Spring 2020

Survival, growth, and radula morphology of postlarval pinto abalone (Haliotis kamtschatkana) when fed six species of benthic diatoms

Lillian Miller Kuehl
Western Washington University, lilliankuehl@gmail.com

SURVIVAL, GROWTH, AND RADULA MORPHOLOGY OF POSTLARVAL PINTO ABALONE (*HALIOTIS KAMTSCHATKANA*) WHEN FED SIX SPECIES OF BENTHIC DIATOMS

By

Lillian Miller Kuehl

Accepted in Partial Completion of
the Requirements for the Degree
Master of Science

ADVISORY COMMITTEE

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Dr. Brian Bingham

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GRADUATE SCHOOL

David L. Patrick, Interim Dean

MASTER'S THESIS

Lillian Miller Kuehl

April 25, 2020

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A Thesis
Presented to
The Faculty of
Western Washington University

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ABSTRACT

Haliotis kamtschatkana Jonas (pinto or northern abalone) is the only abalone native to the Pacific Northwest of North America. *Haliotis kamtschatkana* populations are in decline, and current restoration efforts in Washington State rely on out-planting hatchery-produced juveniles. Although several other abalone species are cultured extensively, little information exists on the cultivation of *H. kamtschatkana*, and hatchery production of this species has largely been a matter of trial and error. Hatcheries report highest mortalities in the postlarval stage, especially the first 3 to 6 months. Postlarvae feed on films of benthic diatoms, and the purpose of this study was to test 6 benthic diatom species as suitable diatom diets for *H. kamtschatkana*. Diatom diet suitability might rely on several factors, including morphology of the radula. The radula is a crucial feeding structure for gastropods and may display morphological plasticity, but it has never been characterized in *H. kamtschatkana* postlarvae. We investigated survival, growth, and radula morphology of *H. kamtschatkana* postlarvae when fed one of 6 benthic diatom species for 61 days post-settlement. *Amphora salina* best supported survival, especially in the first 20 days post-settlement (mean of 60% [SD, 22%] at day 20, mean of 47% [SD, 16%] at day 61), and *Achnanthes brevipes* yielded exceptionally low survival (mean of 12% [SD, 13%] and day 20, mean of 1% [SD, 3%] at day 61). Postlarvae fed *Cylindrotheca closterium* grew fastest among treatments (linear mixed model shell length = $293 * e^{0.021t}$, measured 1,110 µm [SD, 244 µm] at day 61), followed by postlarvae fed *Amphora salina*, *Navicula incerta*, or *Nitzschia laevis* (no significant difference between these diets; linear mixed model shell length = $302 * e^{0.018t}$, measured 894 µm [SD, 132 µm] at day 61).

We found no effect of diatom diet on radula morphology, but morphology was similar to that of other abalone species, with similar correlations between morphological characteristics and

shell length. We recommend that radula development of other species may be used as a proxy for *H. kamtschatkana* radula development, in the absence of further investigation.

We recommend *A. salina* as a suitable diet for newly settled *H. kamtschatkana* postlarvae, and that a combination of *A. salina* and *C. closterium* be investigated to support both survival and growth.

ACKNOWLEDGEMENTS

I would like to thank Puget Sound Restoration Fund for their donation of abalone and algae to this project, and particularly Josh Bouma for invaluable abalone advice and Stuart Ryan for algal culture. I received generous funding from the Pacific Northwest Shell Club Malacological Scholarship, the National Shellfisheries Association Michael Castagna Student Grant for Applied Research, and Western Washington University's Fund for the Enhancement of Graduate Research. I would like to thank my fellow graduate students, thesis committee, and adviser for academic support and feedback in all stages of my project. Much thanks for the support of my friends, family, and coworkers.

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INTRODUCTION

Haliotis kamtschatkana kamtschatkana Jonas (pinto or northern abalone, hereafter referred to as *H. kamtschatkana*) ranges along the west coast of North America from Point Conception, California to southern Alaska, and is the only abalone found north of Oregon (Paul and Paul 1981; Geiger 2000). Combinations of poaching, commercial fishing, and recreational overharvesting severely diminished all populations of the species (Campbell 1999; Wallace 1999; Rothaus et al. 2008; Neuman et al. 2018; Carson and Ulrich 2019). Since abalone aggregate and broadcast spawn, adult density must be sufficiently high for gamete densities that allow fertilization (Allee 1949; Rothaus et al. 2008). Despite over two decades of harvest closure, persistently low adult *H. kamtschatkana* densities in the Salish Sea have caused reproduction and recruitment failure, leading to overall population decline that is unlikely to be reversed without human intervention (Rothaus et al. 2008; Bouma et al. 2012; Carson and Ulrich 2019).

Haliotis kamtschatkana restoration efforts in Washington State currently depend on hatchery production of juveniles by Puget Sound Restoration Fund. Restoration workers condition and spawn broodstock, rear larvae, induce settlement, and grow postlarvae first on diatom films, then on macroalgae (Vadopalas and Watson 2014). Juveniles are released at subtidal sites in the San Juan Archipelago when they are 8 to 45 mm in shell length (Vadopalas and Watson 2014). Hatchery workers have reported that low survival and slow growth make the early postlarval stage a bottleneck in efficient hatchery production of *H. kamtschatkana* (2015 conversation with Joshua V Bouma, unreferenced). Most mortality in abalones occurs in the first month post-settlement (Ebert and Houk 1984) and, anecdotally, *H. kamtschatkana* is more negatively affected by handling and experiences lower survival generally compared to other abalone species

(2015 conversation with Joshua V Bouma, unreferenced). Research specific to *H. kamtschatkana* postlarval cultivation is limited. Caldwell (1981) reported that "the overall survival [of *H. kamtschatkana*] would not lend itself well to a commercial operation", and qualitatively described *H. kamtschatkana* postlarval growth rate as two-thirds that of *H. rufescens* Swainson, with high variability among individuals.

Abalone postlarvae begin to graze on benthic diatoms shortly after settlement, and the success of postlarvae depends on various diatom characteristics (Roberts, Kawamura, and Nicholson 1999; Gordon et al. 2006; Xing et al. 2007; Correa-Reyes et al. 2009). Depending on their size, postlarvae may graze on diatoms' polysaccharide-based extracellular mucus (Hoagland et al. 1993; Kawamura and Takami 1995), consume whole diatoms (Kawamura, Roberts, and Nicholson 1998; Roberts, Kawamura, and Nicholson 1999), or rupture diatoms and consume the contents (Kawamura and Takami 1995). As postlarvae age and grow, they can consume different diatoms because the radula has larger teeth with steeper clearance angles that can break open diatoms that are tightly adhered or that have strong frustules (Kawamura, Roberts, and Nicholson 1998; Kawamura et al. 2001). The concurrent development of the digestive glands and stomach may also contribute to an increased ability to digest different diatoms (Takami et al. 1998).

Several factors influence which diatoms should be used in a hatchery to optimize abalone growth and survival. Diatom nutritional value, ingestibility, and growth rate depend on age of diatom film (Kawamura, Roberts, and Takami 1998), light intensity (Searcy-Bernal and Gorrostieta-Hurtado 2007), water flow (Searcy-Bernal and Gorrostieta-Hurtado 2007), inoculum density (Courtois de Vicose, Porta, et al. 2012), species of diatom (Roberts, Kawamura, and Nicholson 1999; Daume et al. 2000; Gordon et al. 2006; Xing et al. 2007; Correa-Reyes et al.

2009), and biochemical composition (Gordon et al. 2006; Correa-Reyes et al. 2009; Courtois de Vicose, Porta, et al. 2012). Lacking research on the dietary value of different diatoms fed to *H. kamtschatkana* postlarvae, restoration hatcheries of this species have two choices: feed natural diatom film, which varies dramatically in quality and composition, or feed cultivated diatoms based on research of other abalone species. The latter can be problematic because *H. kamtschatkana* is found in cooler waters than other abalone species (Paul and Paul 1981; Paul and Paul 1998; Bouma 2007), and temperature affects diatom growth and survival, as well as the natural diatom communities in which *H. kamtschatkana* evolved. Thus, a diatom that works well for cultivation of one abalone species may not be appropriate for *H. kamtschatkana*.

The radula is important to postlarval success, because it affects the efficiency of food consumption, and improves digestion if it ruptures diatoms during consumption (Kawamura et al. 1995). Abalone have rhipidoglossan radulae, which are flexible and have many outer marginal teeth that sweep the substratum like a broom, in addition to a small number of more sturdy teeth in the center of each row (the rachidian tooth and lateral teeth; Steneck and Watling 1982; Ponder and Lindberg 1997). When postlarvae graze, the radula interacts directly with the diatoms, so radula morphology might be an important factor in diatom ingestibility (Roberts et al. 2001). Radula development has been characterized in several species of abalone (Roberts, Kawamura, and Takami 1999; Kawamura et al. 2001; Kawamura et al. 2001; Onitsuka et al. 2004; Johnston et al. 2005), but has not been examined in *H. kamtschatkana*. Differences in tooth number and rate of development are apparent between abalone species, but the basic process of development is the same: rows of teeth are formed at the posterior end of the radula, and worn teeth are shed from the anterior end (Moss 1999; Roberts, Kawamura, and Takami 1999; Kawamura et al. 2001; Takami et al. 2003; Onitsuka et al. 2004; Takami et al. 2006). As abalone age, the number of rows per radula increases, the number of marginal and lateral teeth per row increases, and the shapes of the teeth change. An understanding of *H. kamtschatkana* radula development might indicate changes in the radula commensurate with changes in diatom diet as abalone grow, e.g. changes in tooth angle correlate with an ability to consume larger and more adhesive diatoms (Takami and Kawamura 2003) and teeth that are more numerous, more blunt, and larger correlate with the transition to macroalgae (Takami et al. 2003; Onitsuka et al. 2004). In addition, some species of Littorinidae have demonstrated radula plasticity in response to diatom diet type, but this has not been investigated in Haliotidae.

Abalone produce lecithotrophic larvae, which survive on energy reserves from egg yolk during development. These energy reserves are also available to postlarvae, demonstrated by the fact that starved postlarvae can survive for 12 days (*H. discus hannai*; Takami et al. 2000) or for 29 days (*H. iris*; Fukazawa et al. 2005), albeit with slowed growth (Takami et al. 2000; Roberts et al. 2001). Thus, even though diatom species identity (Kawamura, Roberts, and Takami 1998; Courtois de Vicose, Viera, et al. 2012) and density (Gorrostieta-Hurtado and Searcy-Bernal 2004) affect postlarval growth and survival, these changes might be undetectable until two weeks post-settlement if growing postlarvae are also relying on yolk reserves. There are no yolk reserve studies on *H. kamtschatkana* postlarvae, but we assume that this species is similar to others in that regard.

In the present study we fed *H. kamtschatkana* postlarvae one of 6 diatom species and measured survival, growth, and radula morphology at intervals over 61 days post-settlement. The species of diatoms were *Achnanthes brevipes, Amphora salina*, *Amphiprora paludosa*, *Cylindrotheca closterium*, *Navicula incerta*, and *Nitzschia laevis*, in addition to a starvation control. The survival and growth component of this project aimed to provide useful information for *H. kamtschatkana* hatcheries when choosing species of diatoms as feeds. The radula component of this project aimed primarily to describe the morphology of the postlarval radula in *H. kamtschatkana* in the context of diet suitability and nutrient access, and secondarily to observe whether diatom diet can induce radula plasticity in the postlarval stage.

MATERIALS AND METHODS

We reared *H. kamtschatkana* postlarvae for 61 days post-settlement and fed them *ad libitum* on one of 6 species of benthic diatoms or in a starvation control. We counted survival at days 20, 26, 38, 49, and 61 post-settlement, measured growth at days 7, 20, 42, and 61 post-settlement, and collected postlarvae for radula analysis every other week. To investigate one basic component of nutrition, we measured the carbon-nitrogen ratio of the diatoms. We dissected radulae from animals, imaged them using scanning electron microscopy, and measured radula and tooth sizes and positions from the images. We investigated radula morphology over time and by size, and plastic response of radula morphology to diatom diet type.

