Electronic Supplementary Information

Farmed Jumbo Shrimp Molts: An Ionic Liquid Strategy to Increase Chitin Yield per Animal while Controlling Molecular Weight

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MATERIALS AND METHODS

Chemicals. Ionic liquid 1-ethyl-3-methylimidazolium acetate ([C_2mim][OAc]) was obtained from Proionic (Grambach, Austria), Lot# 17PI250_F3, water content 0.11%, 917 kg batch. Toluene for light scattering instrument calibration was of HPLC grade (purity 99.85%) manufactured by Acros Organics and obtained from VWR (West Chester, PA), catalog number 26837-0010, Lot# B0537317. Toluene was distilled prior to use, cooled to room temperature (RT), and stored under nitrogen over 3 Å molecular sieves dried at 300 °C.

Sodium hydroxide (pellets, ACS grade, catalog number 97064-498) and hydrochloric acid (ACS Grade, Catalog # BDH3026-500MLP) were obtained from VWR (VWR International Avantor Sciences, Radnor, PA). Deionized (DI) water was obtained from Evoqua Water Technologies' Purification System LDIRS03 (Richmond, VA).

Biomass. All molts and shrimp were obtained from Global Blue Technologies' (GBT) indoor farm located in Taft, TX. The species was Hybrid (H1) Pacific whiteleg shrimp (*Litopenaeus vannamei*) and grown in a zero-water-exchange modular biosecure system (closed loop Recirculating Aquaculture System or RAS) which provided temperature-controlled water to the shrimps' environment. Molt biomass was from the same species which were collected from organized raceways at the bottom of the ponds which were designed to collect and organize the molts by ABW/age as they fell to the bottom of the ponds. The molts collected were from shrimp with

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Average Body Weight (ABW) animals from 5-10 g (ABW5-10), 10-20 g (ABW10-20), and 30 – 40+ g (ABW30-40+). The molts were washed with DI water, dried at 80 °C in a convection oven until constant weight was obtained, and shipped to Mari Signum Mid-Atlantic, LLC, in Richmond, VA. For comparison, shells from ABW 30-40 g Hybrid (H1) Pacific whiteleg shrimp (*Litopenaeus vannamei*) were peeled from the animals (Peels), collected, and treated as above for the molts.

Biomass Preparation. All ABW categories of molt shells were ground separately in 3-4 g batches using a Werke M20 Universal grinder from IKA (Wilmington, NC) and sieved to a particle size of < 250 μm using a set of ISO test stainless steel sieves with wire mesh (Gilson, Lewis Center, OH). The < 250 μm biomass obtained was stored in 50 mL plastic centrifuge tubes. Before each laboratory experiment, the shrimp shells were additionally ground using an electric lab mill (Model M20 S3, IKA, Wilmington, NC), and separated by particle size using a set of ISO test stainless steel sieves with wire mesh (Gilson, Lewis Center, OH) decreasing in size (1000, 500, and 250 μm) into a collecting pan. Particle sizing was carried out in small batches of *ca*. 1−2 g, until a sufficient amount of ground shrimp shells with desired particle sizes (< 250 μm) was obtained.

Similarly, the peeled shells from animals with ABW 30-40 g were inspected to make sure no obvious shrimp meat was left, and the back and tail of the shells were collected. The shells were washed five times with tap water, one time with DI water, and then dried in an oven (Precision Econotherm Laboratory, Winchester, VA) at 80 °C for 2 days. After drying, the grinding and sieving process was conducted in the same fashion as for the molts.

Determination of Chitin Content Using the Black and Schwartz Method.[1] To determine the amount of chitin in each shell type, the Black and Schwartz method was carried out. Exactly 2.000 g of ground and sieved shell was weighed into a 100 mL round-bottom flask equipped with a Teflon coated magnetic stir bar and a condenser and 48 mL of 1 M hydrochloric acid was added and the mixture was heated for 1 h at 90 ^{o}C in a thermocouple monitored oil bath under reflux with stirring via an IKA RCT basic hotplate (Wilmington, NC) at 250 rpm. After 1 h, the flask was removed from the oil bath and cooled to room temperature. The reaction mixture was transferred to centrifuge tubes and centrifuged at 3800 rpm for 10 min in a Thermo Scientific Sorvall Legend XTR TX-1000 centrifuge (Waltham, MA).

The supernatant was then carefully separated from the precipitate using a disposable plastic pipette. Subsequently, ca. 45 mL of fresh DI water was added, and the resultant suspension centrifuged. Centrifugation, aqueous phase decantation, and fresh DI water addition steps were consecutively repeated until the washings were no longer acidic (pH 7 determined by pH paper). The remaining solid was transferred to the original round-bottom flask and 48 mL of 1.25 M aqueous NaOH and the mixture heated under reflux with stirring for 1 h at 90 ^{o}C as noted in the acid step. After 1 h, the mixtures were removed from the oil bath, cooled to room temperature, transferred to centrifuge tubes, and centrifuged for 10 min at 3800 rpm. The supernatant was decanted and discarded, and the remaining solid was washed with addition of fresh DI water followed by centrifugation at 3800 rpm for 10 min. The solid was repeatedly washed and centrifuged with fresh DI water (8 more times) until the supernatant was neutral as shown by pH paper (pH 7). The supernatant was decanted and discarded, and the remaining solid was transferred onto a Petri dish and dried overnight at 80 ^{o}C in the oven. The final mass of the dried solids were used to calculate the percent chitin **Table S1**.

Table S1. Percent Chitin in Molts and Peels Determined by the Black and Schwartz Method

	ABW5-10	**ABW10-20**	**ABW30-40+**	**Peels**
Trial 1	18.9	19.8	22.6	29.1
Trial 2	19.0	19.4	22.1	28.9
Trial 3	18.3			28.9
Average	**18.7(4)**[a]	**19.6(3)**[a]	**22.4(4)**[a]	**29.1(2)**[a]

[a]The standard deviation for the listed average value is shown in parentheses.

Statistical Analysis of Chitin Content. Statistical calculations were carried using the Sigma Plot software (SYSTAT™, Erkrath, Germany).[2] Since only normally distributed data can be analyzed by analysis of variance (ANOVA), first, a normality test was used to test the null hypothesis that "the samples come from a normal distribution" against the alternative hypothesis "the samples do not come from a normal distribution." In the case of a normal distribution, a test of homogeneity of variances was used to test the null hypothesis that each group's variance was the same for each type of biomass. If the differences in the adjusted means among the treatment groups were found to be greater than would be expected by chance, a statistically significant difference ($P = <0.001$) between the groups is noted. Since ANOVA tests indicate whether there is an overall difference between the groups, but not which specific groups differed, we conducted a Holm-Sidak test in

order to compare the amount of chitin in the different molt and peel groups. The results are presented below.

A. Comparison Between Molts

- **Normality Test**: Test passed (P = 0.108).
- **Equal Variance Test** for the null hypothesis that two normal populations have the same variance: Test passed (P = 0.774).

