Journal of *Marine Science and Engineering*

MDPI

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Diet and Temperature Effects on the Survival of Larval Red Deep-Sea Crabs, *Chaceon quinquedens* (Smith, 1879), under Laboratory Conditions

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Abstract: Declines in commercial crustacean species (such as lobsters, king crab, etc.) have caused an increased interest in the harvest of the red deep-sea crab *Chaceon quinquedens*. The red deep-sea crab is a federally managed fishery; however, little is known about the species' general biology, especially the conditions required for larval survival. We aimed to answer two main questions about the life history of the red deep-sea crab. First, is there a common larval hatching pattern between adult female crabs? Specifically, our inquiries are about the duration of the hatching process, daily peak hatching time, and the relationship between female morphometry and the total larvae hatched. Second, which are the factors affecting the survival and development of larval red deep-sea crabs? In order to answer these research questions, we studied the effects of diet (rotifers, *Artemia* sp., algae, and unfed), temperature (9 °C, 15 °C, and 20 °C), and aquaculture settings. Ovigerous females were obtained from commercial traps and transported to the NOAA James J. Howard Laboratory, NJ. They were placed in the Females Husbandry and Hatching Collection System (FHCS), where the larvae hatched. Hatching of adult females was monitored and measured by volume. A simple linear regression (SLR) was calculated to predict the number of larvae hatched based on the measured volumes, and it was significant (F = 1196; df = 1, 13; R^2 = 0.9892, p = 3.498 × 10^{-14}). Duration of hatching period showed an approximate 30 days for adult females red deep-sea crabs, with a common daily maximum hatching time at 22:00 hrs (hatching time seem to follow the sun cycle and the first hours after sunset, Perez, pers. observation). Linear polynomial quadratic regressions were conducted for both years with an interaction term for the two continuous variables (diet and temperature), and were used to model the proportion of larval survival through time. In both years, a highly significant difference was obtained (F = 56.15; df = 4, 2134; R^2 = 0.09353; p = < 2.2 × 10^{-16}). There is an effect of diet and temperature in the survival of red deep-sea crabs, but not a combined effect of them.

Keywords: crab fisheries; red deep-sea crab; larval development; rearing systems; aquaculture; crab feeding

Citation: Pérez-Pérez, N.M.; Poach, M.; Stevens, B.; Smith, S.L.; Ozbay, G. Diet and Temperature Effects on the Survival of Larval Red Deep-Sea Crabs, *Chaceon quinquedens* (Smith, 1879), under Laboratory Conditions. *J. Mar. Sci. Eng.* **2023**, *11*, 1064. https://doi.org/10.3390/jmse11051064

Academic Editor: Ka Hou Chu

Received: 17 January 2023
Revised: 2 May 2023
Accepted: 3 May 2023
Published: 17 May 2023

1. Introduction

The red deep-sea crab, *Chaceon quinquedens* [1] is a large brachyuran crab from the Geryonidae family that can be found along the continental shelf and slope of the western Atlantic, from Nova Scotia, Canada to the Gulf of Mexico, at depths between 200 and 1800 m [2–4]. Red deep-sea crabs (RDSCs) have been economically and commercially important since the 1970s, when a small fishery was established in New England and the Mid-Atlantic states, which has persisted as the oldest fishery of geryonid crabs in the

Atlantic [5]. In US waters of the Atlantic Ocean, the RDSC is managed as a single stock with an undetermined minimum legal size, maximum sustainable yield, and overfishing limit due to a lack of critical biological information. However, a fishery management plan (FMP) was established in 2002 by the New England Fishery Management Council (NEFMC) mainly focusing on quota restrictions and fishing effort controls because of the limited biological knowledge available for RDSCs [6] (A total allowable catch was only established in 2002 [7], when the most recent total allowable landing was determined to be 1775 t for the male-only directed fishery [8]. On average, the red crab fishery in the Mid-Atlantic Bight catches about 32 t of live crabs per trip, year-round, in water depths >600 m.

Recently, knowledge of embryonic development and fecundity as well as the physiological and sexual maturity of female RDSCs, which is vital to improving management strategies, has been published [9–11]. Physiological and behavioral sexual maturity of red deep-sea crabs were determined using morphological features and ovary and oocyte development to estimate female size at 50% sexual maturity [11]. Physiological size at 50% sexual maturity varied geographically and was estimated to be 61.2 mm carapace length in the Hudson Canyon and 70.8 mm in the Baltimore/Norfolk canyons. Behavioral size at 50% sexual maturity decreased with latitude and was estimated to be 53.9 mm, 62.5 mm, and 65.5 mm carapace length in the Hudson, Baltimore, and Norfolk canyons, respectively [11]. Similarly, Martínez-Rivera and Stevens [10] described the stages and seasonality of red deep-sea crab embryonic development and determined size-specific fecundity. A positive correlation between fecundity and female body size as carapace length was determined via linear regression. The number of eggs per brood ranged from 324,729 to 34,691 for females ranging in size from 106.2 mm to 62.6 mm carapace length [10]. While understanding the reproductive output of female *C. quinquedens* is critical to fisheries' management, we must also understand the development of the early life stages of larval red deep-sea crabs. As a commercially harvested species, it is also important to understand the conditions necessary for the survival, growth, and development of target species' early life stages.

C. quinquedens has a classic Brachyuran cold-water crab life cycle. Brood incubation, zoeal, and larval periods are longer than those for similar species from warm water environments [12]. Larvae usually hatch in spring/summer in shallower waters (200–1800 m) in comparison to their adult counterparts [4]. Larvae are planktonic and develop through six stages: pre-zoea, four zoeal stages, and one megalops stage before juvenile settlement near the mid-slope. This developmental cycle can be completed in 26 days under temperatures ranging from 18 to 21 °C, accelerating development in laboratory conditions where average stage durations were 7 d from zoeal I to zoeal II, 6 d from II to III, 5 d from III to IV, 7 d from IV to megalopa, and finally 14 d from megalopa to first crab [13].