Abalone for feeding trials

We obtained *H. kamtschatkana* larvae at 7 days post-fertilization from the Puget Sound Restoration Fund hatchery in Mukilteo, Washington. We transported them in a 4 L glass jar of 1 µm filtered seawater (FSW) within a cooler to Shannon Point Marine Center, Anacortes, Washington. Larvae were in transit for 3.5 hours and upon arrival at the marine center we allowed them to acclimate to the experimental incubator for 20 hours (12 °C, 12:12 light cycle, illuminated by four cool white fluorescent bulbs). We used UV-sterilized 0.2 µm FSW for all rinsing and rearing after larvae arrived at the laboratory.

To prepare the abalone for the feeding trial, we drained larvae onto a 60 µm Nitex mesh screen and rinsed them with FSW into a 1 L plastic beaker of 500 ml FSW. We then pipetted exactly 10 larvae into each well of 21 6-well culture plates and added FSW to a final volume of 14 ml. We arranged all diatom diets (treatments) such that every 7 wells contained a full set of all diets, randomly arranged (Figure 1). Thus, our experimental unit was a well and we had 18 wells per diatom diet. To induce the larvae to settle and undergo metamorphosis, we added gamma aminobutyric acid (GABA) to each well to a final concentration of 8 μM (Paul Pratt, personal communication, unpublished manuscript). After 104 min (SD, 19 min), we conducted two 85% water changes in succession to remove the GABA, for a final volume of 10 ml of seawater in each well.

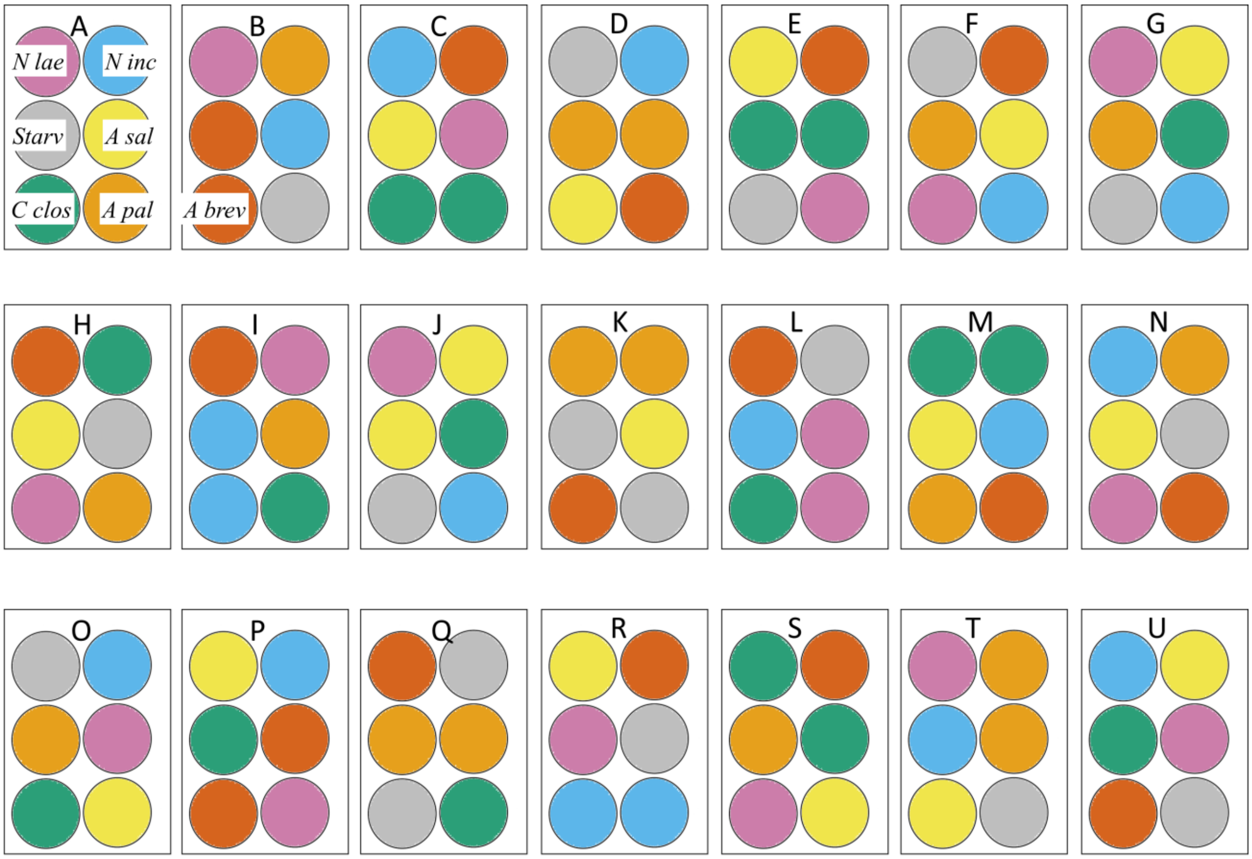

Figure 1: Arrangement of diets (treatments) in 6-well plates. Plate ID letter is at the top of each plate. Each row of plates in this figure was on a different shelf of the rearing incubator. Species are labeled for the first diet set, indicating the color coding of this figure. Abbreviations: A brev = *Achnanthes brevipes*, A pal = *Amphiprora paludosa*, A sal = *Amphora salina*, C clos = *Cylindrotheca closterium*, N lae = *Nitzschia laevis*, N inc = *Navicula incerta*, Starv = starvation control.

Diatoms for feeding trials

We purchased 6 species of benthic diatoms: *Achnanthes brevipes* Agardh (CCMP 100), *Amphiprora paludosa* Van Heurck (CCMP 125; synonym: *Entomoneis paludosa*), *Amphora salina* W. Smith (CCMP 1119; synonyms: *Amphora coffeaeformis, Halamphora covfefe, Halamphora coffeaeformis*), *Cylindrotheca closterium* (Ehr.) Reimann et Lewin (CCMP 340; synonym: *Nitzschia closterium*), *Navicula incerta* Grunow (CCMP 542), and *Nitzschia laevis* Hustedt (CCMP 559), from the National Center for Marine Algae and Microbiota (Bigelow Laboratory, East Boothbay, Maine). We chose these species based on size, shape, preferred temperature range, and information from other feeding studies on abalone postlarvae.

Prior to inoculation into wells containing abalone postlarvae, we cultured the diatoms at 17 °C on a 16:8 hour light:dark cycle in axenic conditions in 1 L flasks of approximately 600 ml Proline F/2 media with added sodium metasilicate (Aquatic Eco-Systems Inc., Apopka, FL). Sixteen hours after the abalone settled, we added diatoms at 1 cell mm^{-2} of wetted well surface area (2,200 cells per well) to the wells, held at 12 °C on a 12:12 hour light:dark cycle. Thereafter, we visually observed that diatoms grew at a rate greater than or equal to postlarval grazing rates and needed no further supplementation. We conducted 80% FSW changes 3 times per week in a 12 °C cold room with 5 ml glass pipets. When diatoms appeared to be growing more than one cell deep, at day 45, we thinned them by brushing well bottoms with fringed waterproof paper.

To determine C:N ratios, we allowed diatoms to grow in wells in the same conditions for one week after postlarvae were removed on day 61. During this week, we conducted water changes as previously described. We then dislodged diatoms and stirred them into suspension in the wells using strips of silicone cut from a 3/8” hose. We pipetted the diatom suspension into a syringe fitted with a 0.7 µm glass fiber filter and placed the filters into tin capsules held in a 24-well culture plate. We dried the filters at 50 °C for 36 hours, then folded the tin capsules closed. Samples were analyzed for ^{13}C and ^{15}N isotopes at the University of California Davis Stable Isotope Laboratory (Davis, California), using an Elementar Micro Cube elemental analyzer interfaced to an Isoprime VisION IRMS (Elementar Analysensysteme GmbH, Hanau, Germany).

We determined mean C:N ratio from each diatom diet and related it qualitatively to survival and growth of postlarvae fed the different diatom diets.

Survival

We counted the number of abalone alive in each well at days 20, 26, 38, 49, and 61 post-settlement. Prior to each count, we used a 1 ml micropipetor to flush water across each postlarva to dislodge the dead and leave the live postlarvae in place.

Because most mortality occurred before our first measurement of survival on day 20, we were unable to fit a Survival Analysis curve. Instead we analyzed percent survival at day 20 and day 61. For both periods, we tested for effect of diatom diet using a one-way ANOVA, followed by pairwise contrasts (Tukey HSD) to compare individual diets to each other. We omitted the starvation control and *A. brevipes* from the day 61 analysis because of extremely low survival (see Results). Since there were no *a priori* hypotheses about how diatom diets would compare to each other, we used pairwise contrasts rather than special contrasts. We chose the Tukey HSD test because it is widely used, we wanted a more sensitive test than a Bonferroni, and we had too many comparisons to use a Fischer's LSD test.

We checked for homogeneity of variance using Levene's test. Variance of survival data was homogenous ($p = 0.55$ for day 20; $p = 0.11$ for day 61). We checked for normality using the

Shapiro-Wilk test with a threshold of $p \geq 0.01$. Two treatments did not meet this normality threshold (*A. brevipes*, $p = 0.0097$ for day 20; *C. closterium* $p = 0.007$ for day 61). To reduce the risk of false positives due to non-normality, we chose an α level of 0.01 for our Tukey HSD test.

Growth

We measured growth of postlarval abalone at days 7, 19, 20, 42, and 61 post-settlement. Day 7 measurements were only of postlarvae residing on the well bottoms, because animals on the well sides were too delicate at this age to dislodge for measurement. Beyond day 7, postlarvae typically resided on the walls of wells and were rarely observed on the bottoms, but were robust enough to be moved to the bottom of the well for measurement. We dislodged all postlarvae in each well using thin grass stems, then manipulated them onto their foot on the bottom center of the well. We dipped the stems in boiling water to prevent diatom contamination between wells. We photographed the postlarvae using a stereoscope equipped with a camera (optical lens magnification 8×; Leica Microsystems, Buffalo Grove, IL). We then measured shell length using ImageJ image analysis software (Rasband 1997).

To compare growth among diatom diets, we used a linear mixed model fit by maximum likelihood and with effective degrees of freedom calculated by Satterthwaite's method (Bates et al. 2015; Kuznetsova et al. 2017). Linear mixed models are appropriate for data that include repeated measurements and have missing data. We calculated the mean shell length of all postlarvae in each well on each measurement day, then natural-log transformed the shell length data so that it fit a linear relationship with age. We first fit the full model, containing the fixed factors diatom diet, age, and diatom diet-age interaction, and the random factors diet set, culture plate, and well (experimental unit). Well was required as a random factor because each well was measured repeatedly throughout the experiment. We then compared the full model to reduced models using the Akaike information criteria and an ANOVA.

After developing the growth models for diets, we tested whether size of postlarvae was influenced by the number of surviving postlarvae in a well, e.g. by competition and crowding. We used data from the four diets with highest survival, because the data were more robust. For each of these diets, we fitted a linear mixed model containing the fixed factors age and count, and the random factor well (experimental unit). Age was transformed as $e^{0.02*\text{day}}$, based on our knowledge from the diet-growth model results, and was required in the model because shell length is highly dependent upon age. Well was required as a random factor because each well was measured repeatedly throughout the experiment.

Radula morphometry

During the feeding trial, we removed one diet set of postlarvae at days 5, 20, and 33 post-settlement, and preserved postlarvae in deionized water in a -20 °C freezer for radula analysis. At day 61, we preserved all surviving postlarvae from the feeding trial using the same methods. In addition, we obtained a few juvenile (9 month old) animals from Puget Sound Restoration Fund for radula dissection and comparison. We extracted each radula by thawing the abalone, then adding 6% sodium hypochlorite to dissolve soft tissues. We used a pipet to flush the radula free of remaining soft tissues, then rinsed it thoroughly with deionized water. Once each radula was rinsed, we placed it on a thin plastic sheet in a droplet of water. Once the radula was dry, we transferred it to a scanning electron microscope aluminum stub by touching it gently with double-stick carbon tape.