Table S2. ANOVA Results for Comparison between Molts

Group Names	N	Missing	Mean[a]	Std Dev	SEM[b]
ABW5-10	3	0	18.733	0.379	0.219
ABW10-20	2	0	19.600	0.283	0.200
ABW30-40+	2	0	22.350	0.354	0.250
Source of Variation	**DF[c]**	**SS[d]**	**MS[e]**	**F[f]**	**P[g]**
Between Groups	2	16.177	8.088	65.804	<0.001
Residual	4	0.492	0.123		
Total	6	16.669			

[a]The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). Power of performed test with alpha = 0.050 - 1.000; [b]SEM - standard error of the mean; [c]DF – degrees of freedom; [d]SS - the sum of the squares of the deviations from the means (variation); [e]MS - within groups mean square; [f]F - test statistic; [g]P - probability of observing a result.

Table S3. All Pairwise Multiple Comparison Procedures (Holm-Sidak Test)

Comparison	Diff of Means[a]	t[b]	Unadjusted P[c]	Critical Level[d]	Significant
ABW30-40+ vs. ABW5-10	3.617	11.300	<0.001	0.017	Yes
ABW30-40+ vs. ABW10-20	2.750	7.844	0.001	0.025	Yes
ABW10-20 vs. ABW5-10	0.867	2.708	0.054	0.050	No

[a]Diff of Means – test differences between two means; [b]t – value of t-test that examines whether group means differ from one another; [c]unadjusted P - unadjusted P-value; [d]critical level - critical values for an ANOVA hypothesis. Overall significance level = 0.05.

B. Comparison Between all Molts and Peels

- **Normality Test:** Test passed (P = 0.313)
- **Equal Variance Test:** for the null hypothesis that two normal populations have the same variance: Test passed (P = 0.745)

Table S4. ANOVA Results for Comparison between Molts and Peels

Group Name	N	Missing	Mean[a]	Std Dev	SEM[b]
ABW5-10	3	0	18.733	0.379	0.219
ABW10-20	2	0	19.600	0.283	0.200
ABW30-40+	2	0	22.350	0.354	0.250
Peels	3	0	28.967	0.115	0.0667
Source of Variation	**DF[c]**	**SS[d]**	**MS[e]**	**F[f]**	**P[g]**
Between Groups	2	184.482	61.494	711.826	<0.001
Residual	4	0.518	0.0864		
Total	6	185.000			

[a]The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). Power of performed test with alpha = 0.050 - 1.000; [b]SEM - standard error of the mean; [c]DF – degrees of freedom; [d]SS - the sum of the squares of the deviations from the means (variation); [e]MS - within groups mean square; [f]F - test statistic; [g]P - probability of observing a result.

Table S5. All Pairwise Multiple Comparison Procedures (Holm-Sidak Test)

Comparison	Diff of Means[a]	t[b]	Unadjusted P[c]	Critical Level[d]	Significant
ABW30-40+ vs. ABW5-10	3.617	13.479	<0.001	0.017	Yes
ABW30-40+ vs. ABW10-20	2.750	9.356	<0.001	0.025	Yes
ABW10-20 vs. ABW5-10	0.867	3.230	0.018	0.050	Yes
Peels vs. ABW5-10	10.233	42.642	<0.001	0.009	Yes
Peels vs. ABW10-20	9.367	34.910	<0.001	0.010	Yes
Peels vs. ABW30-40+	6.617	24.660	<0.001	0.013	Yes

[a]Diff of Means – test differences between two means; [b]t – value of t-test that examines whether group means differ from one another; [c]unadjusted P - unadjusted P-value; [d]critical level - critical values for an ANOVA hypothesis. Overall significance level = 0.05.

Ionic Liquid Extraction of Chitin from Biomass. Two grams of shells were dissolved in 198 (molts) or 98 (peels) g of [C_2mim][OAc] and the mixtures were heated via domestic microwave (Sunbeam, 900 W, Model SGB8901) at full power for 1 min with 3 s pulses with vigorous swirling after three rounds, followed by 2 min with 3 s pulses with vigorous swirling after two rounds, following by an additional 3 min with 2 s pulses with vigorous swirling after every two rounds

Samples were centrifuged for 10 min at 3800 rpm in a Thermo Scientific Sorvall Legend XTR TX-1000 centrifuge (Waltham, MA) to remove calcium carbonate, and kept warm in an 80 ^{o}C Cole-Parmer StableTemp gravimetric convection oven, item number EE5241283 (Charleston, SC). The chitin was coagulated by adding the solution to 600 mL of DI water and the solid chitin was washed 14 times with 1 L of DI water each to remove any remaining IL. The chitin was then dried overnight in an 80 ^{o}C in the oven and weighed the next day (**Table S6**). The hardened chitin was then ground by hand with mortar and pestle and sieved with wire mesh stainless steel sieves to ≤ 250 μm diameter particles for subsequent dissolution.

Table S6. Percent Chitin Extracted

	Peels		**Molts**					
			ABW5-10		**ABW10-20**		**ABW30-40+**	
	% of Biomass	*% of Available Chitin*	*% Biomass*	*% of Available Chitin*	*% of Biomass*	*% of Available Chitin*	*% of Biomass*	*% of Available Chitin*
Trial 1	19.1	65.6	12.1	64.7	10.3	52.6	10.8	48.2
Trial 2	18.2	62.5	12.3	65.8	10.6	54.1	16.1	71.9
Trial 3	17.5	60.1	14.0	74.9	12.9	65.8	17.3	77.2
Trial 4	ND	ND	ND	ND	15.0	76.5	ND	ND
Trial 5	ND	ND	ND	ND	17.4	88.8	ND	ND
Average	**18.3(7)**[a]	**63(2)**[a]	**12.8(9)**[a]	**68(5)**[a]	**13(3)**[a]	**68(14)**[a]	**15(3)**[a]	**66(13)**[a]

[a]The standard deviation for the listed average value is shown in parentheses.

Powder X-Ray Diffraction (PXRD). Data were collected on a Bruker AXS, Inc. (Madison, WI) D2 Phaser Powder X-ray diffractometer. Approximately 20 mg of extracted chitin was ground using mortar and pestle (particle size < 250 μm) and placed on a low background, airtight specimen holder silicon wafer with high-index surface orientation without cavity (available from Bruker AXS, Catalog #: A100B36). Data were recorded in continuous mode at room temperature in Bragg-Brentano geometry using Cu-Kα radiation with instrument parameters set to 1.0 s per step with a total of 3748 steps (0.01°/s) over the range of 2-40° 2θ.

