Galeolaria caespitosa* larvae. Feeding rates were measured for the trochophore, intermediate, and metatrochophore stages ($n = 10$ larval cultures per stage). From a single larval culture, four vial replicates were set up: one for each of the four algal treatments, *Nannochloropsis oculata* (blue), *Tisochrysis lutea* (yellow), *Dunaliella tertiolecta* (green), and *Rhodomonas salina* (red). To each vial (middle row), 2 mL of larvae and 1 mL of a single algal species were added. The final algal concentration was $4.5 \times 10^7 \mu\text{m}^3$, with 300 larvae. Larvae were left to feed for 6 hours. Total cell counts were measured at the start and end of the experiment using flow cytometry and converted to biovolume for analyses.

is 1 if larvae eat each algal species in equal proportion, which would suggest that larvae are generalists. A niche breadth value less than 1 suggests that larvae consume certain algal species more than others. Through ontogeny, if niche breadth increases, this means that niche width broadens across larval stages; larvae consume more algal species in equal proportion.

Statistics

All analyses were done in R (v. 4.0.4; R Core Team, 2016), and linear model assumptions were checked using Q-Q (quantile-quantile) plots, histograms, and boxplots of residuals. We used a linear model to test the effect of the categorical predictor stage (fixed, three levels: trochophore, intermediate, metatrochophore) on the response variable niche breadth. We then used a Wald test to test whether the coefficients were significantly different from 1 (*i.e.*, whether larvae from each stage were eating the four phytoplankton species in equal proportion).

We then used the lme4 package (Bates *et al.*, 2015) to run a linear mixed model that included the factors stage and species (fixed, four levels: *Nannochloropsis*, *Tisochrysis*, *Dunaliella*, and *Rhodomonas*) to explain the response variable final biovolume, which is the final algal biovolume in the vial after larvae fed for 6 h. While we minimized variation in starting biovolume in each vial, we still included starting biovolume (fixed, continuous) as a covariate in the model because we expected some slight variance across treatments and days. We also initially included larval density and the interaction between starting biovolume and larval density in the model but found neither were significant, so both were removed from the subsequent analyses. For our experimental design, each replicate within stage ($n = 10$) was split into the four algal treatments, meaning the four vials coming from the same replicate were not independent (Fig. 2). To account for nonindependence of vials across algal treatments, we included the random factor replicate nested within stage. We tested a full model with all interactions and found that the three-way interaction between species, stage, and starting biovolume was not significant ($P > 0.9$), so we removed this term from the final model. Because final biovolume is a measure of the algae that is remaining in the vial, rather than what was removed by larvae, a large final biovolume means that larvae had low feeding rates, whereas diminished final biovolume means high consumption. We used the emmeans package to estimate all marginal means and their standard errors (Lenth, 2021).

One vial from the trochophore stage and three vials from the intermediate stage were lost due to clogging of the flow cytometer. Two of the vials came from the same replicate, so for the niche breadth analysis, $n = 9$, 8, and 10 replicates in the trochophore, intermediate, and metatrochophore stages, respectively, and $n = 39$, 37, and 40 vials analyzed for the final biovolume analysis.

Results

We found that larvae become more specialized in their diet through time ($F_{2,24} = 39.247$, $P < 0.001$). The niche breadth of the trochophore (estimate \pm SE: 0.988 ± 0.02) and intermediate (0.963 ± 0.021) stages did not differ and were close to 1, but metatrochophore larvae had a narrower niche breadth compared to the other stages (0.764 ± 0.019 ; linear model,