We coated the samples with gold-palladium sputter (60 seconds, 26 kV; Polaron Range; Quorum Technologies, East Sussex, England) then used a scanning electron microscope (Vega TS 5136MM, TESCAN, Kohoutovice, Czech Republic) to obtain an image of each whole radula at 1,000× and an image of 5 to 10 rows in the middle of each radula at 2,000× to 6,000×.

We conducted two types of morphometric analysis: traditional and geometric. For traditional morphometric analysis, we measured radula length, radula width, gap length (distance between rows measured from rachidian base to rachidian base), number of rows, buccal cartilage position (row number at which the posterior ends of buccal cartilages terminate), rachidian height (base to fold), rachidian cusp length (fold to tip), rachidian base width, number of lateral teeth per row, and number of marginal teeth per row (Figure 2). We plotted these measurements against shell length, grouped by diatom diet, following procedures in Avaca et al. (2010). We fit simple linear regressions of traditional morphometric measurements predicted by shell length. We described tooth development with age (rather than with size) qualitatively, because of the limited numbers of postlarvae sampled earlier than 61 days post-settlement.

We observed no difference in radula morphology between diets or wells, and had a limited number of successful dissections, so we treated radulae as individual units, rather than averaging characteristics within wells. For all radula measurements except for radula length, width, and buccal cartilage position, we measured multiple rows or rachidian teeth and averaged them into a single datum per radula.

Our geometric morphometric analysis was based on 2-dimensional landmarks. We described overall shape of rachidian teeth using 7 landmarks, and used 13 landmarks to describe the relative positions of the middle 5 teeth within a row: the rachidian tooth and the first 2 lateral teeth (L1 and L2) on either side of it (Figure 3). We aligned and scaled sets of landmarks using Procrustes General Analysis, which centers, scales, and then rotates shapes until the sum of squared distances among them is minimized. We estimated positions of missing landmarks using multivariate regression estimates (Adams et al. 2019), then averaged the coordinates of landmarks from different rows within each radula. Bilateral symmetry was not assumed for abalone radulae (Hickman 1981; Geiger 1999). We then compared radula landmarks between diatom diets using Procrustes ANOVA (Adams et al. 2019).

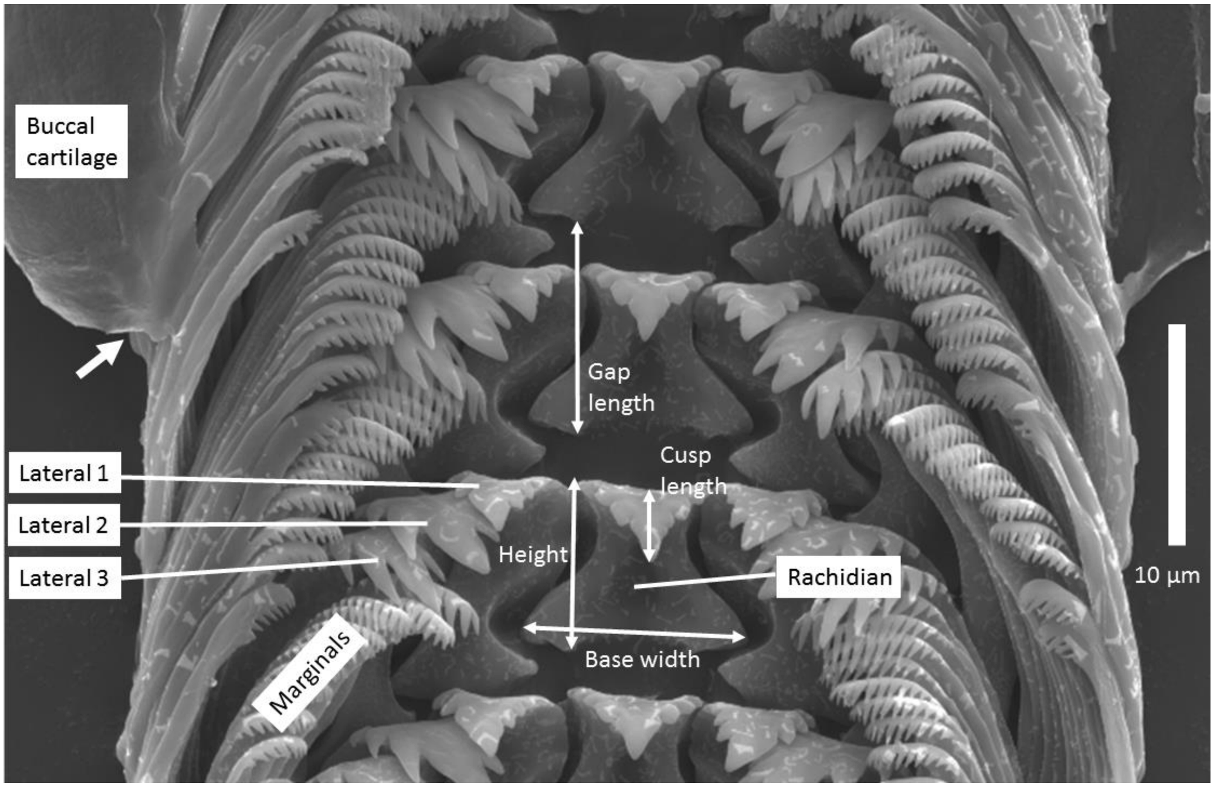

Figure 2: Radula tooth types and measurements. Arrow at left indicates base of row where buccal cartilage ends, as measured in this study.

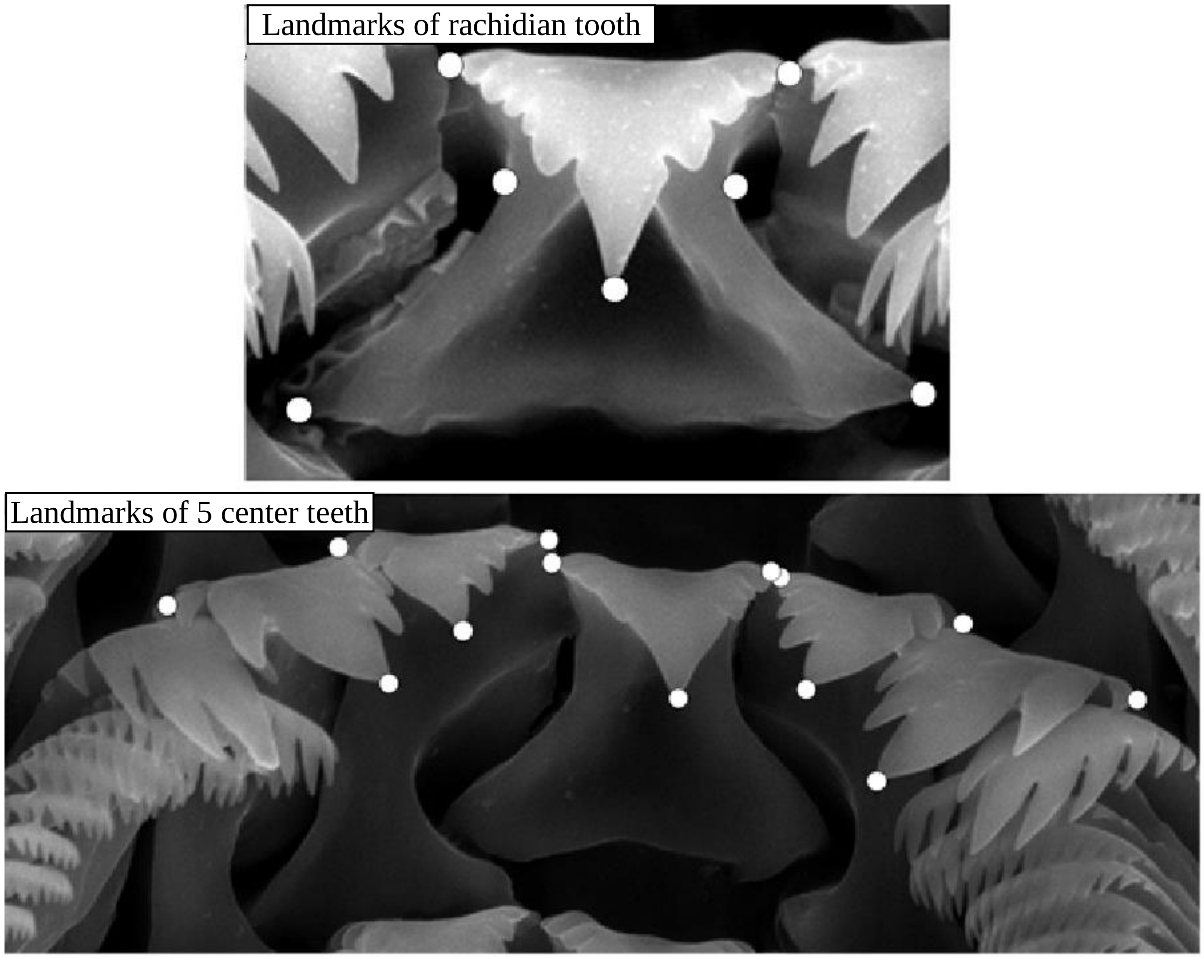

Figure 3: Placement of radula landmarks used to analyze radula morphology. Above: rachidian: 7 landmarks (n = 394 rachidian teeth from 78 radulae; 76 missing landmarks from 49 radulae were estimated). Below: the 5 center teeth: L2-L1-rachidian-L1-L2: 13 landmarks (n = 215 rows from 86 radulae; 64 missing landmarks from 45 radulae were estimated).

RESULTS

Survival

Amphora salina yielded the highest survival (Tukey HSD, $p < 0.03$), in a statistically homogenous subgroup with *N. incerta*, *A. paludosa*, and *C. closterium*. *Navicula incerta*, *A. paludosa*, *C. closterium*, *N. laevis*, *A. brevipes*, and starvation formed a second homogenous subgroup (Tukey HSD $p > 0.05$, Figure 4). *Nitzschia laevis* yielded mediocre survival at day 20, and poor survival at day 61. *Achnanthes brevipes* yielded survival lower than starvation at day 20, and very poor survival at day 61 (Figure 4).

Diatom diet had a significant effect on postlarval survival at both day 20 (ANOVA, $F_{6,95} = 8.60$, $p < 0.001$) and day 61 (ANOVA, $F_{6,66} = 9.70$, $p < 0.001$). At our first measurement of survival, on day 20, all diets yielded less than 40% survival, except *A. salina* with 60%. We did not measure settlement or metamorphosis success separately from survival, so both larvae that failed to settle and postlarvae that died after settlement reduced the percent survival. Therefore, survival at day 20 may be due to the diatoms' suitability as a feed very early in life, suitability for settlement and metamorphosis prior to feeding, or a combination.

For postlarvae fed *N. incerta*, *A. salina*, *A. paludosa*, or *C. closterium*, survival increased substantially after day 20. Of postlarvae fed these diets and alive on day 20, 78% were still alive on day 61 (Figure 4). Differences in survival between these diatom diets from days 0 to 61 were driven by early survival to day 20, indicating that either it is a refuge age of higher survival, or that these diets are poorly suitable for settlement and metamorphosis, thus causing early death. Our last observation of a living starved postlarva was on day 26.