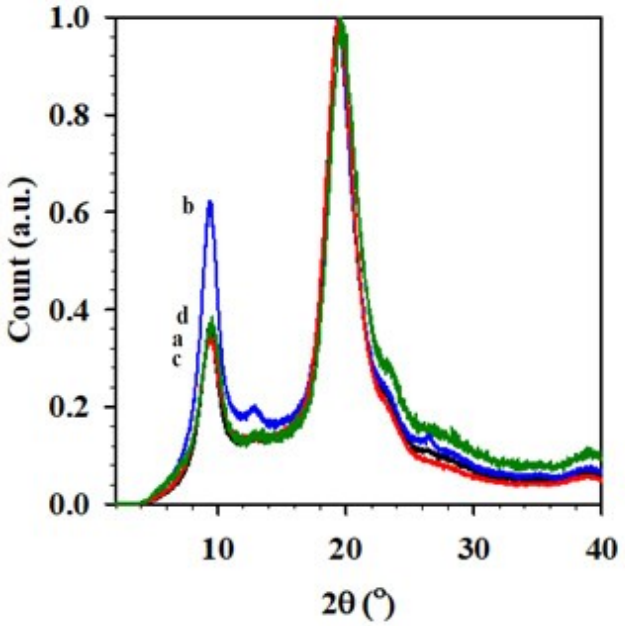

Figure S1. Powder X-ray diffractograms of chitin extracted from molts, ABW5-10 (black; a), ABW10-20 (blue; b), and ABW30-40+ (red; c), and Peels (green; d).

Fourier Transform-Infrared Spectroscopy (FT-IR). FT-IR spectra of chitin extracted from molts and peels were recorded uwsing a Bruker Alpha II FT-IR instrument, Bruker Scientific LLC (Billerica, MA, USA) equipped with a diamond crystal Attenuated Total Reflection (ATR) capability. All spectra were recorded as an average of 64 scans in the range of 400-4000 cm^{-1} (**Figures S2-6**).

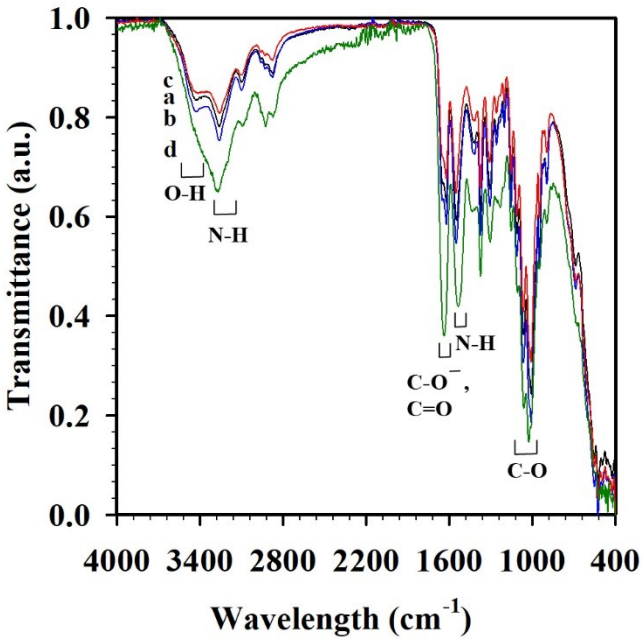

Figure S2. FT-IR spectra of chitin extracted from ABW5-10 (black; a), ABW10-20 (blue; b), and ABW30-40+ (red; c) molts and Peels (green; d).

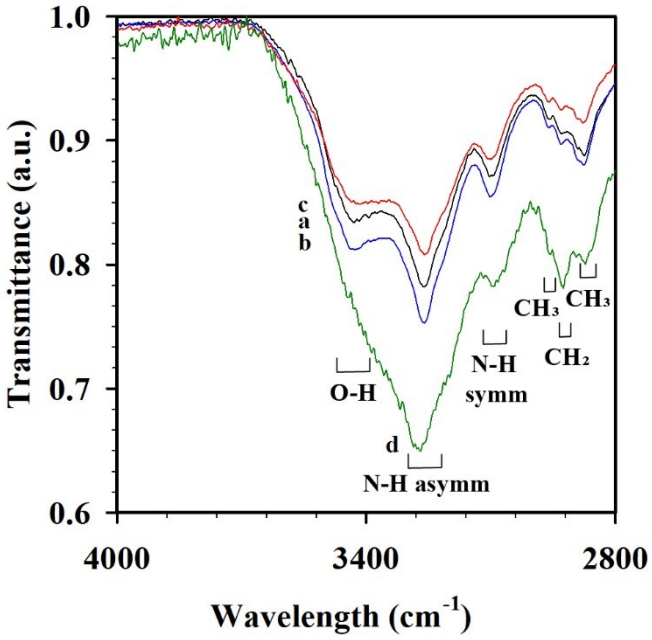

Figure S3. FT-IR spectra from 4000 to 2800 cm^{-1} of chitin extracted from ABW5-10 (black; a), ABW10-20 (blue; b), and ABW30-40+ (red; c) molts and Peels (green; d).

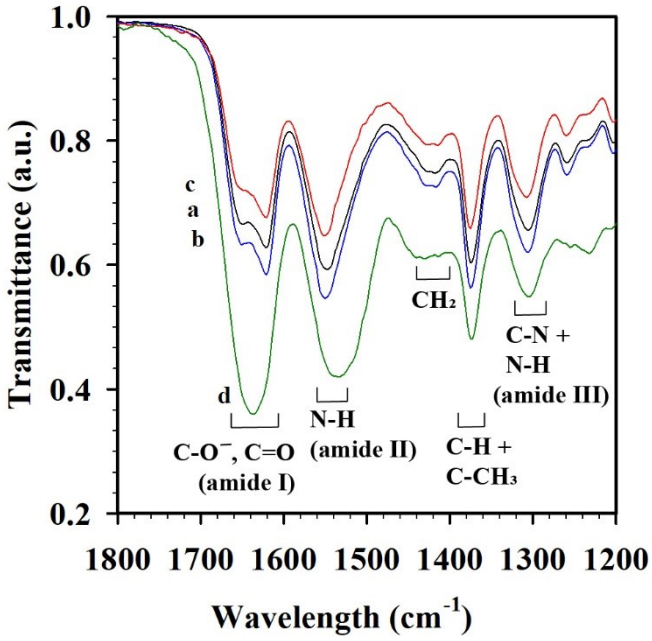

Figure S4. FT-IR spectra from 1800 to 1200 cm^{-1} of chitin extracted from ABW5-10 (black; a), ABW10-20 (blue; b), and ABW30-40+ (red; c) molts and Peels (green; d).

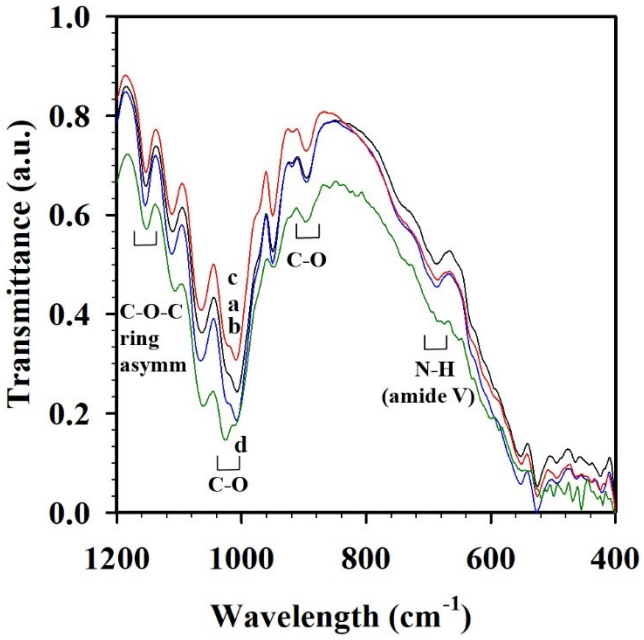

Figure S5. FT-IR spectra from 1200 to 400 cm^{-1} of chitin extracted from ABW5-10 (black; a), ABW10-20 (blue; b), and ABW30-40+ (red; c) molts and Peels (green; d).