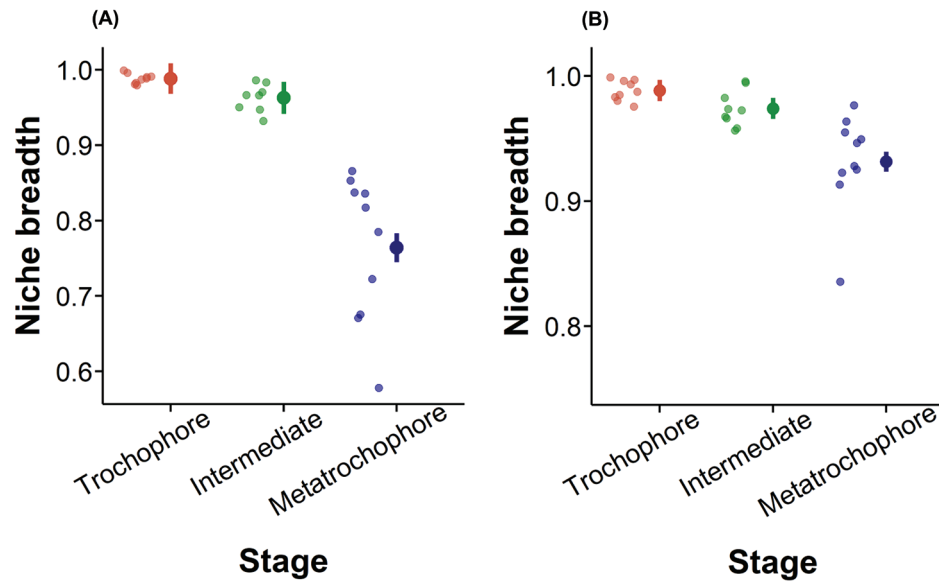


Figure 3. Niche breadth across the larval phase in *Galeolaria caespitosa* when larvae were fed the algal species *Nannochloropsis*, *Tisochrysis*, *Dunaliella*, and *Rhodomonas*. Niche breadth measures the relative abundances of four algal species in the diet of trochophore, intermediate, and metatrochophore larvae. A value of 1 indicates that larvae feed on all four algal species equally, while a value <1 indicates that larvae consume certain species over others. Because *Nannochloropsis* was consumed at low rates across all stages, niche breadth was calculated (A) including all four algal species and (B) excluding *Nannochloropsis* to make sure low consumption on this species was not driving changes in niche breadth. Large dots show linear model estimates and standard error, and smaller points are raw data. There was a greater change in niche breadth when four species were included in the analysis (notice that y-axes across panels are not the same). However, qualitatively, metatrochophores had a significantly narrower diet, even when *Nannochloropsis* was excluded.

$F_{2,24} = 39.247$, $P < 0.001$; Fig. 3A), which was significantly less than 1 (Wald test, $t_{24} = 12.421$, $P < 0.001$).

For the smallest alga, *Nannochloropsis*, the final biovolumes were close to the target starting biovolume ($4.5 \times 10^7 \mu\text{m}^3$), indicating that larvae consumed little of this species regardless of stage. Because feeding rates on *Nannochloropsis* were low across all three stages, we reran our analyses without this species, to test whether it was driving the patterns we observed. Results changed quantitatively but not qualitatively when *Nannochloropsis* was removed from the analysis; niche breadth still narrowed during ontogeny (linear model, $F_{2,25} = 13.156$, $P < 0.001$; Fig. 3B). Niche breadth of trochophores (0.988 ± 0.01) and intermediate larvae (0.974 ± 0.01) did not differ, but metatrochophore larvae had a narrower niche breadth compared to the other stages (0.931 ± 0.01), which was significantly less than 1 (Wald test, $t_{25} = 6.9$, $P < 0.001$). There was also still a significant interaction between larval stage and algal species on final biovolume concentration (Table 1). Overall, our results with four algal species were not driven by low consumption of *Nannochloropsis*.

Consumption rates changed across stages and depended on phytoplankton species, which is consistent with our finding that niche breadth narrowed (Table 1; Fig. 4). For trochophore larvae, the ratio of final biovolume for *Tisochrysis*, *Dunaliella*, *Rhodomonas*, and *Nannochloropsis* was 1:1.06:1.16:1.2, meaning that larvae consumed the algal species in roughly equal proportion. The ratio became in-

creasingly unbalanced with each stage: for intermediate larvae, the ratio was 1:1.14:1.29:1.49, and by the metatrochophore stage, it was 1:1.15:1.58:2.87, meaning *Tisochrysis* was eaten about three times as much as *Nannochloropsis*. Overall, metatrochophore larvae were feeding primarily on *Tisochrysis* and *Dunaliella*, and this bias toward medium-sized cells was driving their narrow niche breadth.