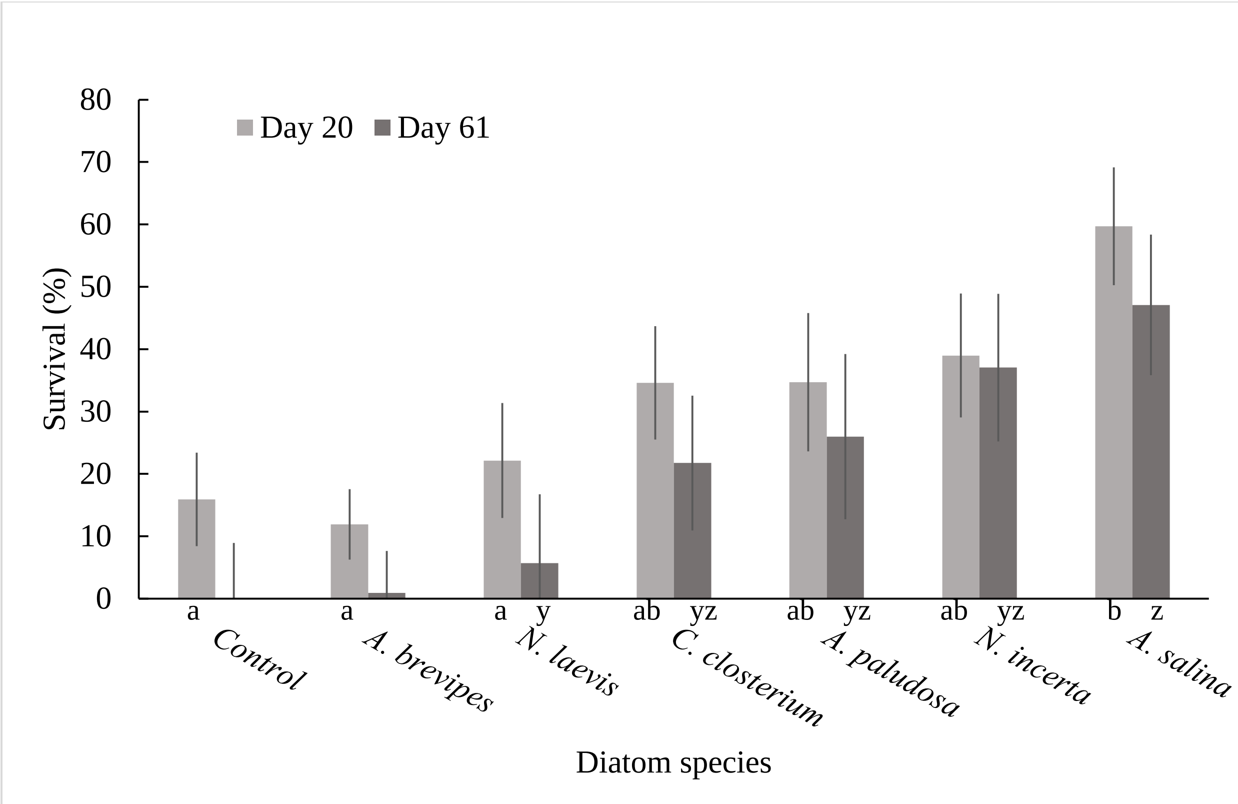

Figure 4: Effect of diatom diet on the survival of postlarval *H. kamtschatkana*. Error bars represent 90% confidence interval. Letters below each bar represent statistically homogeneous sub-groups (ANOVA p > 0.05): letters a and b for day 20; letters y and z for day 61.

Growth model selection

The most parsimonious linear mixed model to predict growth of postlarvae included diatom diet, postlarval age, and diet-age interaction as fixed predictive factors, and well (experimental unit) as a random factor with both a random slope and a random intercept (Table 1). Random factors of culture plate and diet set added little to the models and were left out of the final model (Table 1).

There was a significant difference in modeled initial shell length (intercept) of postlarvae fed *A. salina* compared to starting size of postlarvae fed other diets, even though the actual initial shell length was not different between diets. We viewed this as an unavoidable and insignificant artifact of model fitting.

Survival rates of postlarvae fed *A. brevipes* or the starvation control were too low for statistical analysis of growth, so they were left out of the analysis. Number of surviving postlarvae per well did not affect subsequent growth, indicating that there were no density effects, and space and food were not limiting resources (Table 2).

Table 1: Growth model selection table. All models include a random slope across all ages for each random factor. Diet = diatom treatment. Age = days post-settlement. Well and plate = growing containers. † no significant difference between models, ANOVA $p > 0.05$

	Model #	Predictive factors included in model:	Factors for which a random slope is included across all ages:	df	AIC
†	1)	diet, age, diet-age interaction	well, plate	21	-478
†	2)	diet, age, diet-age interaction	well	18	-477
†	3)	diet, age, diet-age interaction	well, diet set	21	-473
†	4)	diet, age, diet-age interaction	well, diet set, plate	24	-472
	5)	diet, age	well, plate	15	-468
	6)	diet, age	well	12	-467
	7)	diet, age	well, diet set	15	-464
	8)	diet, age	well, diet set, plate	18	-462
	9)	age	well	6	-442
	10)	diet, age, diet-age interaction	plate	18	-418
	11)	diet, age, diet-age interaction	diet set	18	-413
	12)	diet, age, diet-age interaction	diet set, plate	21	-412
	13)	diet, age	plate	12	-389
	14)	diet, age	diet set	12	-386
	15)	diet, age	diet set, plate	15	-383

Table 2: Number of surviving abalone per well does not affect growth. Data are values for shell length within each diet, described by the equation SL = $\beta_0 + \beta_{age}A + \beta_{number}N + e_{well} + \varepsilon_i$, where SL is shell length, A is postlarval age, N is number of postlarvae per well, *e* is the random effect of well, and ε_i is error. df = Satterthwaite's effective degrees of freedom.

				Statistics for β_{number}		
Diet	Intercept	β_{age}	β_{number}	df	t-value	p-value
A. paludosa	25	275	0.15	53	0.045	0.97
A. salina	228	218	-10	57	-1.7	0.09
C. closterium	-56	347	0.99	43	0.16	0.87
N. incerta	103	237	-2.4	43	-0.87	0.39

Growth of postlarvae

Between most diets, there was no significant difference in growth of postlarvae. The exception to this was *C. closterium*, which yielded significantly faster growth than *A. salina* or *N. incerta* (LMM p = 0.001 and 0.018, Satterthwaite effective df = 59 and 64, respectively), and nearly significantly faster than those fed *A. paludosa* or *N. laevis* (LMM p = 0.075 and 0.088, Satterthwaite effective df = 61 and 70, respectively; Figure 5).

The growth of *H. kamtschatkana* postlarvae when fed *C. closterium* is described by the equation

$$shell\ length\ (\mu m) = 293 * e^{0.021t}$$

where t = days post-settlement.

There was no significant difference in growth rate between postlarvae fed *A. paludosa*, *A. salina*, *N. incerta*, or *N. laevis* (LMM p > 0.10, Satterthwaite effective df range = 63 to 75). The growth of postlarvae fed these diatom diets was:

$$shell\ length\ (\mu m) = 302 * e^{0.018t}$$

By day 61, mean shell length among all postlarvae regardless of diet was 944 µm (SD, 186 µm; range of individual postlarvae: 558 to 1,390 µm), and none of the postlarvae developed a respiratory pore within the course of our experiment.

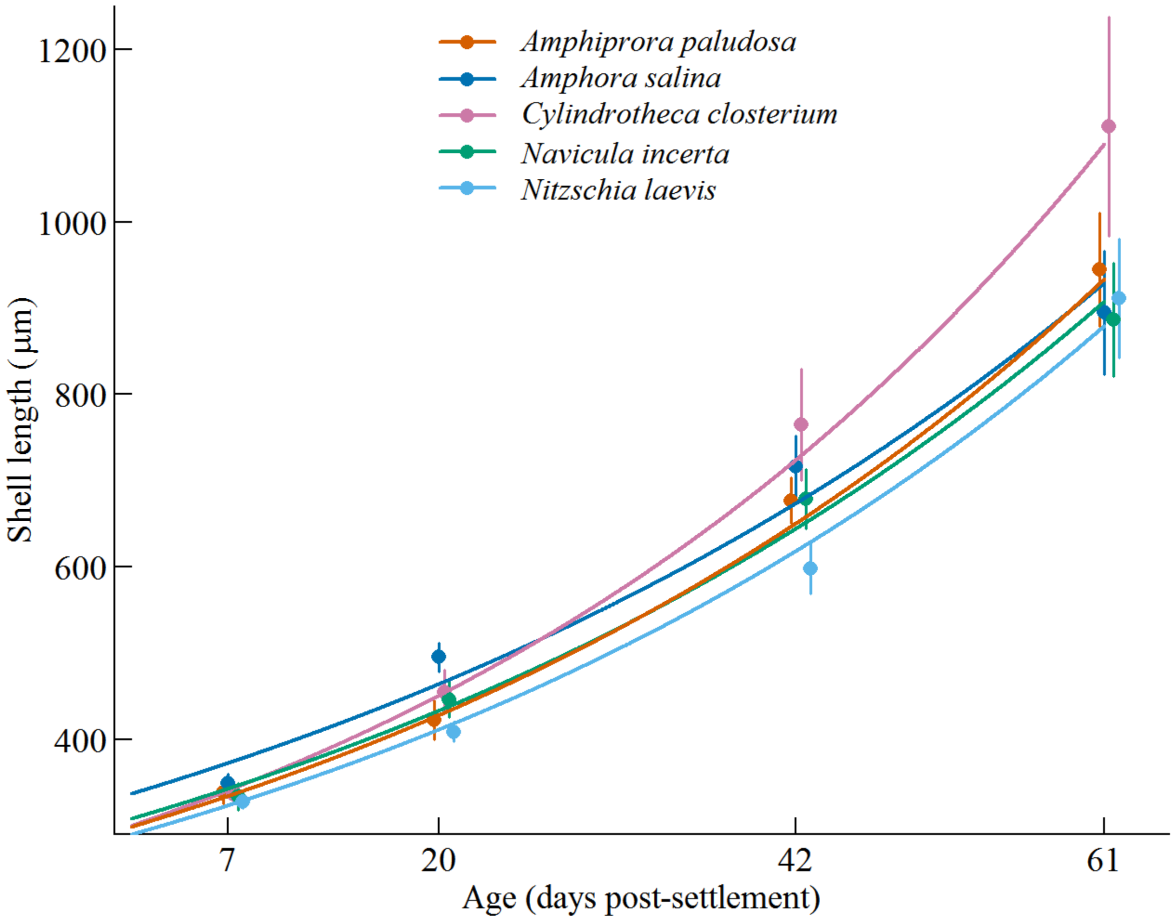

Figure 5: Effect of diatom diet on the growth of postlarval *H. kamtschatkana*. Error bars represent one standard error of the mean. Curves are of the linear mixed model of shell length in response to postlarval age, diatom diet, and age-diet interaction, with well (experimental unit) as a random factor.

Diatom characteristics and carbon:nitrogen ratio

We measured C:N ratio for all diatoms, measured length and width of 4 of the 6 diatoms, and noted growth patterns and shape. C:N values had low variance within diatom diets (Table 3), and there was no clear relationship between C:N ratio and either survival or growth of postlarvae. Likewise, there were no apparent relationships between diatom size and survival or growth of postlarvae

We observed that *A. paludosa* adhered very loosely to container surfaces, almost like a planktonic diatom. *Nitzschia laevis* tended to clump, while *A. salina*, *C. closterium*, and *N. incerta* formed uniform films. *Achnanthes brevipes* and *N. laevis* appeared to grow more slowly than the other diatoms tested, but all diatoms used in this study grew well at 12 ºC. This temperature was within the reported range of all diatom diets except for *C. closterium*, which had a reported temperature range of 18 to 26 ºC (NCMA 2019a).

Table 3: Success of postlarval *H. kamtschatkana* on different diatom diets, and diatom characteristics. Diatom sizes are as measured in the present study and as reported by the Bigelow Laboratory (NCMA 2019b). One standard deviation is given after ±; nd = no data. Sample size for diatom length and width is >20 per diatom; n refers to number of wells that were analyzed for C:N ratio.