Thermogravimetric Analysis (TGA). Thermal data were collected using a TGA 5500 (TA instruments, New Castle, DE, USA) with built-in microbalance, under N_2 and air flow. A sample of chitin extracted from ABW10-20 molts (0.911 mg) was placed into platinum pan and heated from room temperature to 75 ^oC at a rate of 2 ^oC/min, followed by a 30 min isotherm at 75 ^oC under N_2 flow. The heating was then continued to 100 ^oC at the same heating rate. At 100 ^oC the heating rate was increased to 5 ^oC/min and heating continued to 600 ^oC. At 600 ^oC, the heating rate was increased further to 10 ^oC/min, the purge gas switched to air, and heating continued to 1000 ^oC. The resulting data were analyzed using TRIOS Software 5.0.0.44616 provided by TA Instruments.[3]

The TGA (**Figure S6**) indicates a protein decomposition step with ~0.7% loss at T_{onset} = 178.0 ^oC. Chitin decomposition involved two steps, with $T_{1onset\ CH}$ = 306.8 ^oC; the fastest decomposition matched 50% decomposition which took place at $T_{1\ 50\%}$ = 332.9 ^oC. Transition into the second step occurred at 370.4 ^oC, with $T_{2\ 50\%}$ of 537.0 ^oC, and fastest decomposition taking place at 609.0 ^oC. There was <0.5 wt% ash.

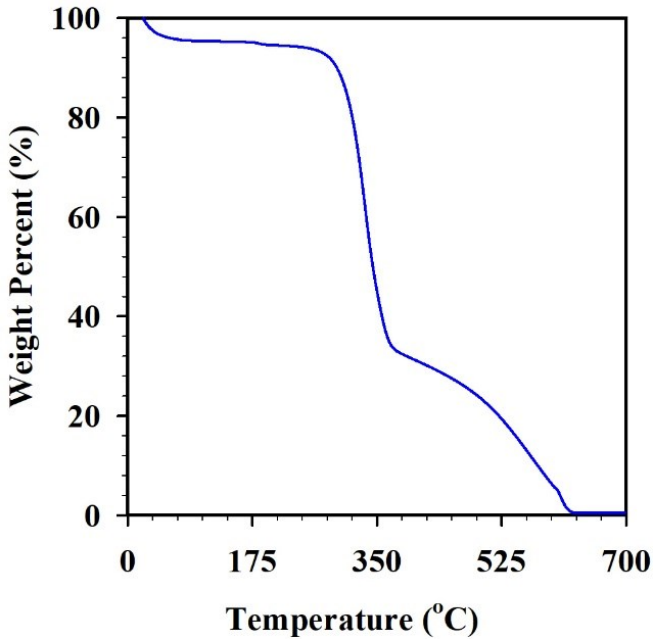

Figure S6 TGA analysis of chitin extracted from ABW10-20 molts.

Degree of Acetylation. The degree of acetylation of the extracted chitin was calculated using a method adopted from the literature.[4] For this, FT-IR spectra (64 scans) were recorded using a Bruker Alpha II FT-IR instrument (Bruker Scientific LLC, Billerica, MA, USA). Then, an 11-point Savitzky-Golay digital filter was applied to a set of digital data points for the purpose of smoothing the data and increasing the precision without distorting the signal intensity using Origin Software.[5] The first derivative of the resultant filtered FT-IR was found and the intensity of the following peaks, MB_1 (1383 cm^{-1}), MB_2 (1327 cm^{-1}), and RB (1163 cm^{-1}) were determined. The %DA was calculated using eq. S1:

$$\%DA = \frac{\frac{(MB1 + MB2)}{RB} - 0.487\%}{0.0157} \quad \textbf{(S1)}$$

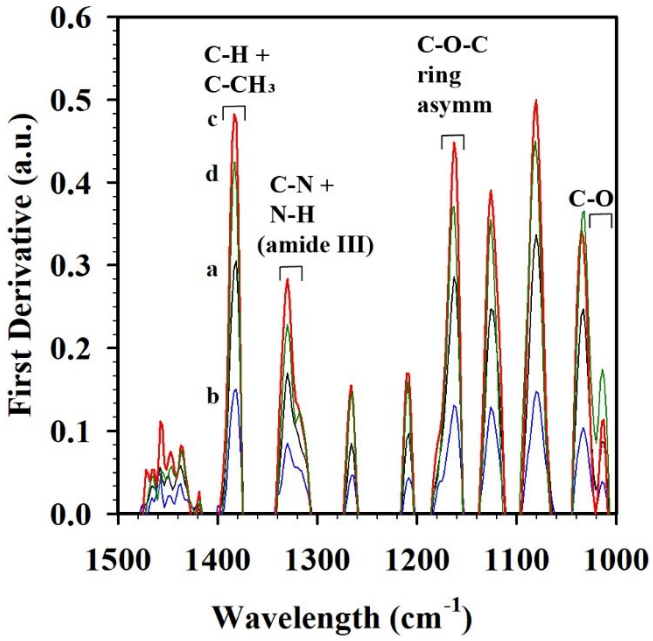

Figure S7. First Derivative of the FT-IR spectra in the region from 1500 to 1000 cm^{-1} of chitin extracted from ABW5-10 (black; a), ABW10-20 (blue; b), and ABW30-40+ (red; c) molts and Peels (green; d).

Table S7. Calculated Percent Deacetylation for Chitin Extracted from ABW5-10 Molts

	Trial	MB_1	MB_2	RB	calc DA (%)
	1	0.306	0.170	0.276	78.8
	2	0.587	0.340	0.543	77.7
	3	0.196	0.114	0.182	77.5
Average					78.0
St. Dev.					0.7

Table S8. Calculated Percent Deacetylation for Chitin Extracted from ABW10-20 Molts

	Trial	MB_1	MB_2	RB	calc DA
	1	0.150	0.085	0.129	85.0
	2	0.577	0.335	0.563	72.2
	3	0.342	0.201	0.325	75.4
Average					77.5
St. Dev.					6.7

Table S9. Calculated Percent Deacetylation for Chitin Extracted from ABW30-40+ Molts

	Trial	MB_1	MB_2	RB	calc DA
	1	0.475	0.284	0.432	80.9
	2	0.340	0.192	0.323	73.9
	3	0.417	0.221	0.378	76.5
Average					77.1
St. Dev.					3.5

Table S10. Calculated Percent Deacetylation for Chitin Extracted from Peels

	Trial	MB_1	MB_2	RB	calc DA
	1	0.425	0.228	0.371	81.1
	2	0.183	0.086	0.156	78.8
Average					80.0
St. Dev.					1.6