Discussion

Whether fundamental niche changes in filter feeders and within the larval phase has been largely unexplored in the context of ontogenetic niche theory. The plankton was once considered a homogenous resource (Buss and Jackson, 1981), so some assumed that the filter-feeding niche did not change through the life history (Werner and Gilliam, 1984). However, we found evidence in *Galeolaria caespitosa* larvae that ontogenetic niche theory applies to filter-feeding organisms: fundamental niche changed during the larval phase, meaning that feeding proficiency varied across stages and across phytoplankton species. Overall, niche breadth decreased across larval stages, meaning that niche narrowed. Our results are consistent with other studies that find that larvae can feed on particular algal species at different rates (Baldwin, 1995; Rico-Villa *et al.*, 2006; Rosa and Padilla, 2020), but we provide a new framework to quantify how fundamental niche changes under the ontogenetic niche theory.

Table 1

Linear mixed model analysis for larval feeding rates

Test	df	F-ratio	P-value
Four species			
Stage	2, 95	0.646	0.526
Species	3, 86	4.791	0.004
Starting biovolume	1, 95	11.372	0.001
Stage × species	6, 78	33.833	<0.001
Stage × starting biovolume	2, 94	2.202	0.116
Species × starting biovolume	3, 87	6.911	<0.001
Three species			
Stage	2, 66	0.347	0.708
Species	2, 60	4.384	0.017
Starting biovolume	1, 68	1.679	0.2
Stage × species	4, 54	3.095	0.023
Stage × starting biovolume	2, 65	0.33	0.72
Species × starting biovolume	2, 60	3.554	0.035

Three larval stages (trochophore, intermediate, and metatrochophore) of the marine polychaete *Galeolaria caespitosa* were fed one of four phytoplankton species (*Nannochloropsis oculata*, *Tisochrysis lutea*, *Dunaliella tertiolecta*, and *Rhodomonas salina*). Two tests were conducted: one including all four algal species and one omitting *Nannochloropsis* to see whether low consumption rates on this species were driving patterns observed. The response variable for each model is the final alga biovolume in each vial. Algal species, larval stage, and starting biovolume within each vial were modeled as fixed effects. Each replicate within stage was split into the four algal treatments, meaning the four vials coming from each replicate were not independent, so the random effect replicate nested within stage was included. A full model with all interactions was tested. The three-way interaction between the fixed effects was not significant ($P > 0.9$), so the term was removed from the final model. Bold values are significant, $P < 0.05$.

Feeding rates and niche breadth can be measured many ways (Slatyer *et al.*, 2013; Rosa and Padilla, 2022). Niche breadth is often calculated from gut contents as the number of items of each resource in the diet (Levins, 1968; Colwell and Futuyma, 1971; Hurlbert, 1978). This approach has the merit of identifying niche partitioning and overlap within species (Bernal *et al.*, 2013) and among species (Gladfelter and Johnson, 1983; Pusineri *et al.*, 2008). But this approach rarely controls for differences in the supply of each item and may over- or underestimate the importance of certain resources that are rare but targeted. Similarly, estimates of larval feeding rates that provide an equal number of cells across phytoplankton species do not reflect the biovolume they consume (Rosa and Padilla, 2020). These approaches are not more or less appropriate; they just access different questions. In our design, we estimate the relative biovolume of each alga in the diet, which is independent of how the number of consumed cells correlates with algal size (*e.g.*, larvae may eat many cells of a small species, but the total biovolume consumption is low). A drawback to our design is that feeding rates are likely to be density dependent and cell density covaries negatively with size. We assume that the niche usage we observe at high concentrations also occurs at low concentrations, but if there is an interaction between algal concentration and niche usage, this

could change our results. We tried to limit the effect of concentration by providing larvae with an *ad libitum* number of cells across all species and by including the initial biovolume of cells in each vial in our statistical model. We acknowledge that our design is imperfect and does not allow us to tease apart the effects of density dependence, but nevertheless larvae that were fed equal biovolumes across stages and across phytoplankton species fed differently, suggesting that there are factors independent of density that lead to changes in feeding patterns.

We emphasize that by using our no-choice design, we have tested the fundamental niche and not the realized niche—in other words, we have tested feeding proficiency and not preference. Ontogenetic niche theory's core assumption is about the fundamental niche, and the proficiency of an organism to consume a single resource changes through ontogeny, so it was most appropriate to feed larvae one resource at a time. Overall, our niche breadth results were unexpected. We predicted that fundamental niche would expand through ontogeny, as larvae are no longer constrained by their body size. Tests across the whole life history in other systems find results similar to ours: niche breadth can narrow across the life history, for example, in fish (Bonato and Fialho, 2014), frogs (Luria-Manzano and Ramirez-Bautista, 2019), grasshoppers (Dopman *et al.*, 2002), and shrimp (Selvarani, 2009). However, within larval phases, consensus is lacking: feeding niches can broaden through time (Ohba, 2009; Egan *et al.*, 2018) or shift entirely (Bernal *et al.*, 2013; Schriever and Williams, 2013). Such studies remain rare, and more are needed to understand whether the patterns we observe in our study are ubiquitous.