Previous larval development studies under laboratory conditions focused on other red deep-sea crab species, *Chaceon granulatus* [14] and *Geryon trispinosus* [15] and were restricted to descriptions of larval development and included no assessment on rearing system, diet, or temperature.

Both temperature and diet affect larval survival and development of planktonic crab species such as *C. quinquedens* [16–22]. Larval red deep-sea crabs were reared between 12 and 26 °C [18]. In the coldest temperature treatment (12 °C), developmental abnormalities occurred. In addition, larvae survival to the first zoeal stage was higher at warmer temperatures [18]. The temperature range for optimal larval red deep-sea crab growth was determined to be between 9 °C and 15 °C, as temperatures at 6 °C slowed down development [20]. In addition to temperature effects on larval RDSC development, a lack of suitable prey in sensitive early life cycle phases of Brachyuran crabs can cause death due to starvation even if feeding later resumes [23]. However, comparisons of larval development and survival between RDSCs (*Geryon quinquedens*) and a marine shallow water crab (*Menippe mercenaria*) may show some indication that RDSC has some larval nutritional flexibility to survive in a deep-sea environment [19], since there was no difference in the survival of *G. quinquedens* megalop stage between the rotifer and brine shrimp diets. How-

ever, there was evidence of a developmental delay in the larvae reared strictly on the rotifer diet. In contrast, the shallow water crab, *M. mercenaria*, did not survive to the megalop stage under the rotifer diet [19].

Despite a multi-decade fishery for the red deep-sea crab, little is known about the optimal growing conditions for larval crab stages. Additionally, the effects of the interactions between diet and temperature on larval stages of RDSCs have not been previously assessed. There is interest in the potential viability of the RDSC as a candidate for commercial aquaculture; however, that requires cost-effective high survivability rearing methods similar to those mentioned for red king crabs [24]. These methods also require an understanding of the optimal RDSC larval development conditions, such as the interactions between temperature and diet as well as culture method. Here, we present two options for rearing larval red deep-sea crabs and describe the effects of diet and temperature on larval development for *C. quinquedens*.

Our objectives are twofold: (i) to determine the optimal conditions for peak survival and development of deep-sea red crab larvae under laboratory conditions that provide a healthy population, and (ii) to study the factors affecting the survival of the early life stages of the red deep-sea crab in aquaculture settings, such as temperature and diet.

2. Materials and Methods

2.1. Crab Collection and Husbandry

Red deep-sea crab (RDSC) ovigerous females were collected by the Atlantic Red Crab Company from sites within Site 3—south of the RDSC distribution in the USA Western Atlantic (Figure 1—MAP; NEFMC 2014).

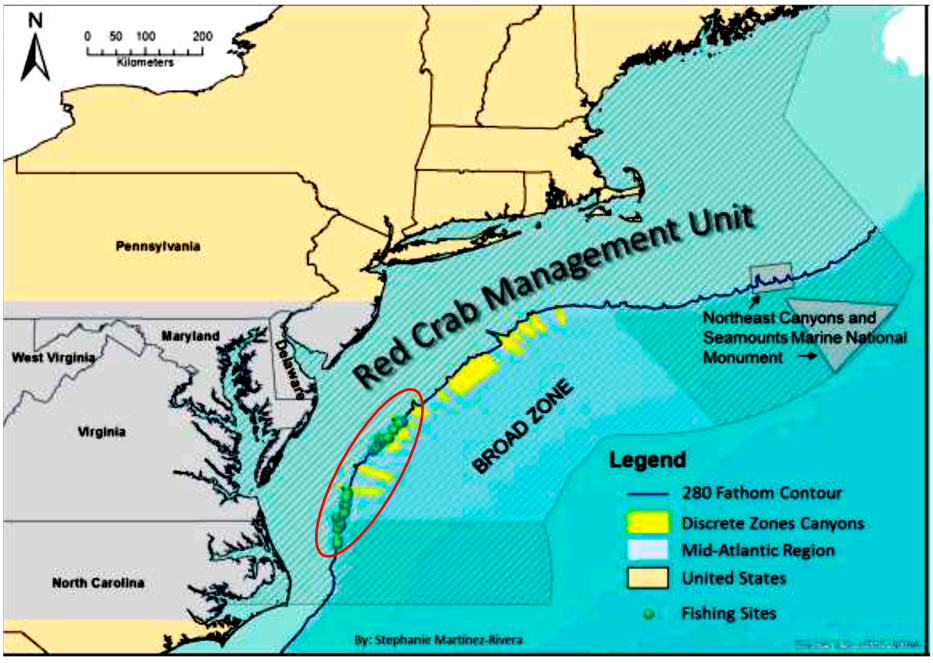

Figure 1. Designated and used fishing/sampling sites at Newport News by the Atlantic Red Crab Company. Map by Stephanie Martínez-Rivera. The fishing locations are the ones marked with the green dots on the map shown in red oval, provisionally.