Diatom species	Length (µm)	Bigelow length (µm)	Width (µm)	Bigelow width (µm)	Survival	Growth	C:N	n
A. salina	nd	24 - 30	nd	6 - 8	Excellent	Fair	30 ± 2	9
N. incerta	18	12 - 16	7.9	4 - 6	Good	Fair	24 ± 3	8
C. closterium	13	18 - 22	4.2	2 - 3	Fair	Excellent	10 ± 2	10
A. paludosa	13	9 - 21	7.9	6 - 9	Fair	Good	10 ± 4	8
N. laevis	15	16 - 20	6.5	4 - 6	Poor	Good	21 ± 3	6
A. brevipes	nd	6 - 20	nd	6 - 13	Poor	Poor/NA	22	1

Radula development with age and size: traditional morphometrics

As postlarvae aged, we observed an increase in rachidian tooth size, radula length and width, number of teeth per row, and number of rows per radula (Table 4). For all ages and sizes of postlarvae in our study, qualitative tooth shape differences were small: tooth angles and shapes were very similar among all postlarval radulae, and the prominent difference was in the size and number of teeth rather than the shape (Figure 6). From day 5 to day 61, the radula gained an average of 10 marginal teeth and 2.7 lateral teeth. Radula length approximately tripled in this time from 68 to 220 μm, and radula width approximately doubled from 17 to 41 μm (Table 4).

In 61-day old postlarvae, shell length was a linear predictor of radula size, rachidian tooth size, gap length, and number of both marginal and lateral teeth per row (Figure 7, Table 5).

The buccal cartilage position did not change with age or shell length, indicating that postlarvae graze using 10 rows (SD, 1.5 rows) of teeth regardless of postlarval age or size. Number of rows per radula increased slightly with age but not with day 61 shell length.

Table 4: Radula measurements by age. Data include all diatom diets. Number of lateral teeth are total per row of the radula; number of marginal teeth are per one side of row. Number of rows are per radula. Buccal cartilage position refers to the number of rows anterior to and adjoining the buccal cartilages; in other words, the row number where the cartilages end. Rach = rachidian tooth. One standard deviation is given after ±; nd = no data. Sample sizes are of individual postlarvae, shown in parentheses. *This shell length is of the study population, because we did not measure individuals prior to dissection before day 61.

Age (d)	Shell length (µm)	No. lateral teeth	No. marginal teeth	No. rows	Buccal cartilage position	Radula length (µm)	Radula width (µm)	Gap length (µm)	Rach. height (µm)	Rach. tip (µm)
5	nd	2 (2)	2 (2)	17 (1)	nd	68 (1)	17 ± 1 (4)	4.5 ± 0.2 (4)	3.8 (2)	1.8 ± 0.2 (3)
20	440 ± 52*	2 ± 0 (11)	4 ± 0.4 (11)	21 ± 3 (9)	10 ± 1 (10)	104 ± 14 (9)	22 ± 3 (11)	4.8 ± 0.4 (11)	3.9 ± 0.3 (9)	2.2 ± 0.4 (9)
33	nd	4 ± 0 (14)	7 ± 1 (12)	23 ± 2 (13)	11 ± 1 (14)	139 ± 17 (14)	28 ± 4 (16)	5.9 ± 0.6 (15)	5.0 ± 0.4 (14)	2.7 ± 0.4 (14)
61	903 ± 179	5 ± 1 (100)	12 ± 2 (91)	23 ± 2 (83)	10 ± 2 (65)	221 ± 49 (80)	41 ± 9 (94)	9.0 ± 1.9 (111)	6.7 ± 1.4 (98)	3.2 ± 0.7 (98)

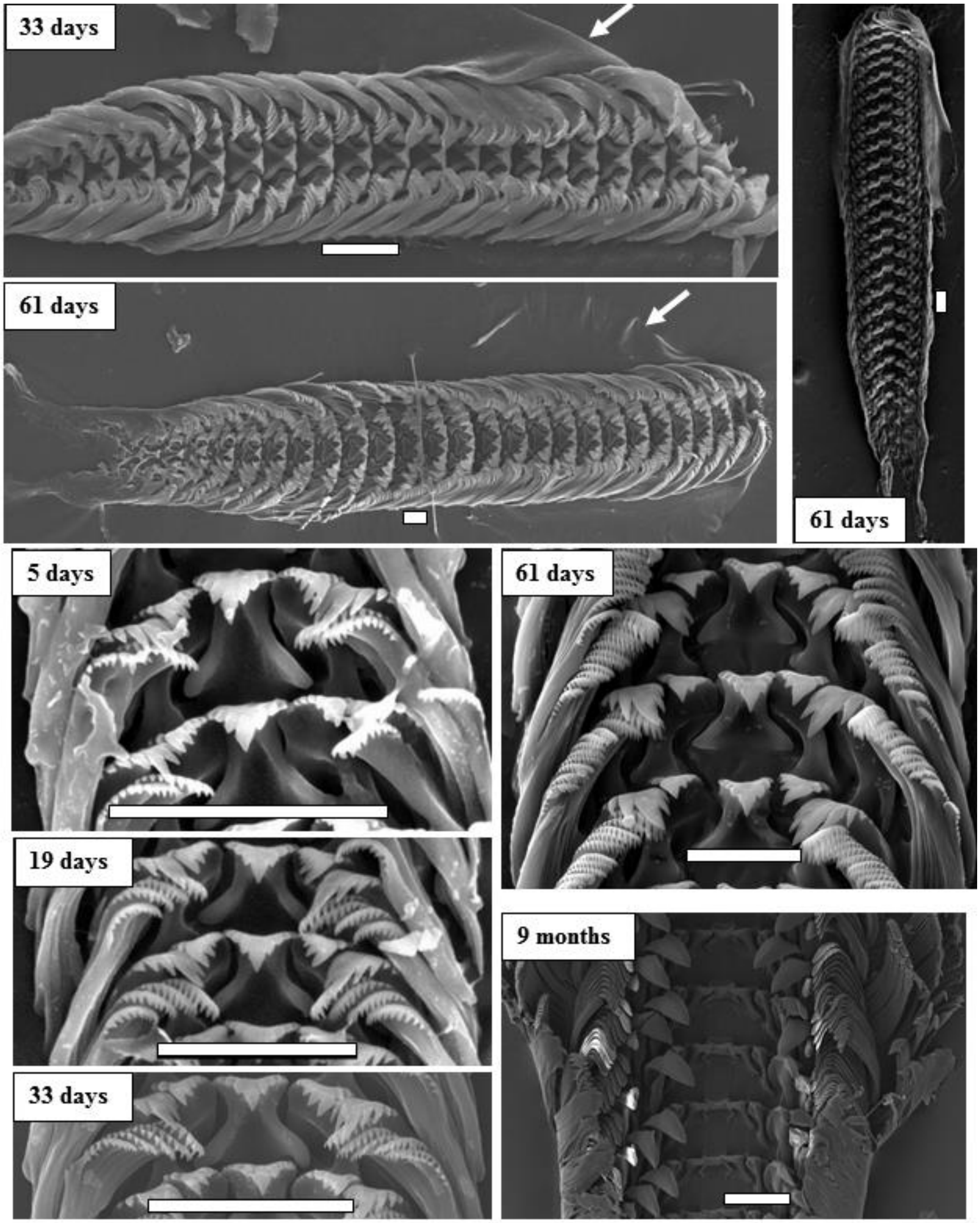

Figure 6: Radula morphology examples: scanning electron microscope (SEM) images of radulae at days 5, 19, 33, and 61 post-settlement, and 9-month old juvenile. Top-right image shows tooth-down view of the radula. Note buccal cartilages in top left images (arrows). Scale bars represent 10 µm, except 100 µm for 9-month old juvenile radula.

Table 5: Regression equations and r^2 values for radula morphology as a function of shell length. The independent variable (x) is shell length (mm). Sample sizes are of individual postlarvae.

Variable	Slope	Intercept	r^2	n
Gap length (µm)	9.0	1.0	0.93	106
Radula width (µm)	42	3	0.93	89
Rachidian height (µm)	6.2	1.1	0.91	93
Radula length (µm)	234	9	0.90	77
Marginal teeth per row	8.4	4.5	0.88	86
Rachidian tip (µm)	2.1	1.3	0.81	93
Lateral teeth per row	3.6	1.6	0.76	95
Rows per radula	-0.6	23	0.19	79
Buccal cartilage position	2.0	8.3	0.01	62
Rachidian ratio	0.4	1.8	0.01	93

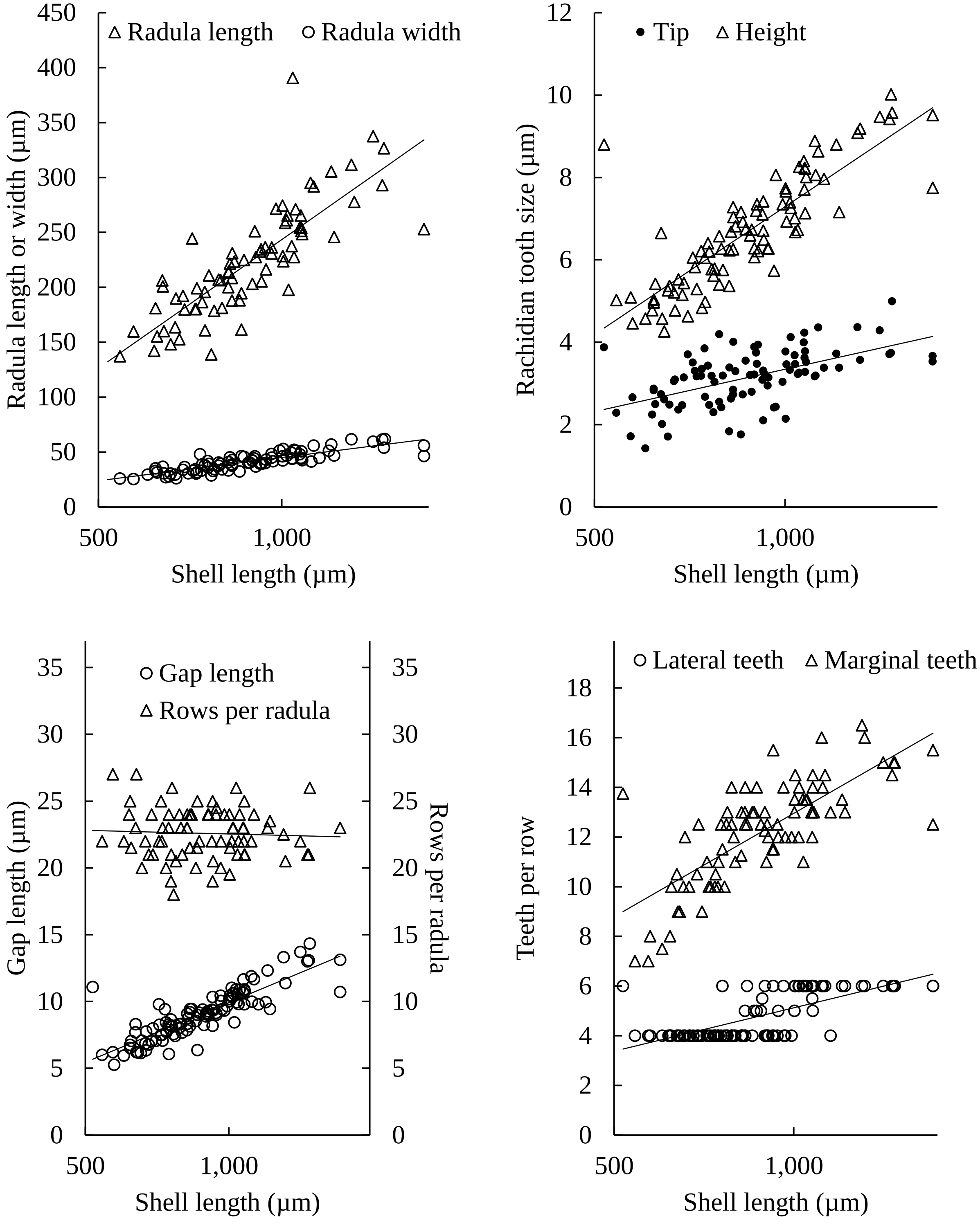

Figure 7: Radula measurements as functions of shell length in 61-day old postlarvae. Data include all diatom diets. Each data point represents one postlarva. Linear regression lines were calculated using the least squares method; see Table 5 for r^2 values and equations of the lines.