The theory of interspecific competition in filter feeders has a particular focus on size: larger species should have a broader niche compared to small species, because they can access both large and small resources (Hall *et al.*, 1976). Classic ontogenetic niche theory also focuses on body size, specifically, how body size scales with niche breadth (Mittelbach, 1981; Werner and Gilliam, 1984; Lundberg and Persson, 1993; Persson *et al.*, 1998). For species with complex life cycles, individuals increase substantially in size, even within the larval phase, so the optimal resource likely changes with body size; a classic example is lepidopterans increasing $\sim 10^3$ in mass (Kivela *et al.*, 2020). For *Galeolaria* larvae, body mass increase is much lower compared to lepidopterans (about sixfold; ELR, unpubl. data), but in contrast to lepidopterans, the metatrochophore stage adds adult features to the larval body, suggesting that larvae approaching metamorphosis may have different requirements and/or optimal resources. Thus, a simple expectation from theory would be an expansion of niche breadth with ontogeny, but we observed niche breadth constriction despite the increase in size, suggesting that the body size–niche breadth relationship is more complex than classic theory has appreciated (MacArthur and Pianka, 1966; Pastorok, 1981; Wirtz, 2012).

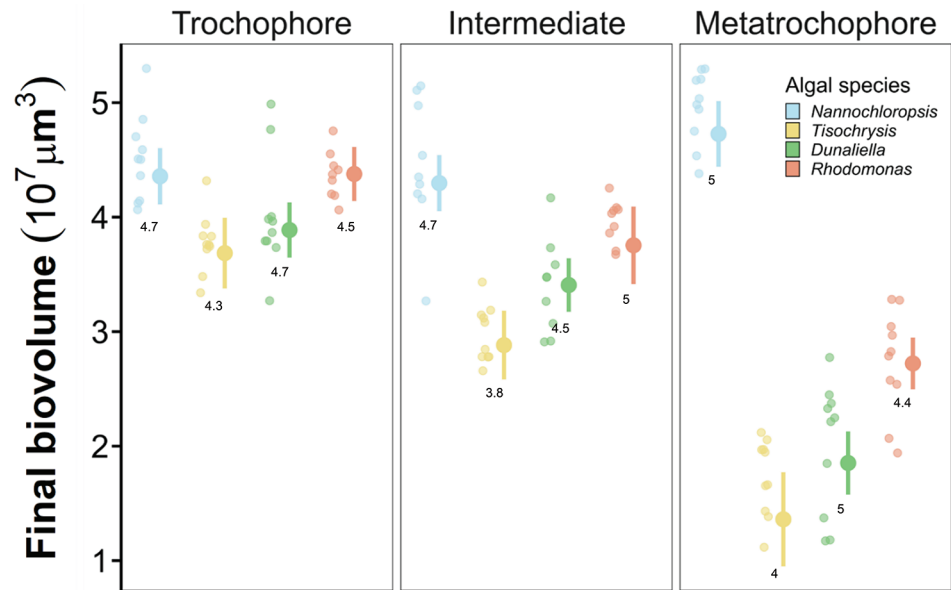


Figure 4. Final biovolume of algae in vials after trochophore, intermediate, and metatrochophore larvae of *Galeolaria caespitosa* fed for 6 h. Large dots show linear model estimates and standard error, and smaller points are raw data. Larvae were fed one of four algal species of equal biovolumes: *Nannochloropsis oculata* (blue), *Tisochrysis lutea* (yellow), *Dunaliella tertiolecta* (green), and *Rhodomonas salina* (red). Average starting biovolumes (10^7) are listed under model estimates. Because the final biovolume of each vial was measured, rather than the biovolume removed by larvae, high values represent species that were eaten less often compared to species with low final biovolume estimates.