The fertilized females were transported in a car, using a cooler with ice packs and wet towels, to the NOAA James J. Howard Sandy Hook lab, New Jersey. Alive females were then measured, examined (number of legs, shell condition), and randomly placed in the Female Husbandry and Hatching Collection System (FHCS). The FHCS was designed to maintain live RDSC adult females as well as allow for the ability to collect, count, measure, and transfer hatchlings. The FHCS is a flow-through system (1.2 L/10 min

adjustable) with chilled seawater (6.9–11.3 °C, salinity 27–30‰) obtained from the main seawater supply of the NOAA James J. Howard Marine Sciences Laboratory at Sandy Hook, New Jersey. Chilled seawater was UV sterilized and filtered with a combination of a 25 µm and two 5 µm or 50 µm, 20 µm, and 5 µm polypropylene cotton filters (Millipore Sigma, Burlington, MA, USA) depending on seawater sedimentation before entering the individual female buckets. Twelve buckets of 15 L each were distributed and placed within two 210 cm × 60 cm rectangular tanks. Buckets had the capacity to hold one living female red deep-sea crab. Each bucket was equipped with an individual air stone, a water inflow hose, and an outflow extended to the exterior of the bucket. The water exiting through the outflow was directed to a filter mesh placed over a collection cup. This collected the hatched larvae for enumeration. The room containing the system was maintained with a 48 lx (light intensity (luminous flow per unit of area) from 8:30 a.m. (day) to 8:30 p.m. (night) with a 30 min transition to each (sunrise and sunset). One week was used as the acclimation period where handling of animals was minimal. After the acclimation period, adult female crabs were fed every 3 days with defrosted and cut herring fillets to maintain the FHCS and keep hatching containers clean.

During the acclimation period, lost eggs and deposited larvae because of handling were discarded from the collectors. Post-acclimation period, each mesh filter collector was removed and replaced with a clean cup unless no larvae were observed in the filter. All larvae and eggs collected in the collector were transferred with a squirt bottle containing UV-filtered seawater and a funnel to a graduated cylinder (25 mL or 50 mL) until they were settled at the bottom.

Eggs were observed using a dissecting microscope to determine their developmental stage (i.e., eyed, not eyed). Hatched larvae were collected every 24 h, when possible, from all females with advanced eggs. Once eggs were released in larger volumes within a 24 h period, larvae were harvested at 4 h intervals (200, 600, 1000, 1400, 1800, and 2200 h) for 3 consecutive days. The contents of each housing bucket were emptied at each 4 h interval through the larvae collector. If larvae hatching volume decreased substantially after the 3 days, larvae were harvested every 12 h until either (1) hatching volume increased as such that 4 h intervals resumed or (2) hatching volume decreased to less than 1 mL, at which point collection frequency went back to every 24 h. Similar patterns have been shown in other crabs [25] that showed higher hatching at dusk.

2.2. Experimental Rearing System Setups

Two different experiment setups were used, LSFI and LSFII, during the red crab larvae feeding experiments. The experimental setup, LSFI (Figures 2 and 3), consisted of a flow-through system (inflow 12 L/5.1 h) that used filtered seawater (salinity 27‰) from the main seawater supply of the NOAA James J. Howard Marine Sciences Laboratory at Sandy Hook, New Jersey.

During the first experiment, water pumped from Sandy Hook Bay passed through a cyclonic filter followed by sock filters, and finally through sand filtration to a reservoir. From there, the filtered water was then run through a UV sterilizer (Lifegard, Pentair) and two 0.35 µm cartridge filters. To raise the pH to more open ocean conditions, dissolved CO_2 was removed by passing the water through two Liqui-Cel® membrane contactors (Membrana-Charlotte, Charlotte, NC, USA) that were run in vacuum mode and with CO_2 free air to assist with CO_2 stripping. Filtered, sterilized, and CO_2-stripped water then proceeded to a head tank, which was bubbled with CO_2 free air to ensure against hypoxia before being gravity fed to the inflow line. pH of the inflow water was measured periodically during the first experiment, and it ranged from 7.88 to 8.11 on the total pH scale. Alkalinity of the incoming water was calculated based on pH and dissolved inorganic carbon using CO_2 SYS (ORNL/CDIAC-105) [27,28]. Calculated alkalinity ranged from 2137 to 2228 µM/kg of seawater. Salinity of incoming water was about 32 ppt. Inflowing seawater was UV sterilized and treated through a filtration system of chemically resistant pleated polyester filtration 100% cellulose-free cartridges (5 µm, 20 µm, and

50 µm) and then distributed into the experimental buckets. Two tanks containing 10 buckets each (19 L) with lids were used to act as chillers, keeping each set of 10 buckets at 9 °C ± 1 °C and 15 °C ± 1 °C, respectively. These temperatures were selected because they are considered the best range of temperatures for rapid juvenile RDSC growth [19]. Each bucket held 3 smaller containers (750 mL) with a capacity to hold 10 living red deep-sea crab larvae (density 10 larvae/750 mL). Larvae containers were designed to allow the flow of diets through, but not the escape of larvae. Three circular holes (5.5 cm in diameter) were cut, then covered with 150 µm or 250 µm nitex screen mesh (6.8 cm in diameter) depending on the cell size of selected diet. Each temperature treatment had 2 buckets randomly assigned per diet treatment, for a total of 60 larvae per diet type. The light cycle within the tank room was 12 day/12 night hours.

Figure 2. (**A**) Diagram of the red deep-sea crab adult female hatching system. (**B**) Picture of the same hatching system used during the study. Diagram by Dr. Jeffrey Pessutti and picture taken by Tanya Breen in 2014 [26].

Figure 3. The LFSI 2014 system had one tank (230 × 60 cm) for each temperature (9 °C and 15 °C), ten buckets in each tank, and three small containers within each bucket in each tank. Each of the three diets were randomly assigned to the buckets and each small container randomly labeled 1 through 3. Each small container held 10 larvae, for a total of 60 larvae per diet per temperature tank.