Radula development with age and size: geometric morphometrics

In addition to traditional morphometric measurements, we analyzed landmark positions of rachidian teeth and of the middle 5 teeth of each radula (Figure 3). Our rachidian tooth landmarks marked the relative tip position, which could indicate tooth contact angle with the grazing surface, and marked three different widths of the hourglass-shaped rachidian teeth, which could reveal changes in overall structure. There were no apparent changes of position of any of these landmarks (Figure 8). Although the number of teeth in each row increased with size and age, the positions of landmarks on the middle 5 teeth did not change relative to each other with age (Figure 8).

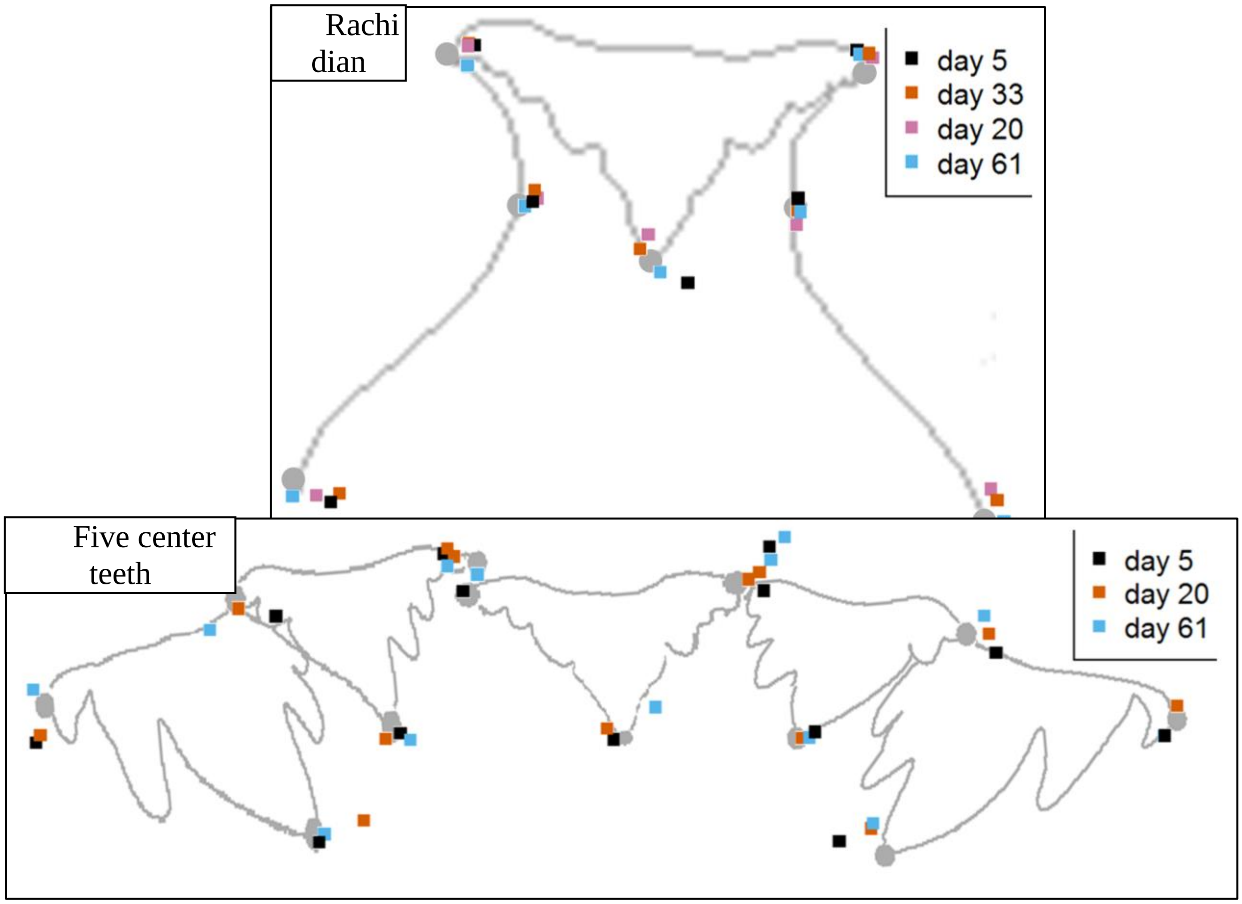

Figure 8: Mean radula landmark positions by age. Colored markers represent landmark positions (Figure 3) at different ages. Landmarks were centered, scaled, and aligned by Procrustes General Analysis. The gray landmark dots and tooth outlines are examples to assist the reader in understanding the landmarks, and not representative of any one result.

Lack of radula plasticity in response to diet

The diatom diets we tested had no effect on any traditional morphometric or landmark measurement of radula morphology. Orientations of landmarks of the rachidian tooth, and landmarks of the middle 5 teeth were not significantly different between diatom diets (Procrustes ANOVA for rachidian $F_{4,97} = 0.68$; $p = 0.8$; for row $F_{4,78} = 1.1$; $p = 0.4$).

Postlarvae fed *C. closterium* and *N. laevis* showed more difference in row-center landmarks than any other pair of diets (Figure 9). Even so, differences between these two diets are so small that many position-change arrows are obscured by the reference points: where two marks exit the reference point, these two marks are the back sides (barbs) of the arrow.

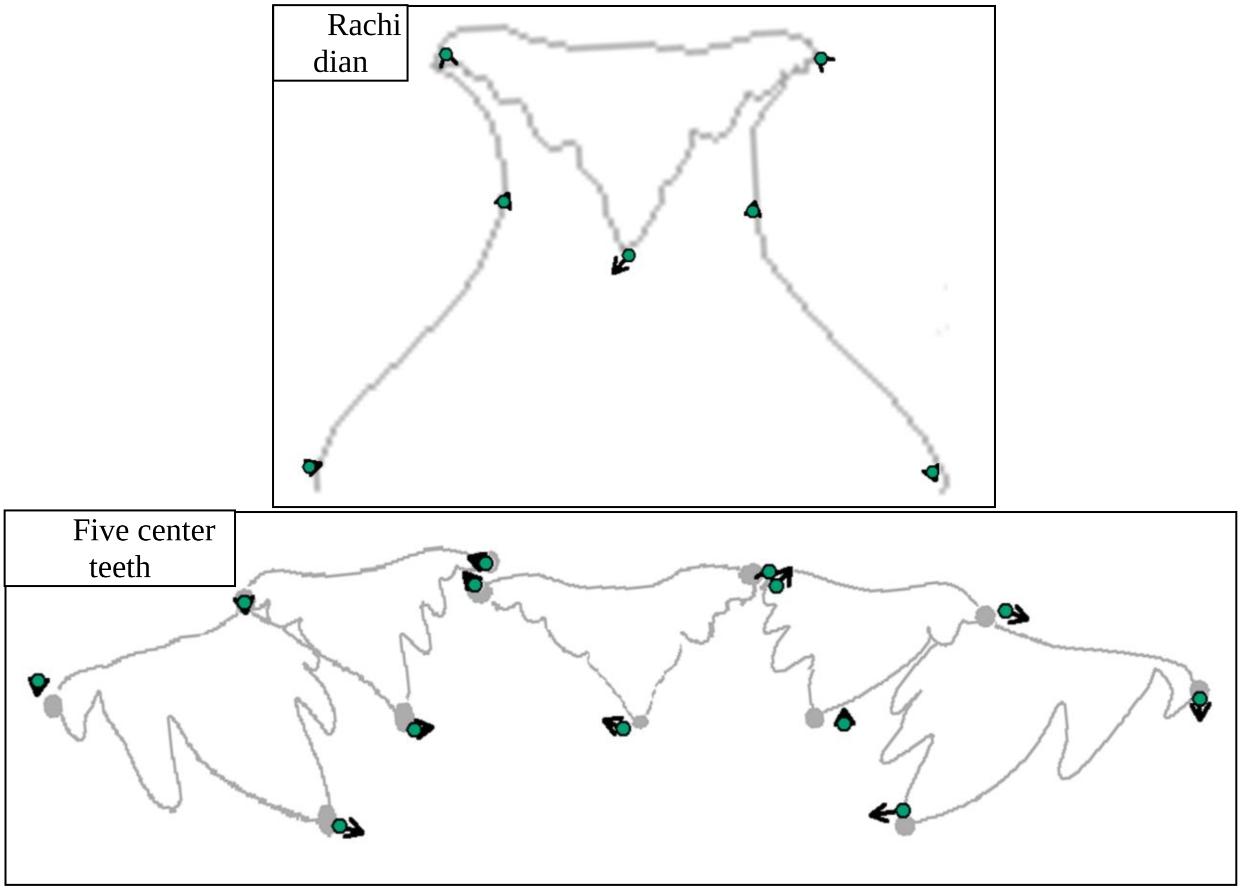

Figure 9: Vector description of the lack of difference between radula landmarks in postlarvae fed two different diets: *C. closterium* (reference points) vs. *N. laevis* (tips of arrows). This is the greatest difference in tooth morphology that we found, indicating a lack of plastic response to diet. Note that some reference points obscure the arrow tips and only the arrow "barbs" are visible. The gray landmark dots and tooth outlines are examples to assist the reader in understanding the landmarks, and not representative of any one result.

DISCUSSION

Effect of diatom diet

Amphora salina was the best diet for survival of postlarval *H. kamtschatkana*. It appears to be a good diet for other abalone species as well, although it appears only twice in abalone feeding literature to date (Table 6): 77% of *H. asinina* Linnaeus fed *A. salina* survived to day 20 post-settlement (Ding et al. 2017), and *H. diversicolor supertexta* postlarvae preferentially consumed *A. salina* from natural mixed diatom films (Zhang et al. 2010). *Amphora salina* grows singly (Wang et al. 2014) or in pairs. Its organic layer is smooth and tight on the silica frustule, and there is an additional glucose polymer present between cells after division (Tesson and Hildebrand 2013), which may contribute to extracellular nutritional availability to small postlarvae. Extracellular polymeric substances may affect food preferences, digestibility, and particle sizes consumed (Joyce and Utting 2015). This could help explain the success of *A. salina* as a diet in our study, in spite of it being the largest of the diatoms that we tested.

Cylindrotheca closterium was the best diet for growth of postlarval *H. kamtschatkana* in our study. *Cylindrotheca closterium* is very well represented in postlarval feeding literature as a successful feed for a variety of abalone species. Its frustule is thin (Reimann and Lewin 1964); this may increase nutritional value because the cell contents can be digested if the frustule breaks during feeding. Postlarvae of *H. rufescens*, and likely other abalone species as well, will pass whole, live diatoms through the digestive system if the diatom frustule is strong enough to remain intact during feeding (Argumedo-Hernández et al. 2010).

Many abalone feeding papers claim *C. closterium* has weak adhesion, but the basis for this traces back to only one paper, which did not directly measure adhesion (Kawamura and Hirano 1992). In our observation, *C. closterium* did not have particularly weak adhesion and instead formed a substantial film that did not dislodge when culture flasks were swirled. Adhesive properties of benthic diatoms depend not only on species, but also on strain, growing conditions (de Brouwer and Stal 2002; Ravizza and Hallegraeff 2015), and surface material (Rasmussen and Østgaard 2001; Holland et al. 2004), any of which might explain this discrepancy. Regardless of the adhesion level, *H. kamtschatkana* postlarvae appeared to have no difficulty consuming *C. closterium*. Near the end of the 9-week experiment we observed that the films of *C. closterium* were nearly grazed clean, and feces were numerous. This indicates a high level of consumption that would have approached food limitation had our experiment continued.