Across all three stages, the medium-sized species, *Tisochrysis* and *Dunaliella*, were consumed at high rates, and consumption increased more steeply across ontogeny compared to the other algae. Thus, the niche of late-stage larvae narrows because they specialize on medium-sized algal species, a finding that is similar to work in bivalve larvae that were fed the same species (Rosa and Padilla, 2020, 2022). The high consumption on *Tisochrysis* and *Dunaliella* may be driven by the larval feeding mechanism: theory and empirical evidence in planktonic midge larvae found that medium-sized prey were optimal, because these maximized capture success relative to encounter rate (Pastorok, 1981), which could help explain why the two medium-sized species in our study were eaten at the highest rates. Consumption for the largest species, *Rhodomonas*, was lower than the medium-sized cells, particularly for trochophores. We think that this may be related to large cell size: the longest dimension of a *Rhodomonas* cell is $\sim 15 \mu\text{m}$, about the same as the diameter of the larva mouth opening of our species, so consumption may be low because larvae are gape limited. Surprisingly, feeding rates on the smallest algal species (*Nannochloropsis*) were low across the whole larval phase. Larvae were unlikely to be constrained by cell size or encounter rate for *Nannochloropsis*, because the cell is small and concentrations were high, suggesting that factors such as food processing (e.g., handling time; Lundberg and Persson, 1993), assimilation rate (Rendleman and Pace, 2018), or food quality (Helm and Bourne, 2004) may explain low feeding rates on this species.

Change in feeding niche is also likely related to nutritional requirements: food quality is important for growth in larval mosquitos (Merritt *et al.*, 1992), flies (Nestel and Nemny-Lavy, 2007), fish (Dabrowski, 1986; Zarantonello *et al.*, 2021), frogs (Venesky *et al.*, 2012), and bivalves (Hendricks *et al.*, 2003; Aranda-Burgos *et al.*, 2014) and can also affect adult fitness (Colasurdo *et al.*, 2009; van Schoor *et al.*, 2020). How nutritional requirements change in the larval phase is less clear, but higher feeding proficiency on a particular resource that also yields greater success in metamorphosis would suggest that later stages need certain resources to prepare for metamorphosis. Complex tissue reorganization during metamorphosis for frogs (Stephens *et al.*, 2017; Zhu *et al.*, 2020, 2021) and fish (Padron *et al.*, 1996; Pfeiler, 1996) relies on fat stores, and food deprivation during metamorphic climax can slow the transition (Wright *et al.*, 2011). Phytoplankton vary in their nutrient content (Helm and Bourne, 2004), so marine invertebrate larvae may have different requirements before settlement; one study found that early-stage larvae selectively consumed different algal species compared to late-stage larvae that were competent for settlement, and feeding rates were lower late in ontogeny (Rosa and Padilla, 2020). In our study, the phytoplankton species that was consumed the least (*Nannochloropsis*) also has the lowest nutritional value relative to its size (Helm and Bourne, 2004; Rosa and Padilla, 2020, 2022). Instead, metatrochophore larvae that need to prepare for settlement (beginning at ~ 8 d), narrowed their niche and increased consumption of the most nutritious

species, *Tisochrysis* and *Dunaliella*. Our data may highlight the importance of niche shifts for larvae when preparing for metamorphosis.

Our results demonstrate that late-stage metatrochophore larvae have a narrower niche breadth compared to early stages—in other words, larvae transition from a generalist to a specialist diet, and this is likely influenced by larval body size and/or phytoplankton size and quality. It is also worth noting that a recent study found that surface charge (*i.e.*, “stickiness”) of the particle affected capture, and this effect was sometimes independent of nutritional content and size (Rosa and Padilla, 2022), suggesting that resource characteristics may contribute to feeding proficiency. Regardless of the mechanism, we show that fundamental niche changes in the larval phase of a filter feeder—a system that is usually excluded from classic niche theory. Adult benthic filter feeders have been shown to affect phytoplankton communities (Hardenbicker *et al.*, 2015; Comerford *et al.*, 2020) and have specific feeding preferences, and our results suggest that similar dynamics occur in the larval phase as well. Now that we know the fundamental niche changes during the larval phase, an interesting next step would be to provide larvae with multiple algal species to characterize how the realized niche (*i.e.*, preference) changes during the larval phase.

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Ethical Care Considerations

Animals were collected under permit from Chelsea Pier, Port Phillip Bay, Victoria, Australia, and maintained in the laboratory for experiments.

Data Accessibility

Data and code are available on Dryad at <https://doi.org/10.5061/dryad.djh9w0w4p>.

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