The LSFII system was used in a second experiment which had custom made upwellers composed of a clear conical hatcher (capacity 2.4 L) (Pentair Aquatic Eco-Systems®, Apopka, FL, USA), a top extension with lid, a base reducer of 4-3 inchesPVC (10.16–7.62 cm), a 1 $\frac{1}{2}$ inches PVC (3.81 cm) outflow pipe, and a rectangular aperture covered with a 250 µm mesh (Figure 4). The total capacity of the upweller units with top extensions was 3.15 L, where the fitted conical hatcher could hold 20 living RDSC larvae. Filtered seawater recirculated into the system from the bottom of the upwellers with a flow of 0.25 L/min, through the inflow reservoir 100 and 25 µm mesh, then pumped into the conical hatchers. Two individual circular temperature tanks with chillers were set at 15 °C ± 1 °C and 20 °C ± 1 °C, where each tank contained 12 upwellers. These temperature treatments were selected based on the reported ranges for rapid larvae/juvenile development as well as recent red deep-sea crab surveys, where a temperature of 14.2 °C was recorded at 135 m depth [29]. Each tank had 2 upwellers randomly assigned to a diet treatment, for a total of 60 larvae per diet type. Again, light cycle within the tank room was 12 day/12 night hours. During the second experiment, the water was prepared using distilled water that was enriched and mixed with a commercial sea salt mixture, Instant Ocean Sea Salt for Aquariums, containing every major, minor, and trace element necessary while allowing pH to remain above 8. The sea salt solution we used was meant to mimic natural seawater conditions during the second experiment with consistent water quality conditions. The systems' water circulations were different: LSFI was flow-through and LSFII was recirculating.

2.3. Larval Crab Feeding Experiments

Both LFSI and LFSII rearing systems were allowed to run for several days prior to the start of the larvae feeding experiments. The mesh collector cups from the FHCS that contained larvae were placed into a cup containing cold UV filtered seawater, so larvae would be suspended and could swim. Actively swimming larvae were collected with a plastic pipette and transferred to small beakers of cold filtered seawater in groups of 10. Each beaker was randomly assigned to both temperature and diet treatments in both the

LFSI and LFSII systems. Algal diet culture cultivation and maintenance can be found in supplementary material 1 of the Master's Thesis of Perez-Perez [26]. Red deep-sea crab larvae were fed diets of rotifers (*Brachionus plicatilis)*, *Artemia* spp., algae (*Tisochrysis lutea*, T-Iso), and a mixed combination diet once every morning (24 h cycle) to determine which diet promoted red deep-sea crab larvae growth and development (Table 1). *B. plicatilis* cultures were started using a live culture obtained from Reed Mariculture Inc. (Campbell, CA, USA). Similarly, *Artemia* cysts were purchased from Inve Aquaculture (Salt Lake City, UT, USA). Finally, algal cultures of *Tisochrysis lutea* (T-Iso) were obtained from NEFSC Milford Laboratory (Milford, CT, USA). The LFSI diet experiment included an unfed control treatment (U), a rotifer-only diet (R), a rotifer and *Artemia* diet (A + R), an Artemia-only diet (A), and finally an *Artemia* and *Tisochrysis lutea* (A + AL) diet treatment (Table 1).

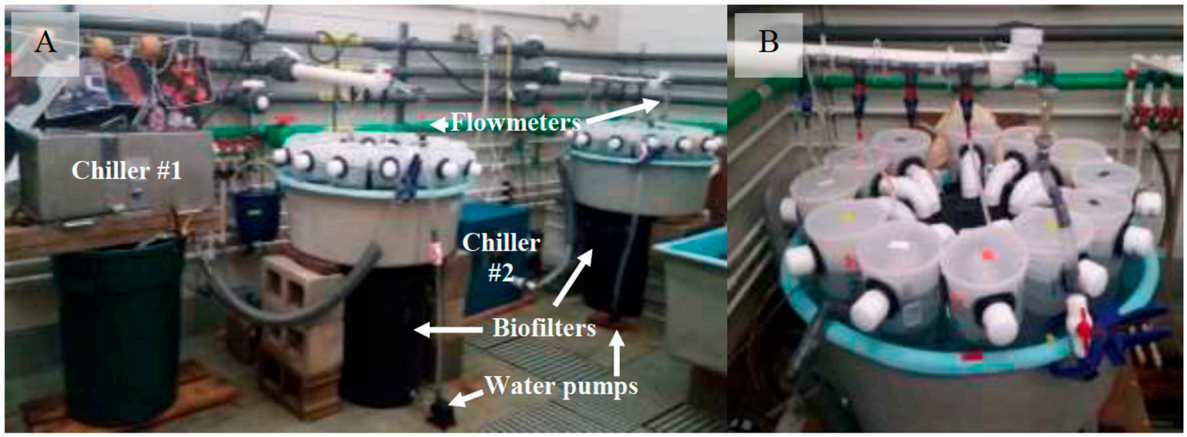

Figure 4. The LFSII 2016 system had (**A**) two circular tanks, one for each temperature (15 °C and 20 °C) and (**B**) 12 custom made upwellers in each tank. Each of the four diets were randomly assigned to the upwellers and each upweller held 20 larvae, for a total of 60 larvae per diet per temperature tank.

Table 1. Diet treatment combinations for both LFSI and LFSII (2014 and 2016, respectively) with the number of larvae per temperature treatment as well as the number of female broods used to obtain larvae. For 2014, there were two diet setups: the first one is above the line, but the A, A + R, and A + AL diets were not successful, so the diets were changed to the ones shown below the line with double the amount of R and AL. * Unfed (U), Rotifers *Brachionus plicatilis* (R), *Artemia* spp. (A), and algae *Tisochrysis lutea* (AL).