Calculation of Chitin Accumulation. Chitin content per molt can be calculated using eq. **S2**:

$$\frac{Chitin\ content}{molt\ cycle} = (Shrimp\ mass, g) * \left(wt\% \frac{molt}{total}\right) * (wt\% \frac{chitin}{total\ molt})$$

(**S2**)

We estimated the mass of the shrimp as a function of age in days using a previously published growth model of *Litopenaeus vannamei*[6] to develop eqs. **S3-S5**.

$$m = 0.007e^{0.102a}; \text{ when } 0 \leq m < 1 \quad (\mathbf{S3})$$

$$m = 0.156a - 7.609; \text{ when } 1 \leq m < 18 \quad (\mathbf{S4})$$

$$m = 0.091a + 1.911; \text{ when } 18 \leq m \leq 35.4 \quad (\mathbf{S5})$$

where m is total mass of shrimp (g), and a is shrimp age (days). A weight percent of molted exoskeleton in relation to the total mass of the shrimp can be approximated using data from another study,[7] in which the average mass of the total shrimp and the shell and tail of *Penaeus notalis* was measured, from which an average weight percent of 14.1% of the total shrimp mass is the shell and tail.

To estimate how often the shrimp molts, we turned to a published study of *Litopenaeus vannamei* over the molt cycle,[8] which indicates the average molt cycle length increases with increasing age in shrimp where a 30 day old shrimp has a molt cycle of 4.6 days/cycle, a 90 day old shrimp was 11.8 days/cycle, and a 180 day old shrimp was 17.2 days/cycle. Because the article does not provide a continuum, we created a plot of growth rate vs. age in days (see **Figure S8**) using these data, and found the equation that fits these data in order to calculate the molt cycle length.

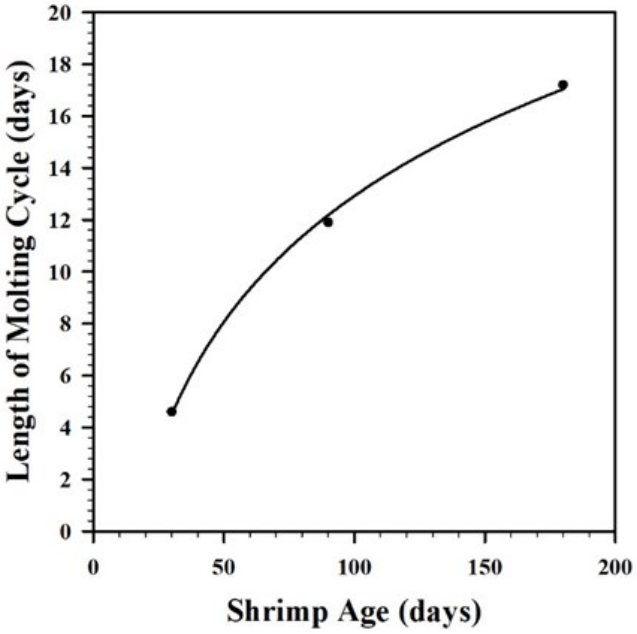

Figure S8. Change in the Length of Molt Cycle.

The length of the molt cycle for a particular shrimp age was estimated using eq. **S6**:

$$L = 6.9887\ln(a)-19.303 \quad \textbf{(S6)}$$

where L is the length of the molt cycle in days and a is the age of the shrimp in days.

Using an arbitrary starting assumption that the shrimp entered the raceway at 20 days old (and 0.05 g according to eq. **S4**), we calculated the length of the molt cycle and determined the number of molts per age/size range. Our calculations suggest 10 molts from 0.05-5 g, 3 from 5-10 g, 5 from 10-20 g, 6 from 20-30 g, and 3 additional molts assuming we arbitrarily end the lifecycle at 1 year. If we then conservatively assume we will start collecting molts after 80.8 days and an ABW of 5 g and harvest the shrimp after 1 year, we can calculate a cumulative chitin resource from molts and peel of 11.49 g of which we can extract and recover 7.58 g. A summary of the results for a one year old *Litopenaeus vannamei* shrimp in terms of number of molts and total obtained chitin is provided in **Table S11**.

Table S11. Total Chitin Amount

Number of Molt Cycles 0.05-5 g	10
Number of Molt Cycles 5-10 g	3
Number of Molt Cycles 10-20 g	5
Number of Molt Cycles 20-30 g	6
Number of Molt Cycles 30-40+ g	3
Total Number of Molt Cycles	**27**
Total Available Molt Chitin	10.08 g
Total IL Extracted Chitin from Molt[a]	**6.65 g**
Total Available Peel Chitin[b]	1.41 g
Total IL Extracted Chitin from Peel[a]	**0.93 g**
Total Available Molt and Peel Chitin	11.49
Total IL Extracted Chitin from Molt and Peel[a]	**7.58 g**

[a]Extractable chitin is calculated from total available chitin using an average experimental extraction yield of 66%; [b]Available peel chitin was calculated from a 12 month old shrimp using an experimentally determined chitin content of 28.9%.

MOLECULAR WEIGHT DETERMINATIONS

Chitin Dissolution and Consecutive Dilutions. A stock solution of chitin in [C_2mim][OAc] (concentration of ~20 mg/g solution) was prepared by weighing 15.00 g of IL into a 20 mL capacity screw top vial (VWR supplies, model number V2757C-FM-SP, catalog number 470146-668) using a microbalance (Secura 125-1S, Sartorius Lab Instruments GMBH, Goettingen, Germany). After weighing the IL, 300 mg of ≤ 250 μm diameter chitin particles were weighed directly into the same vial. Close attention was paid to ensure that no particles adhered to the walls of the vial, and the vial was closed tightly. The resulting mixture was heated via oil bath at 100 ^{o}C for 48 h while stirring with a Teflon coated stir bar on an IKA RCT basic hot plate (Wilmington, NC) to obtain complete dissolution. In the same oil bath at the same time, ~40 mL of IL was heated at 100 ^{o}C for 48 h while stirring in a closed 100 mL round bottom flask. This "heated IL" was used as the solvent to dilute the stock solution into different concentrations.

Consecutive dilutions ranging between 1-15 mg/g were made from each stock solution, with each dataset having 8-10 dilutions less than 6 mg/g occurring in ~0.4 mg/g increments (concentration datasets are shown in **Tables S12-14**). An initial mass of heated IL was weighed into a new 20 mL screw top vial, followed by a known amount of 80 ^{o}C stock solution ensuring

none of the liquid adhered to the sides. Between measurements, the stock solution was stored at 80 °C in a Cole-Parmer StableTemp gravimetric convection oven (Charleston, SC) to decrease the high viscosity of the solution for easier pipette transfer during mass measurement. Once diluted, Teflon coated stir bars were inserted into the vials and the solutions were stirred on an IKA RCT basic hot plate (Wilmington, NC) for 1 h without heating and subsequently degassed by vacuum for 1 h before any instrument measurements were made.