A combination of *A. salina* and *C. closterium* might be favorable to hatchery success, since the former best supported survival and the latter best supported growth, particularly after the first four weeks post-settlement. Diatoms fed in combination can successfully support postlarval settlement, growth, and survival in *H. rufescens* (Araya et al. 2010), *H. fulgens* Philippi (Viana et al. 2007), and *H. discus hannai* Ino (Gordon et al. 2006), but for simplicity most diatom feeding experiments test only single-species diets. When feeding two or more diatoms simultaneously, it is important that both grow well and neither out-competes the other. *Cylindrotheca closterium* has some allelopathic effects against dinoflagellates but these effects have not been shown against diatoms (Xu et al. 2019); for example, it grows viably with *Navicula* sp. (Najmudeen 2017). However, *C. closterium* grows especially quickly during initial surface colonization (Najmudeen 2017), so to co-culture our highest performing diets, *A. salina* must either grow equally quickly during initial surface colonization alongside *C. closterium*, or continue to grow robustly despite this initial competition. Another option is to inoculate *A. salina* in advance so that it can begin colonization in the absence of competition, then add *C. closterium* a few days later.

Table 6: Summary of literature on diatoms as food for abalone postlarvae of shell lengths less than 800 µm. Where growth and/or survival are given as a range, this is often because the experiment was testing other culture parameters (e.g. artificial lighting, water flow). Age refers to days post-settlement. Ages in parentheses are experimental durations, for references where initial age is not given. SL = shell length. Blank fields: information not given in reference.

Diatom	Abalone	Growth (µm d^{-1})	Survival (%)	Initial SL (µm)	Initial Age (d)	Final Age (d)	Temp. (°C)	Reference
Achnanthes brevipes	*H. discus hannai*	57		1,300	28	41	20	Kawamura et al. 1995
Achnanthes longipes	*H. discus hannai*	48		1,300	28	41	20	Kawamura et al. 1995
Achnanthes longipes	*H. discus hannai*	9	70	674		(7)	20	Takami et al. 2003
Amphora angusta	*H. discus hannai*	30		1,300	28	40	20	Kawamura et al. 1995
Amphora luciae	*H. discus hannai*	24	43	280	78	109	22	Gordon et al. 2006
Amphora proteus	*H. discus hannai*	12	35		19	38		Xing et al. 2007
Amphora salina	*H. discus hannai*	19	26		9	28		Xing et al. 2007
Cocconeis scutellum	*H. discus hannai*	8	100	350	11	25	20	Kawamura and Takami 1995
Cocconeis scutellum	*H. discus hannai*	25		350	0	10	20	Kawamura and Takami 1995
Cocconeis scutellum	*H. discus hannai*	41		1,300	28	40	20	Kawamura et al. 1995
Cocconeis scutellum	*H. discus hannai*		0	~350	0	28	20	Takami et al. 1997
Cylindrotheca closterium	*H. discus hannai*	22	63	350	11	25	20	Kawamura and Takami 1995
Cylindrotheca closterium	*H. discus hannai*	36		350	0	10	20	Kawamura and Takami 1995
Cylindrotheca closterium	*H. discus hannai*	50		1,300	28	39	20	Kawamura et al. 1995
Cylindrotheca closterium	*H. discus hannai*	21	90	447	25	32		Takami et al. 2003
Cylindrotheca closterium	*H. discus hannai*	33	88	657	27	34	20	Takami et al. 2003
Cylindrotheca closterium	*H. discus hannai*	44	78	854	21	29	20	Takami et al. 2003
Hantzschia amphioys	*H. discus hannai*	11	47		20	39		Xing et al. 2007
Mix: *Navicula lenzii, Amphora luciae*	*H. discus hannai*	36	49	280	2	33	22	Gordon et al. 2006
Mix: *Navicula lenzii, Nitzschia laevia*	*H. discus hannai*	23	42	280		(31)	22	Gordon et al. 2006

Diatom	Abalone	Growth (μm d^{-1})	Survival (%)	Initial SL (μm)	Initial Age (d)	Final Age (d)	Temp. (°C)	Reference
Mix: *Navicula lenzii, Nitzschia laevia, Amphora luciae*	*H. discus hannai*	33	40	280	36	67	22	Gordon et al. 2006
Mix: *Nitzschia laevia, Amphora luciae*	*H. discus hannai*	27	31	280	23	54	22	Gordon et al. 2006
Navicula cf. *lenzii*	*H. discus hannai*	21	40	280		(31)	22	Gordon et al. 2006
Navicula corymbosa	*H. discus hannai*	31	84		10	29		Xing et al. 2007
Navicula parva	*H. discus hannai*	12	74		32	51		Xing et al. 2007
Navicula ramosissima	*H. discus hannai*	16	67	350	11	25	20	Kawamura and Takami 1995
Navicula ramosissima	*H. discus hannai*	18		350	0	10	20	Kawamura and Takami 1995
Navicula ramosissima	*H. discus hannai*	23		1,300	28	41	20	Kawamura et al. 1995
Navicula seminulum	*H. discus hannai*	12	51			(19)		Xing et al. 2007
Navicula sp.	*H. discus hannai*	46	73	360		(40)	22	Pang et al. 2006
Nitzschia laevis	*H. discus hannai*		4	280		(31)	22	Gordon et al. 2006
Nitzschia sp.	*H. discus hannai*	14		1,300	28	40	20	Kawamura et al. 1995
Nitzschia sp.	*H. discus hannai*	35	95		46	65		Xing et al. 2007
Pleurosigma sp.	*H. discus hannai*	24		1,300	28	39	20	Kawamura et al. 1995
Rhaphoneis surirella	*H. discus hannai*	28	86		40	59		Xing et al. 2007
Stauroneis constricta	*H. discus hannai*	19	65	350	11	25	20	Kawamura and Takami 1995
Stauroneis constricta	*H. discus hannai*	26		350	0	10	20	Kawamura and Takami 1995
Synedra investiens	*H. discus hannai*	18		1,300	28	40	20	Kawamura et al. 1995
Navicula incerta	*H. fulgens*	15	>95	~250	0	15	18	Searcy-Bernal et al. 2001
Navicula incerta	*H. fulgens*	33	>95	474	15	60	18	Searcy-Bernal et al. 2001
Cocconeis scutellum	*H. iris*	45	70	650	24	92	18	Roberts, Kawamura, and Nicholson 1999
Cocconeis sp.	*H. iris*	33 - 42	~80	1,400		(14)	18	Uriarte et al. 2006
Cylindrotheca closterium	*H. iris*	40	75	650	33	101	18	Roberts, Kawamura, and Nicholson 1999
Cylindrotheca closterium	*H. iris*	42 - 48	~90	1,400		(14)	18	Uriarte et al. 2006

Diatom	Abalone	Growth (μm d^{-1})	Survival (%)	Initial SL (μm)	Initial Age (d)	Final Age (d)	Temp. (°C)	Reference
Navicula britannica	*H. iris*	25	48	650	28	96	18	Roberts, Kawamura, and Nicholson 1999
Navicula ramosissima	*H. iris*	25	100	650	35	68	18	Roberts, Kawamura, and Nicholson 1999
Nitzschia ovalis	*H. iris*	23 - 45	~90	1,400		(14)	18	Uriarte et al. 2006
Pleurosigma sp.	*H. iris*	1	0	650		(54)	18	Roberts, Kawamura, and Nicholson 1999
Achnanthes brevipes	*H. kamtschatkana*	4	1	332	7	61	12	*present study*
Amphiprora paludosa	*H. kamtschatkana*	11	26	339	7	61	12	*present study*
Amphora salina	*H. kamtschatkana*	10	47	349	7	61	12	*present study*
Cylindrotheca closterium	*H. kamtschatkana*	14	22	337	7	61	12	*present study*
Navicula incerta	*H. kamtschatkana*	10	37	334	7	61	12	*present study*
Nitzschia laevis	*H. kamtschatkana*	11	6	327	7	61	12	*present study*
Cocconeis sp.	*H. rubra*	27	71	400	12	87	17	Daume et al. 2000
Cylindrotheca closterium	*H. rubra*	33	17	400	44	121	17	Daume et al. 2000
Navicula jeffreyi	*H. rubra*	35	63	400	21	98	17	Daume et al. 2000
Navicula sp.	*H. rubra*	39	75	400		(77)	17	Daume et al. 2000
Navicula sp.	*H. rubra*	19	0 - 40	490		(42)	18	Day et al. 2004
Navicula sp.	*H. rubra*	12	20 - 85	663		(21)	18	Day et al. 2004
Navicula sp.	*H. rubra*	8	5 - 10	663	39	60	18	Day et al. 2004
Amphiprora paludosa	*H. rufescens*	26		~300	5	55	18	Correa-Reyes et al. 2009
Navicula incerta	*H. rufescens*	21		~300	5	55	18	Correa-Reyes et al. 2009
Navicula incerta	*H. rufescens*	48 - 62	27 - 68	490	9	72	18	Anguiano-Beltran and Searcy-Bernal 2013
Navicula incerta	*H. rufescens*	31 - 38	52 - 80	332	6	50	17	Searcy-Bernal & Gorrostieta-Hurtado 2007
Navicula incerta	*H. rufescens*	15 - 22	54 - 67	1,250		(30)	16	Uriarte et al. 2006
Nitzschia laevis strain “B”	*H. rufescens*	13		~300	5	55	18	Correa-Reyes et al. 2009
Nitzschia laevis strain “C”	*H. rufescens*	19		~300	5	55	18	Correa-Reyes et al. 2009
Nitzschia laevis strain “D”	*H. rufescens*	20		~300	5	55	18	Correa-Reyes et al. 2009
Nitzschia cf. fonticola var. *pelagica*	*H. rufescens*	12		~300	5	55	18	Correa-Reyes et al. 2009

Diatom	Abalone	Growth (µm d^{-1})	Survival (%)	Initial SL (µm)	Initial Age (d)	Final Age (d)	Temp. (°C)	Reference
Nitzschia frustulum var. *perminuta*	*H. rufescens*	16		~300	5	55	18	Correa-Reyes et al. 2009
Nitzschia thermalis var. *minor*	*H. rufescens*	29		~300	5	55	18	Correa-Reyes et al. 2009
Wild diatoms (*Navicula* sp. and *Cocconeis* sp. predominant) on macroalgae	*H. rufescens*	1.9%	29	<400	0	30	15	Muñoz et al. 2012
Wild diatoms (*Navicula* sp. and *Cocconeis* sp. predominant) on plastic plates	*H. rufescens*	0.8%	40	<400	0	30	15	Muñoz et al. 2012
Amphora sp.	*H. tuberculata coccinea*	50	~75	241	0	70	21	Courtois de Vicose et al. 2012
Navicula incerta	*H. tuberculata coccinea*	46	~75	241	0	70	21	Courtois de Vicose et al. 2012
Nitzschia sp.	*H. tuberculata coccinea*	43	~75	241	0	70	21	Courtois de Vicose et al. 2012
Proschkinia sp.	*H. tuberculata coccinea*	38	~75	241	0	70	21	Courtois de Vicose et al. 2012

Achnanthes brevipes was a remarkably poor diet and should be avoided for very young *H. kamtschatkana*. We saw no indication that postlarvae grazed on this diatom, and postlarvae died as quickly as those in the starvation control. At one point we observed an individual postlarva moving about its container but making no effort to feed on the *A. brevipes* cells over which it was moving. This was not due to cell size, as *A. brevipes* cells were not larger or smaller than other diatom diets tested. *Achnanthes brevipes* cells grow on short mucilage stalks (Toyoda et al. 2005), which might make them too difficult for *H. kamtschatkana* to consume, or the postlarvae may be responding to chemical cues indicating that the diatom is toxic or otherwise not suitable for consumption.

In comparison, *A. brevipes* may be a suitable feed for other, larger abalone species. *Haliotis diversicolor aquatilis* Reeve postlarvae under 1,000 µm shell length (Onitsuka et al. 2007) and *H. discus hannai* postlarvae less than 1,200 µm shell length (Takami et al. 2003) cannot detach the cells of related diatom *Achnanthes longipes* Agardh. At 4 weeks post-settlement with shell lengths of approximately 1,400 µm, however, *H. discus hannai* fed *A. longipes* and *A. brevipes* do grow in shell length (Kawamura et al. 1995). The greatest shell length of *H. kamtschatkana* in the current study was slightly less than 1,400 µm at 8.5 weeks, and no postlarvae fed *A. brevipes* survived to that point, let alone grew to that size.