Experiment	* Diet	Temperature	Number of Larvae	Number of Broods
2014 LFSI Flow-Through	U	9 °C, 15 °C	60	1
	R	9 °C, 15 °C	60	1
	A	9 °C, 15 °C	60	1
	A + R	9 °C, 15 °C	60	1
	A + AL	9 °C, 15 °C	60	1
	U	9 °C, 15 °C	60	1
	R	9 °C, 15 °C	120	1
	AL	9 °C, 15 °C	120	1
2016 LFSII Recirculating	U	15 °C, 20 °C	60	7
	R	15 °C, 20 °C	60	7
	A	15 °C, 20 °C	60	7
	A + R	15 °C, 20 °C	60	7

In 2016, the LFSII experiment included an unfed control (C), a rotifer-only diet (R), an *Artemia*-only diet (A), and an Artemia and rotifer diet (A + R) (Table 1). Initial treatments

that included *Artemia* spp. nauplii > 48 h old were too large for red deep-sea crab larvae predation and contributed to their death after 48 h. Therefore, additional diet experiments were used which included unfed (U), a rotifer (R), and *T. lutea* (AL) groups (Table 1). Diet concentrations varied per diet type and treatment based on larval density (Table 2). Survival rates, as the percentage of larvae alive at a specific time, were recorded daily as well as zoeal stage at death for development over time.

Table 2. Diet concentrations and volumes fed to the larvae per container ranges for both larval rearing experiments. For 2014, there were two diet setups: the first one is above the line, but the A, A + R, and A + AL diets were not successful, so the diets were changed to the ones shown below the line with double the amount of R and AL.* U—Unfed; R—Rotifers; A—Artemia; A + R—Artemia + Rotifers; A + AL—Artemia + Algae. ** Substituted by an additional rotifer and two algae treatments. Final diet treatments for 2014 are shown below.

Experiment	* Diet	Concentration (Number of Diet/mL)	Amount Fed per Container (mL)
2014 LFSI Flow-Through	U	0	0
	R	5–3	40–7
	** A	5	20
	** A/R	2.5/5	20/range from 40 to 25
	** A/AL	2.5/10,000,000	20/range from 50 to 20
	U	0	0
	R	range from 5 to 3	range from 40 to 7
	AL	10,000,000	range from 30 to 10
2016 LFSII Recirculating	U	0	0
	R	range from 7 to 4	range from 18 to 2
	A	range from 0.7 to 0.5	range from 7 to 3
	A/R	range from 0.4 to 0.3/from 3.5 to 2.5	range from 4 to 1.5/from 9 to 1

2.4. Statistical Analysis

Survival analysis was calculated as the number of surviving larvae within a given treatment weighted by the number of deaths. To assess the effects of diet, temperature, and zoeal stage on the survival of the red deep-sea crab a linear mixed effects approach was used. Daily recorded larvae mortality was analyzed using linear mixed effects models (LME) from the 'lme' package [30] within the R statistical framework (R-4.2.2)). Survival time in days was standardized using the 'scale' command within the R statistical framework to convert days into standard deviations from the mean. Linear mixed effects models are an extension of simple linear models to allow both fixed and random effects, and are particularly used when there is non-independence in the data, such as arises from a hierarchical structure, in our case larval survival within each treatment group and across the treatments.

To compare survival differences between LFSI and LFSII systems, only the 15 °C unfed and rotifer treatments from both systems were used. Survival time in days was converted to standard deviations from the mean, and One-Way Analysis of Variance (ANOVA) was conducted using diet (unfed or rotifers) and larval rearing system (LFSI or LFSII) as factors, as well as the interaction between them. All ANOVA statistical analysis was conducted within the R statistical framework (R-4.2.2). A Kruskal–Wallis Test was used to evaluate differences in larval survival within each treatment tank (A–B) and container (I-III) in Larvae Feeding Experimental System 2014 (LFS I). Kruskal–Wallis Test was also used to evaluate differences within each treatment tank's upwellers (A–C) in Larvae Feeding Experimental System 2016 (LFS II).

In order to obtain the hatched estimation from measure volumes, a simple linear regression with a set intercept on cero was used to describe the relationship between number of larvae and volume and obtained the line formula for estimation. Then, their homogeneity of variances was tested using the Levene's test, histograms, and a QQ plot.

Levene's test with center = mean was used. Previous Monte Carlo studies indicated that using the mean provided the best power when the underlying data followed a symmetric or moderate-tailed distribution [31].

3. Results

3.1. RDSC Larval Survival

Daily larval survival was calculated as the number of larvae surviving within a given treatment weighted by the number of deaths. In the LFSI system, larvae fed rotifers at both 9 and 15 °C survived the longest, with 20% survival around the 30 day mark (Figure 5). Both the unfed and algae-fed treatments at 9 °C had larvae survive until roughly the 25 day mark, whereas the larvae in the unfed and algae-fed treatments at 15 °C all expired around 20 days (Figure 6). In the LFSII system, larval survival in nearly all diet treatments responded similarly, except for the *Artemia* spp. at 15 °C fed larval RDSC, which had higher overall survival between days 15 and 18 (Figure 6). Overall, RDSC larvae survived longer in the LFSI system at cooler temperatures, reaching 20% survival at 30 days, whereas 20% survival was hit at the ~17 day mark in the LFSII system. The effects of diet, temperature, and their interactions on survival differed between larval rearing systems and are shown in statistical outcomes (Tables 3 and 4).