Table S12. Sample preparation of Chitin Extracted from ABW5-10 Molts by Serial Dilution[a]

Sample	Mass of Stock Solution (g)	Mass of Heated IL (g)	Concentration of Chitin in Solution (g/g)
1	0.11865	2.01262	0.00109
2	0.14879	2.00587	0.00136
3	0.20658	2.00251	0.00184
4	0.26714	2.00889	0.00231
5	0.54522	3.01082	0.00301
6	0.49403	2.00361	0.00389
7	0.59022	1.99685	0.00448
8	1.11928	3.01404	0.00532
9	1.35535	2.99213	0.00613
10	0.93849	2.00600	0.00626
11	1.16669	2.00993	0.00722
12	0.55469	0.75831	0.00830
13	1.03249	0.99137	0.01002
14	1.59075	0.49565	0.01498

[a]Stock concentration: 0.019651 g/g (prepared by dissolution of 0.299 g chitin in 14.900 g IL).

Table S13. Sample preparation of Chitin Extracted from ABW10-20 Molts by Serial Dilution[a]

Sample	Mass of Stock Solution (g)	Mass of Heated IL (g)	Concentration of Chitin in Solution (g/g)
1	0.10706	2.00839	0.000979
2	0.15360	2.00088	0.001380
3[c]	0.19356[c]	2.00431[c]	0.001704[c]
4	0.3058	2.00444	0.002562
5	0.62582	3.00657	0.003334
6[c]	0.52828[c]	1.99576[c]	0.004051[c]
7	0.58844	2.00485	0.004391
8	1.09344	3.01028	0.005157
9	1.18399	3.00604	0.005469
10	0.88997	1.99969	0.005960
11	1.21601	1.99324	0.007333
12	0.74425	0.99891	0.008263
13	1.01913	1.00043	0.009766
14[d]	1.33452[d]	0.76240[d]	0.012317[d]
15	1.47918	0.50169	0.014452

[a]Stock concentration: 0.019353 g/g (prepared by dissolution of 0.29602 g chitin in 15.00006 g IL); [b]Excluded from SLS measurements due to significant color difference; [c]Excluded from refractive index measurements due to significant color difference.

Table S14. Sample preparation of Chitin Extracted from ABW30-40+ Molts by Serial Dilution[a]

Sample	Mass of Stock Solution (g)	Mass of Heated IL (g)	Concentration of Chitin in Solution (g/g)
1	0.14144	3.01772	0.000877
2	0.26166	3.00789	0.001568
3	0.37954	2.99529	0.002203
4	0.50104	3.01823	0.002789
5	0.62226	3.01025	0.003356
6	0.74450	2.99302	0.003902
7	0.88062	3.00471	0.004440
8	0.97028	2.99903	0.004789
9	1.22392	2.99211	0.005687
10	1.67060	3.01390	0.006986
11	1.85033	3.00408	0.007467
12	1.07774	1.01908	0.010069
13	2.76771	1.00376	0.014376

[a]Stock concentration: 0.019590 g/g (prepared by dissolution of 0.39949 g chitin in 19.99287 g IL).

Measurement of Refractive Index Increment dn/dc. The refractive index of chitin solutions was measured at 23 ^{o}C using a Refractometer Abbemat 500 (Anton Paar, Ashland, USA). The sample cell was first rinsed with DI water and acetone, then dried and calibrated using only air. Solutions measured were ensured to be equal in volume and then inserted onto the optical portion of the sample cell to cover it completely during the measurement. The samples were allowed to equilibrate with the set temperature (23 ^{o}C) of the refractometer before taking measurements repeatedly in live mode over an hour until the change in refractive index per minute was consistently under 0.0000008 nD/min. The refractive indices were plotted as a function of concentration to determine the refractive index increment (dn/dc; the slope of the linear best fit) as shown in **Figures S9-11**.

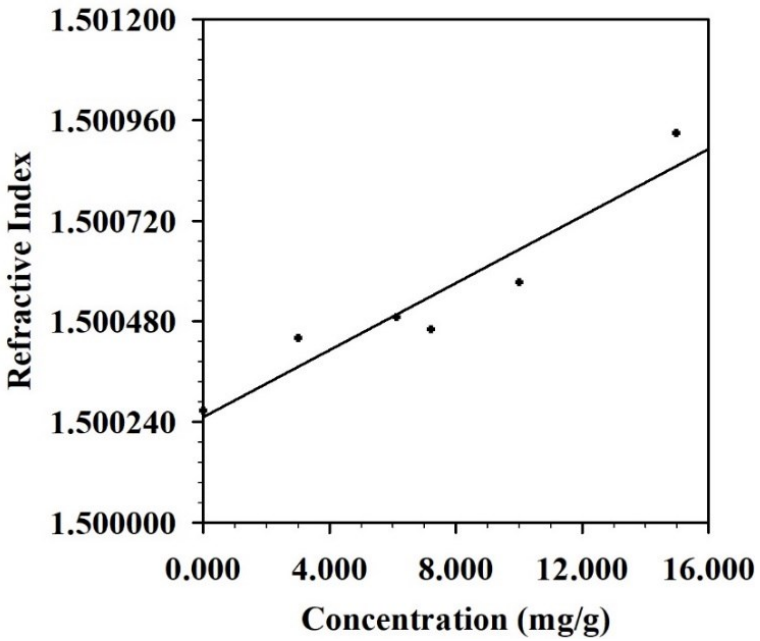

Figure S9. Plot of dn/dc of solutions of chitin extracted from ABW5-10 molts (y = 3.99E-05x + 1.5003; R^2 = 0.9041).

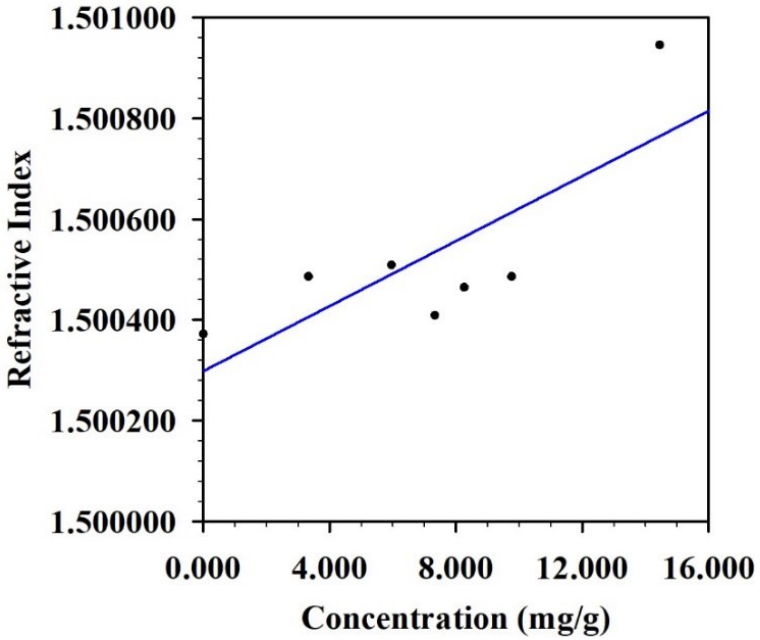

Figure S10. Plot of dn/dc of solutions of chitin extracted from ABW10-20 molts (y = 3.23E-05x + 1.5003; R^2 = 0.6056).