Amphiprora paludosa, *N. incerta*, and *N. laevis* yielded moderate survival and growth. These three diatoms are all represented in previous literature but with variable performance as diets (Table 6). This may be related to cell density (Correa-Reyes et al. 2009). *Navicula incerta* is commonly a reference diet against which other diatom species are compared, or used as a fixed diet in tests of growing conditions for abalones, such as light intensity and seawater flow rate (Searcy-Bernal and Gorrostieta-Hurtado 2007; Correa-Reyes et al. 2009; Anguiano-Beltran and Searcy-Bernal 2013). *Nitzschia laevis* was a more suitable food for *H. kamtschatkana* in our study than it is for *H. discus hannai* postlarvae, which cannot survive on it (Gordon et al. 2006).

Culture conditions and strain ID influence myriad diatom characteristics (e.g. Guerrini et al. 2000), and it is rare for any two studies to compare the same set of diatoms. Diatom adhesive strength is primarily measured in the context of testing anti-fouling coatings for underwater surfaces. These tests are focused on differences between coatings, not between diatoms, so their applicability to abalone postlarvae feeding constraints is limited. Likewise, studies of diatoms' organic composition often focus on only one or two diatoms at a time.

No previous work on yolk reserves in *H. kamtschatkana* exists. In our study, 15% of starved postlarvae survived to day 20, but only a single larva survived to day 26. Bacteria may have been present, but no diatoms or other algae. Even if bacteria were nutritionally available to postlarvae in the starvation control, without mucus secretions from diatoms, the bacteria would have limited growth media.

We used carbon-nitrogen ratio as a simple proxy for nutritional value of food. However, in this study we found no relationship between this ratio and postlarval survival or growth rates. In the snail *Potamopyrgus jenkinsi*, C:N ratios up to 15:1 were positively and tightly correlated with growth, but for higher ratios, the correlation was loose and negative (Dorgelo and Leonards 2001). In our study, only 2 diatoms had ratios of 15:1 or less and they did not follow this pattern: *C. closterium* (10.5:1 [SD, 3.7]) yielded higher growth than *A. paludosa* (9.7:1 [SD, 1.6]). For extensive consideration of the use of C:N ratios in molluscan mariculture, see Bayne (2009).

We recommend that future work on feeds for *H. kamtschatkana* postlarvae consider lipid and amino acid compositions. *Haliotis rufescens* postlarval growth rate correlates with amino acid content but not fatty acid profiles (Correa-Reyes et al. 2009). In a study of 4 diatom diets, including *N. incerta*, *Amphora* sp. had the highest lipid and protein content, the lowest carbohydrate content, and yielded fastest growth of *H. tuberculata coccinea* Reeve postlarvae (Courtois de Vicose, Viera, et al. 2012). *Navicula incerta* yielded moderate growth, and showed moderate lipid, protein, and carbohydrate content; fatty acid profiles varied between all 4 diatoms in the study. Formulated feeds may be used to test different levels of biochemical components for abalone postlarval nutrition (Montaño-Vargas et al. 2005).

Unlike the other diatoms tested, *A. salina* was isolated from the middle of the geographic range of *H. kamtschatkana* (Saanich Inlet, British Columbia; NCMA 2019c), so we suggest that future workers test more diatoms isolated from this area. However, geography does not necessarily correlate with diet success, since *H. kamtschatkana* postlarvae grew well when fed *C. closterium* isolated from the Sargasso Sea in the North Atlantic open ocean (NCMA 2019a). *Navicula incerta* was isolated from San Francisco, near the southern end of *H. kamtschatkana*'s range (NCMA 2019d). The lowest performing diets in this experiment, *A. brevipes*, *A. paludosa*, and *N. laevis*, were all isolated from Nantucket Bay and Nantucket Sound, Massachusetts (NCMA 2019e; NCMA 2019b; NCMA 2019f).

***Haliotis kamtschatkana* growth rates**

Our study is the first quantitative account of growth rates of *H. kamtschatkana* postlarvae. Caldwell (1981) qualitatively described their growth as much slower than *H. rufescens*, which is a closely related species (Gallardo-Escarate et al. 2004; Crosson and Friedman 2018). In our study, mean shell length of postlarval *H. kamtschatkana* increased 11 µm day^{-1} on average (range 4.2 to 20 µm day^{-1}) from days 7 to 61. In comparison, growth rate of postlarval *H.*

rufescens is approximately 10 to 30 µm day^{-1} in the first 30 days post-settlement, and up to 125 µm day^{-1} at day 60 post-settlement (Martinez-Ponce and Searcy-Bernal 1998; Gorrostieta-Hurtado and Searcy-Bernal 2004; Uriarte et al. 2006; Searcy-Bernal et al. 2007; Searcy-Bernal and Gorrostieta-Hurtado 2007; Correa-Reyes et al. 2009; Muñoz et al. 2012; Anguiano-Beltran and Searcy-Bernal 2013). *Haliotis kamtschatkana* postlarvae fed *C. closterium* in our study grew at 20 µm day^{-1} once they were above ~800 µm shell length; postlarvae of *H. discus hannai*, a species extensively cultivated in Asia and Chile, grow about 20 to 40 µm day^{-1} when fed *C. closterium* at shell lengths of 800 to 1,200 µm (reviewed by Takami and Kawamura 2003), and postlarvae of *H. iris* are double the length of *H. kamtschatkana* at 60 days post-settlement when fed *C. closterium* (2,700 µm vs. 1,360 µm, respectively; Roberts, Kawamura, and Takami 1999). We also found that abalone growth rate increased exponentially with age over the course of our experiment. For example, from our model for *A. salina*, *N. incerta*, or *N. laevis*, growth rate was 6.2 µm day^{-1} at day 7 and 16 µm day^{-1} at day 61. Oddly, few other studies report growth as an exponential equation and instead give linear measures of growth, sometimes at different ages.

Shell length predicts soft tissue mass in gastropods generally (McKinney et al. 2004; Mehler et al. 2015) and in haliotids specifically (Najmudeen 2015). Strength of such relationships is variable both within and between species, and between size classes, but shell length is nonetheless very commonly used as a metric of abalone size. Shell length is suitable to our work because our goal is to support hatchery production of *H. kamtschatkana* up to a shell size suitable for restoration out-planting, in contrast to work increasing meat yields for abalones grown for food.

The radula

Radula development in *H. kamtschatkana* is similar to other species: the number of lateral teeth increases slowly; the number of marginal teeth increases rapidly; and shell length has a strong linear relationship with radula width, radula length, gap length between rows of teeth, and rachidian tooth size in postlarvae of *H. kamtschatkana* (Figure 7), *H. discus hannai* (Kawamura et al. 2001), *H. diversicolor aquatilis* (Onitsuka et al. 2004), and *H. iris* (Roberts, Kawamura, and Takami 1999). There were small differences between *H. kamtschatkana* and other species at the same shell lengths; for instance, the number of lateral teeth per row was slightly higher than it is in *H. discus hannai* (Kawamura et al. 2001), and the radula was narrower than it is in *H. iris* (Roberts, Kawamura, and Takami 1999). At shell lengths of 750 to 1,200 µm, the *H. kamtschatkana* radula was approximately one-quarter of the length of the shell, but in *H. iris* and *H. diversicolor aquatilis* of the same size it was approximately one-third of the length of the shell. (Roberts, Kawamura, and Takami 1999; Onitsuka et al. 2004).

Larval veliger age is strongly correlated with number of rows of teeth per radula (in *H. australis*, Moss 1999; in *H. discus hannai*, Takami et al. 2006), but rate of gain of tooth rows may (in *H. discus hannai*; Takami et al. 2006) or may not (in *Haliotis australis* Gmelin; Moss 1999) increase when a larva settles and metamorphoses. At day 10 post-fertilization, *H. australis* postlarvae consistently have 16 rows of teeth (Moss 1999). In our experiment, postlarvae at day 12 post-fertilization (day 5 post-settlement) had 17 rows of teeth, and, throughout our trial, grew approximately three-quarters as quickly as *H. australis* (Moss 1997).

Three measured characteristics did not increase with *H. kamtschatkana* shell length: buccal cartilage position, number of rows of teeth per radula, and the ratio between rachidian cusp length and rachidian height (Figure 7 and Table 5). Buccal cartilage position is associated with the number of rows of teeth being used for feeding. Teeth posterior to the buccal cartilages are not in use but ready for deployment when anterior teeth wear out and are shed. Therefore, it appears that both number of teeth in use and teeth in standby are relatively constant at any given time in *H. kamtschatkana* postlarvae. This might indicate that rate of tooth wear is the same regardless of age or size in the first 60 days. The buccal cartilage position of other abalone species is unknown.

The ratio between rachidian cusp length and rachidian height is a proxy for rachidian tooth contact angle with the substratum. This ratio did not change with postlarval age or size (Figure 7, Table 5), nor did geometric morphometrics of landmark positions on teeth reveal any quantitative changes in tooth shape or relative position over time (Figure 8).

In general, as abalone age, individual teeth become gradually less serrated. However, we observed reduced serrations only in a 9-month old *H. kamtschatkana* juvenile outside of our feeding trial, not in postlarvae up to 61 days post-settlement, (Figure 7). Reduced tooth serrations are first visible at 1,200 µm shell length and 53 days post-settlement in *H. iris* (Roberts, Kawamura, and Takami 1999), 1,800 µm shell length in *H. diversicolor aquatilis* (Onitsuka et al. 2004), and 1,890 µm shell length and 49 days post-settlement in *H. discus hannai* (Kawamura et al. 2001). *Haliotis discus hannai* postlarvae at 17 days post-settlement and 1,145 µm shell length have teeth of similar size to our *H. kamtschatkana* postlarvae at 61 days post-settlement and similar shell length, but the former have teeth with a more delicate appearance and deeper serrations (Kawamura et al. 2001). If *H. kamtschatkana* tooth morphology mirrors that of other species, our study may have ended at around the time that decreased tooth serration would be noticeable.

Diatom shape, size, and adhesion did not seem to affect postlarval growth or survival in our study. The diatom with weakest adhesion, *A. paludosa*, resulted in unexceptional growth and survival. Film appearance and cell size were similar between *N. incerta*, *N. laevis*, and the near-deadly *A. brevipes*. We do not know the relative frustule strength of diatoms in our study, but *C. closterium* is known for a thin frustule (Reimann and Lewin 1964) and led to the fastest growth in our study. Our radula measurements did not explain success or failure of any of our diatom diets—radula morphology of *H. kamtschatkana* was comparable to other abalones that have succeeded when fed these same diets.

We found no evidence for morphological plasticity of the radula in response to diet. Plastic response to diet is known to occur in radulae of adult Littorinidae (Padilla 1998; Padilla 2004; Andrade and Solferini 2006; Molis et al. 2015) and other gastropods (e.g. *Placida dendritica*, Bleakney 1990; *Elysia viridis*, Jensen 1993); however, in some groups such plasticity does not occur (e.g. *Theodoxus fluviatilis*, Zettler et al. 2004; *Buccinanops globulosus*, Avaca et al. 2010; the polyplacophoran *Leptochiton asellus*, Sigwart and Carey 2014). Our abalone may have been too young to display plasticity, the study may have been too short, the diatoms too similar, or perhaps abalone in general do not have morphological plasticity of the radula.

Conclusion

We recommend that *H. kamtschatkana* hatcheries feed *A. salina* immediately after settlement, possibly paired with *C. closterium* to support growth. Future work should investigate diatom diets for *H. kamtschatkana* postlarvae during and immediately after settlement, and at 2 to 4 months post-settlement. This work should consider biochemical composition, and should reiterate diets with attention to whether it is the diatom species or the growing conditions that drive diet success. *Amphora salina* may also be of interest to those studying settlement and metamorphosis success, because of the especially high early survival of postlarvae grown on it. Radula development in *H. kamtschatkana* is very similar to other abalone species, albeit slower, in tandem with overall growth. However, radula morphology did not illuminate why postlarval growth and survival were higher on some diatom diets than on others. *Haliotis kamtschatkana* postlarval success seemed driven mostly by abalone age and differences between diatom species, rather than any nuances of teeth.

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