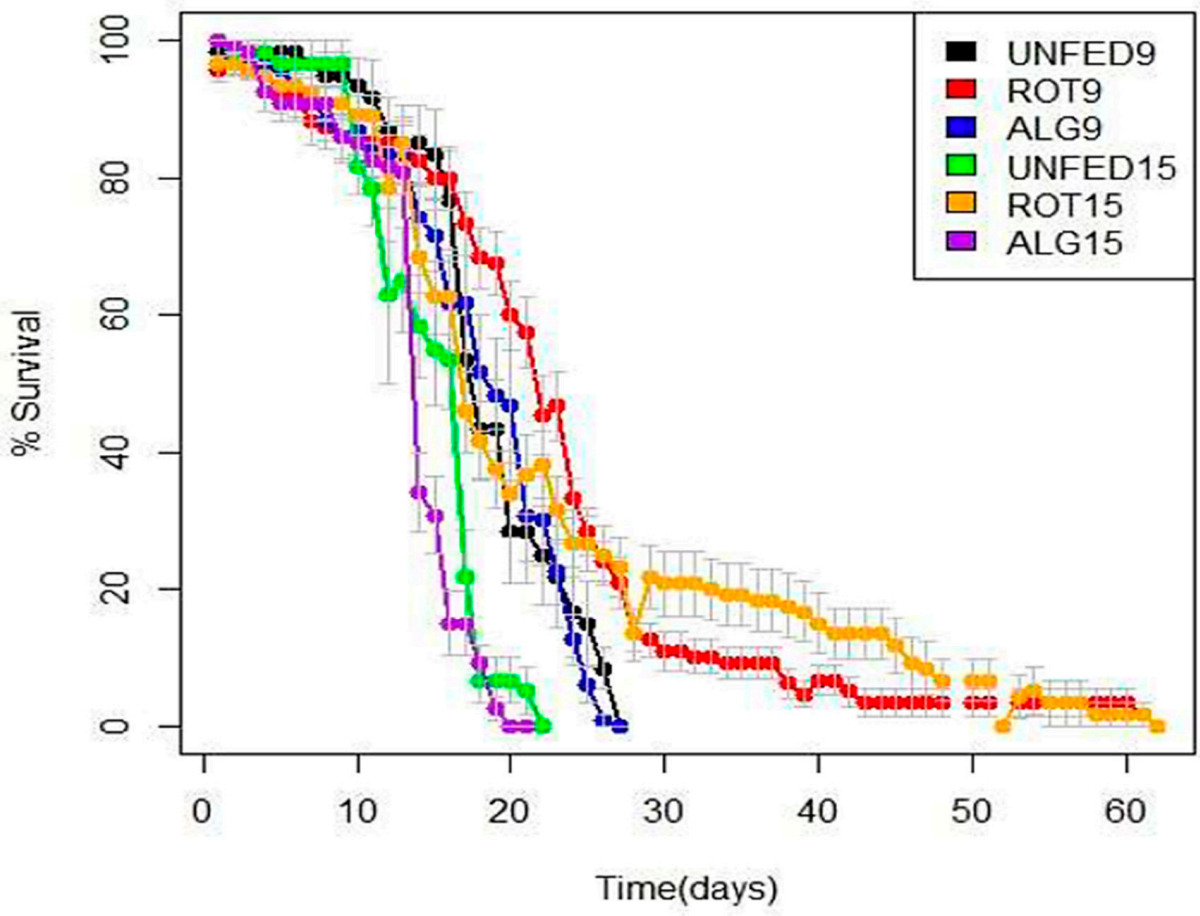

Figure 5. RDSC larval percent survival over time for all diet/temperature treatment types in the LFSI system.

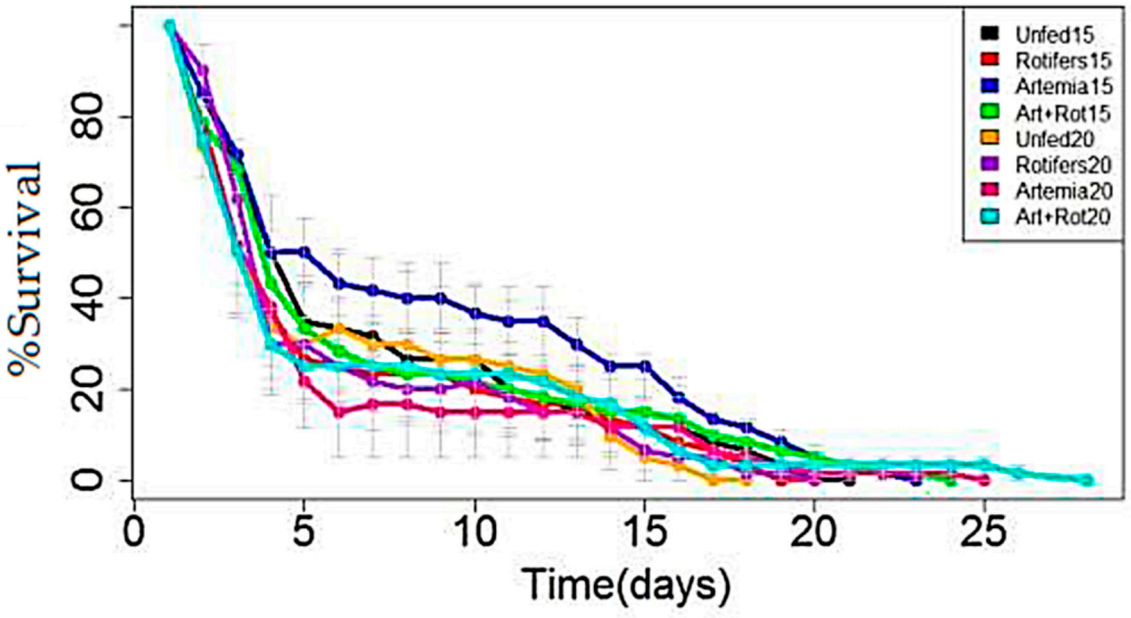

Figure 6. RDSC larval percent survival over time for all diet/temperature treatment types in the LFSII system.