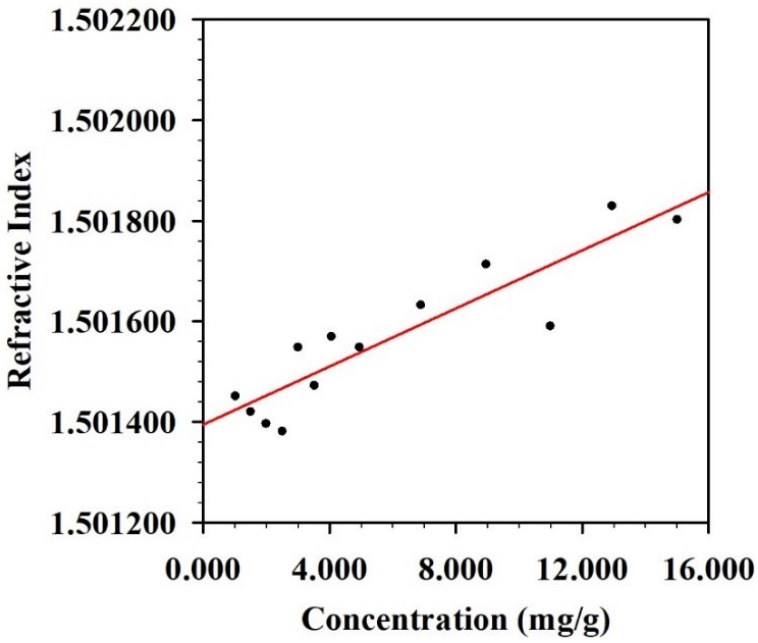

Figure S11. Plot of dn/dc of solutions of chitin extracted from ABW30-40+ molts (y = 2.89E-05x + 1.5014; R^2 = 0.8288).

Measurement of Viscosity. Solutions of chitin samples, all in [C_2mim][OAc], were prepared at 1.0, 1.5, and 2.0 wt% loading by dissolving the chitin in 3 g of [C_2mim][OAc]. The mixtures were heated at 90 °C in an oil bath with magnetic stirring until the solutions appeared clear (12-48 h). The exact procedure for viscosity sample preparation is as follows: chitin was ground to <125 μm, and a standard amount of 3.0 g ionic liquid was used with the necessary mass of chitin for each weight percent. After preparing these solutions, they were placed in an oil bath at 90 °C with stirring for 48 h. Approximately 2 mL of the biopolymer solutions were placed in the sample chamber of a Cambridge Viscosity Viscometer, VISCOlab 3000 (Medford, MA). A corresponding piston (depending on the viscosity value) was selected to obtain the measurement. The viscosity was recorded at 25 °C. The software enables sample measurement multiple times until the standard deviation is less than 0.2% before displaying a viscosity value. Duplicates were recorded for each sample and average values calculated.

Method 2. The viscosity of each chitin dilution (via heated IL solvent) was measured using a PAC Cambridge ViscoLab 4000 (Houston, TX). Approximately 1 mL of prepared solution was transferred into the sample chamber by pipette, and an appropriate piston (depending on the solution viscosity) was inserted. The viscosity measurement of each solution was recorded exactly

when the temperature increased from 24.9 to 25.0 °C. This procedure was repeated with at least 4 total dilutions until a full dataset of solution viscosity was obtained.

Calculation of Relative Viscosity. Relative viscosity was calculated using eq. **S7**:

$$\eta_{rel} = \frac{\eta_s}{\eta_o} \quad \textbf{(S7)}$$

where η_{rel} is the relative viscosity, η_s is the viscosity of the solution, and η_o is the viscosity of the solvent, [C_2mim][OAc], found to be 148 cP (measured by Method 1, above). The results are provided in **Table S15** and **Figure S12**.

Table S15. Results of Viscosity Measurements at 25 °C

Shell Type	1.0 wt% chitin (cP)	Relative Viscosity[a]	1.5 wt% chitin (cP)	2.0 wt% chitin (cP)
ABW5-10	743	5.0	-	-
ABW10-20	810	5.7	1420	2500
ABW30-40+	1693	11.4	-	-
Peels	1350	9.1	1800	8790

[a]Relative viscosity of 1 wt% [C_2mim][OAc] solution, with respect to pure [C_2mim][OAc] (148 cP, measured by the same protocol as other samples).

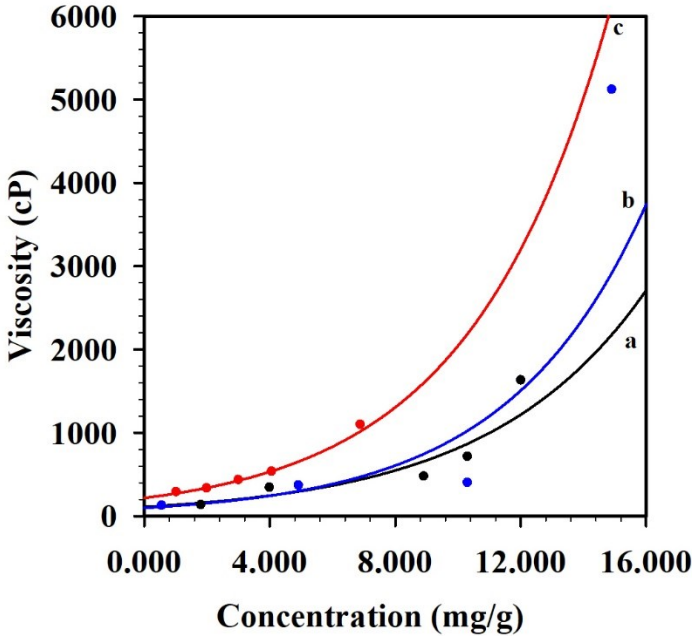

Figure S12. Comparison of chitin solution viscosity vs. concentration of chitin extracted from ABW5-10 (black; a), ABW10-20 (blue; b), and ABW30-40+ (red; c) molts.

Static Light Scattering. The molecular weight of chitin was determined using a method we recently developed,[9] based on a series of measurements from a Zetasizer Nano ZS (Malvern Instruments, Westborough, MA, USA). Measurements were taken at a fixed position (4.65 mm) with a He-Ne laser wavelength of 633 nm and a scattering detection of 173°. The attenuator was set to 8, and measurements were taken using glass cuvettes PCS1115. [C_2mim][OAc] with a viscosity of 135.2 cP and a refractive index of 1.502 was used as the dispersant. The standard operating procedure (SOP) was programmed to prompt users to enter the desired temperature (23 °C), as well as the previously measured viscosity before SLS measurements were taken. For 120 s the sample equilibrated at a specific temperature (23 °C) before being measured 10 times, with each measurement being an average of 10 runs of 10 s each with 10 s delays between each measurement.