Table 3. Kruskal–Wallis Test to evaluate differences in larval survival within each treatment tank (A–B) and container (I–III) in Larvae Feeding Experimental System 2014 (LFS I).

Treatment		*F*	df	*p*
Unfed9	Containers	5.5949	2	0.06097
	Tanks	1.8747	1	0.1709
Unfed15	Containers	3.3506	2	0.1873
	Tanks	0.0047866	1	0.9448
Rotifers9	Containers	3.0138	2	0.2216
	Tanks	6.9637	1	* 0.008318
Rotifers15	Containers	25.956	2	* 2.31×10^{-06}
	Tanks	3.8738	1	* 0.04905
Algae9	Containers	5.5421	2	0.0626
	Tanks	1.0977	1	0.2948
Algae15	Containers	6.5451	2	* 0.03791
	Tanks	0.061819	1	0.8036

* Statistically significant: $p \leq 0.05$.

Differences in proportion of survival between diet and temperature groups (Unfed9, Unfed15, Rot9, Rot15, Algae9, and Algae15) were found to be statistically significant using a Kruskal–Wallis Test (Kruskal–Wallis chi-squared: $H = 189.13$; df = 5, 2139; $p = < 2.2 \times 10^{-16}$). A polynomial quadratic regression with an interaction term for the two continuous variables (diet and temperature) was used to model the proportion of larval survival. A highly statistically significant model was obtained ($F = 56.15$; df = 4, 2134; $R^2 = 0.09353$; $p = < 2.2 \times 10^{-16}$).

Differences in survival between diet and temperature groups (Unfed15, Unfed20, Rotifers15, Rotifers20, *Artemia* sp. 15, *Artemia* sp. 20, Art + Rot15, and Art + Rot20) were found to be statistically significant using a Kruskal–Wallis Test (Kruskal–Wallis chi-squared: $H = 22.89$; df = 7, 534; $p = 0.001781$).

Table 4. Kruskal–Wallis Test to evaluate differences within each treatment tank's upwellers (A–C) in Larvae Feeding Experimental System 2016 (LFS II).

Treatment	*F*	df	*p*
Unfed15	3.2634	2	0.1956
Unfed20	13.975	2	* 0.00092
Rotifers15	10.563	2	* 0.00509
Rotifers20	9.0347	2	* 0.01092
Artemia15	2.5972	2	0.2729
Artemia20	16.506	2	* 0.00026
Art + Rot15	17.881	2	* 0.00013
Art + Rot20	1.5134	2	0.4692

* Statistically significant: $p \leq 0.05$.

A polynomial quadratic regression with an interaction term for the two continuous variables (diet and temperature) was used to model the proportion of larvae that survived. A statistically significant model was obtained ($F = 2.531$; df = 4, 529; $R^2 = 0.01136$; $p = 0.03966$).

A Kruskal–Wallis Test was used to evaluate differences within each treatment tank (A–B) and container (I–III) (LFS I), and they were found to be statistically insignificant in 2014, but within the upwellers (A–C) (LFS II), they were found to be statistically significant for most diets in 2016.

3.2. Larval Development

Levene's test for homogeneity of variances was found to be violated for hatch by group = FemaleID (Levene's test (center = mean): $F = 5.5647$; df = 29, 304; $p = 1.322 \times 10^{-15}$) and group = Day (Levene's test (center = mean): $F = 4.5528$; df = 52, 281; $p = < 2.2 \times 10^{-16}$). QQ plot and histogram showed a skewed distribution. Owing to these results, an *F* statistic not assuming homogeneity of variance was calculated.

In the LFSI system, larvae did not develop past the zoeal I stage in the unfed 9 °C and algae 15 °C treatments (Figure 7). Development to the zoeal II stage occurred in the unfed 15 °C, algae 9 °C, and rotifer 9 and 15 °C treatments (Figure 7). Zoeal stage III development only occurred in both rotifer treatments, and development to stage IV only occurred in the 15 °C rotifer treatment (Figure 7). In the LFSII system, all larvae died in the zoeal I stage in both the 15 and 20 °C unfed and rotifer treatments (Figure 8). Larval progression to zoeal stage II and IV was seen in the *Artemia* spp. 20 °C treatment and only progressed to stage II in the 15 °C treatment (Figure 8). Finally, the combination *Artemia* spp./rotifer diet in the LFSII system showed larval development to the second and third zoeal stage at both 15 and 20 °C (Figure 8). The effects of diet, temperature, and their interactions on zoeal development differed between larval rearing systems and are shown in Figures 7 and 8.

3.3. Comparison of Survival between the LFSI and LFSII Systems

A comparison between both larval systems was made using only the 15 °C unfed and rotifer treatments in each system (Figure 9). ANOVA results showed significance of the interaction between both diet and rearing system at 15 °C ($F = 73.6$; df = 3, 296; $R^2 = 0.4215$; $p \leq 0.0001$). Larval survival at 15 °C was different between the two rearing systems: Larvae Feeding Experimental System 2014 (LFSI) and Larvae Feeding Experimental System 2016 (LFSII).