Calculation of M_W using the Rayleigh Equation. Using the optical constant K (calculated from the measured dn/dc value), concentration c, and the Rayleigh ratio R_θ (the relationship between the Rayleigh scattering of toluene and IL), the Rayleigh equation can be presented graphically as a Debye plot, in which the y-intercept is the inverse of M_W and the slope is the second virial coefficient A_2.[9] A positive value of A_2 indicates that solute particles are more attracted to the

solvent than other solute particles and therefore the solutions have greater stability, while a negative value of A_2 indicates that solute particles are more attracted to other solute particles than the solvent, suggesting that the solutions may be unstable or undissolved.

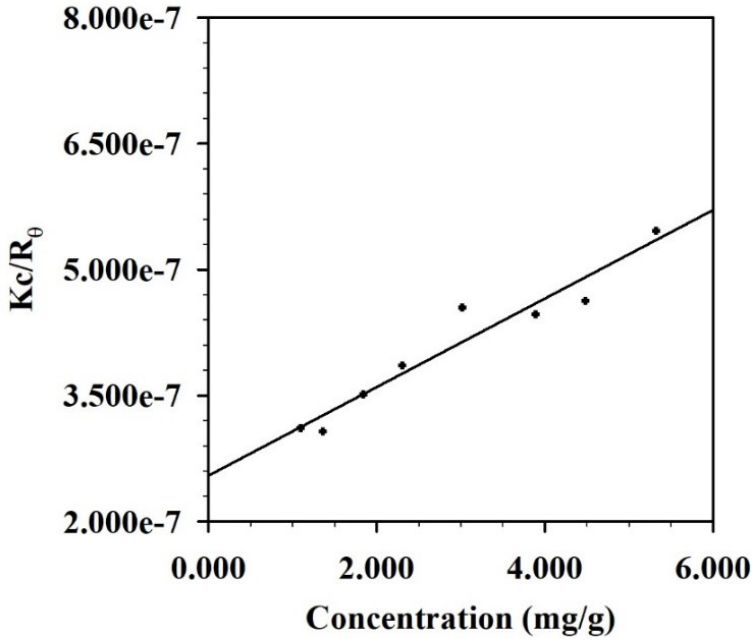

Figure S13. Debye plot of solutions of chitin extracted from ABW5-10 molts (y = 5E-08x + 3E-07; R^2 = 0.9350).

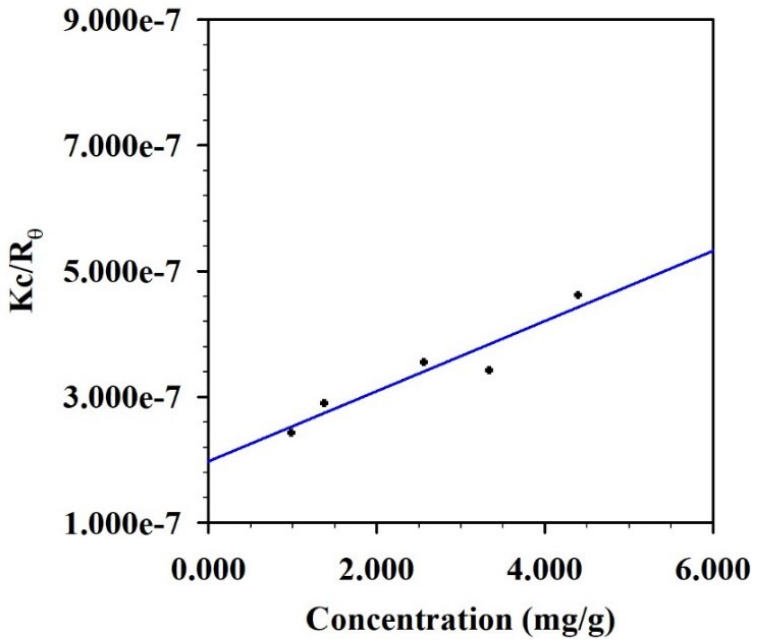

Figure S14. Debye plot of solutions of chitin extracted from ABW10-20 molts (y = 6E-08x + 2E-07; R^2 = 0.9029).

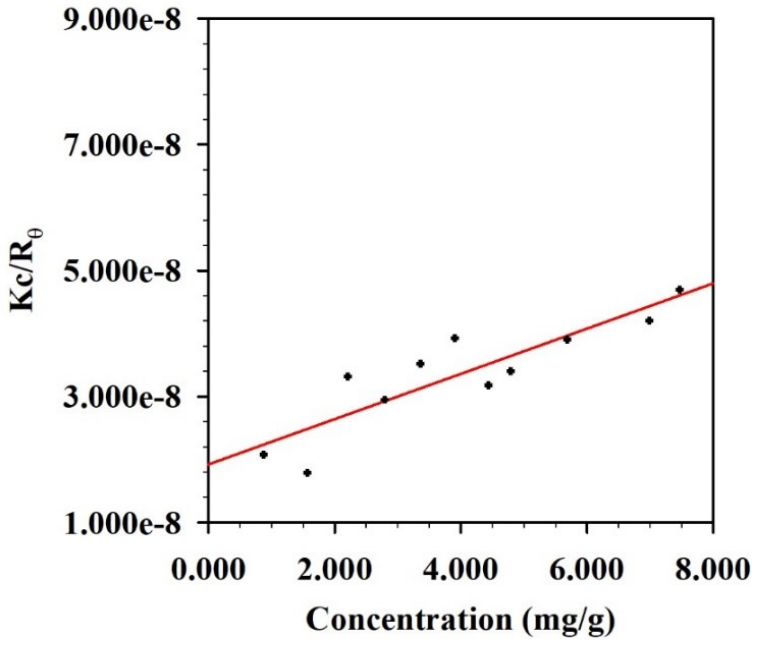

Figure S15. Debye plot of solutions of chitin extracted from ABW30-40+ molts (y = 4E-09x + 2E-08; R^2 = 0.7831).

Table S16. Summary of Preliminary Results

Approximate Age	Extraction Solution[a] (wt%)	Extraction Time[b] (min)	Centrifugation Time[c] (min)	Chitin Yield from Biomass (wt%)	dn/dc	M_W (MDa)
Small (ABW < 15 g)	1	6	20	17.9	0.066	1.7[d]
					0.058	2.1[d]
Small (ABW < 15 g)	1	6	20	16.3	0.066	2.0
Small (ABW < 15 g)	1	5	15	17.4 ± 0.3	0.045	7.1
Medium (ABW > 20 g)	1	6	20	16.5	0.035	7.4
Adult (ABW 10 -15 g)	1	5	15	18.0 ± 1.2	0.023	13.7[e]
					0.027	11.4[e]
Adult (ABW > 40 g)	1	5	15	15.0 ± 0.4	0.034	16.3

[a]Biomass was ground and sieved to a particle size of < 250 μm prior to extraction; [b]Length of microwave-assisted extraction with [C_2mim][OAc]; [c]Centrifugation was performed at a solution temperature of 75 °C and 3000 rpm, with 2 cycles of the length of time listed; [d]M_W was calculated twice from one SLS measurement using different experimentally measured dn/dc values; [e]Both dn/dc and SLS measurements were performed twice.

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