For LFS I, daily recorded larvae survival by diet and temperature was tested for homogeneity of variance using a Levene test, histograms, and QQ plots. Homogeneity of variance was found to be violated by the Levene's test for live larvae proportion by group= Group (Levene's test (center = median): $F = 3.3769$; df = 5, 2133; $p = 0.004834$). Owing to this violated assumption, an *F* statistic not assuming homogeneity of variance

was used. A histogram of larvae survival proportion showed a typical bimodal distribution of the data, and the histogram of larvae survival proportion by days visually presents how larvae died through time. The QQ plot showed a binomial distribution.

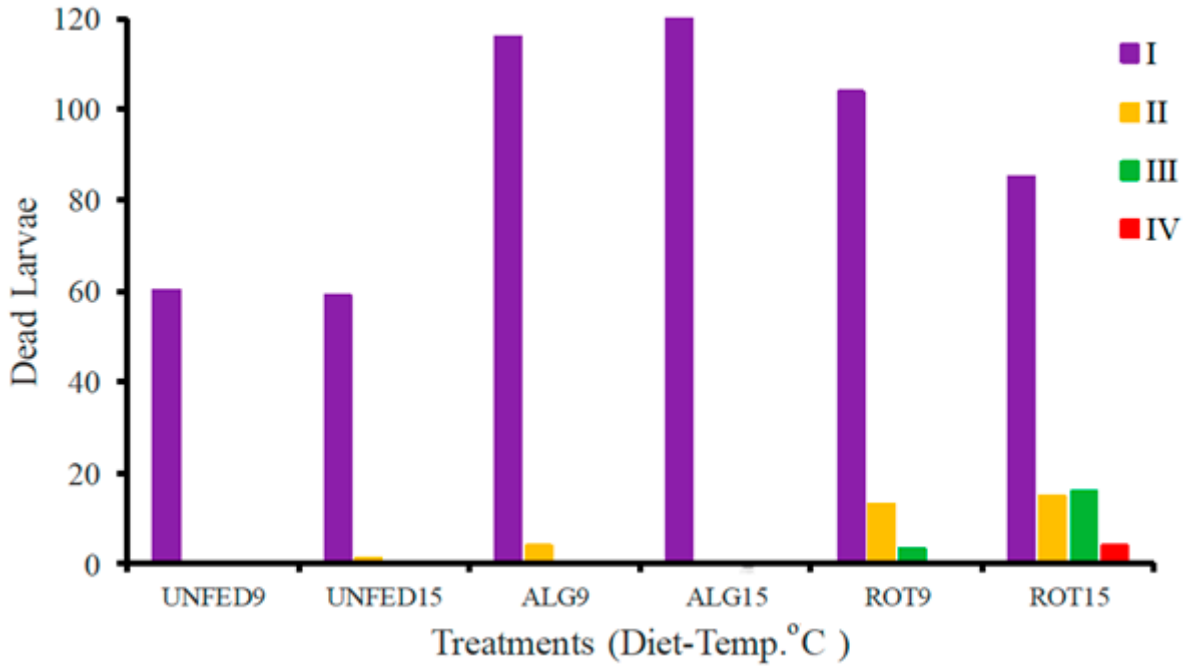

Figure 7. Larval development stage at death per each treatment in the LFSI system. Data are presented as mean values.

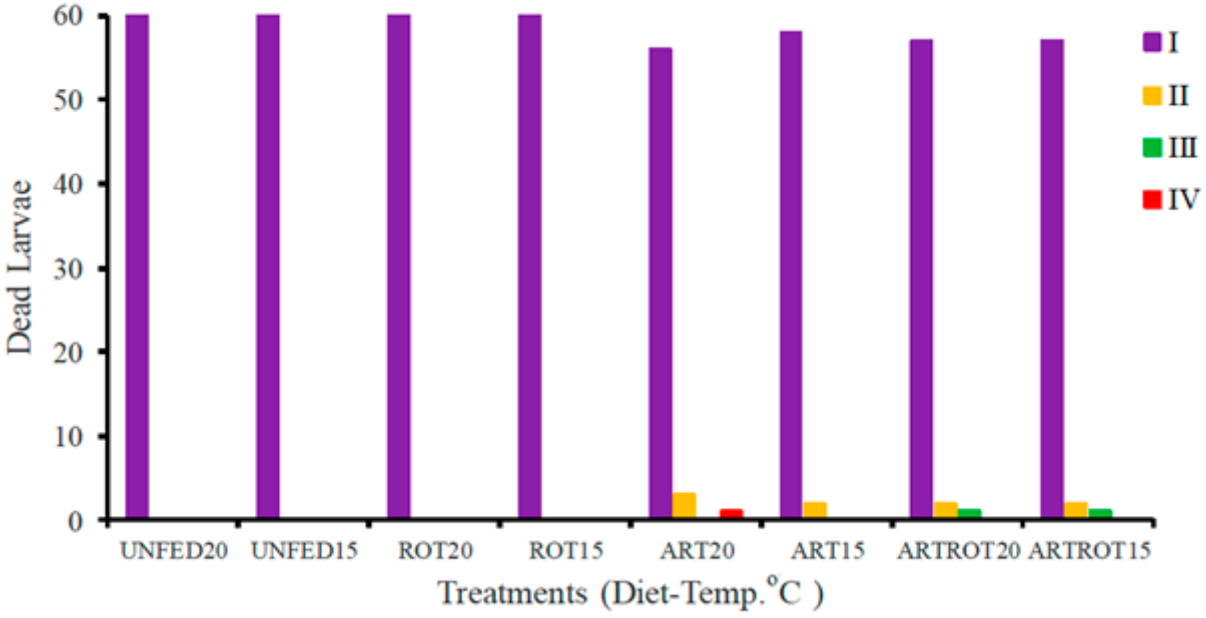

Figure 8. Larval development stage at death per each treatment in the LFSII system. Data are presented as mean values.

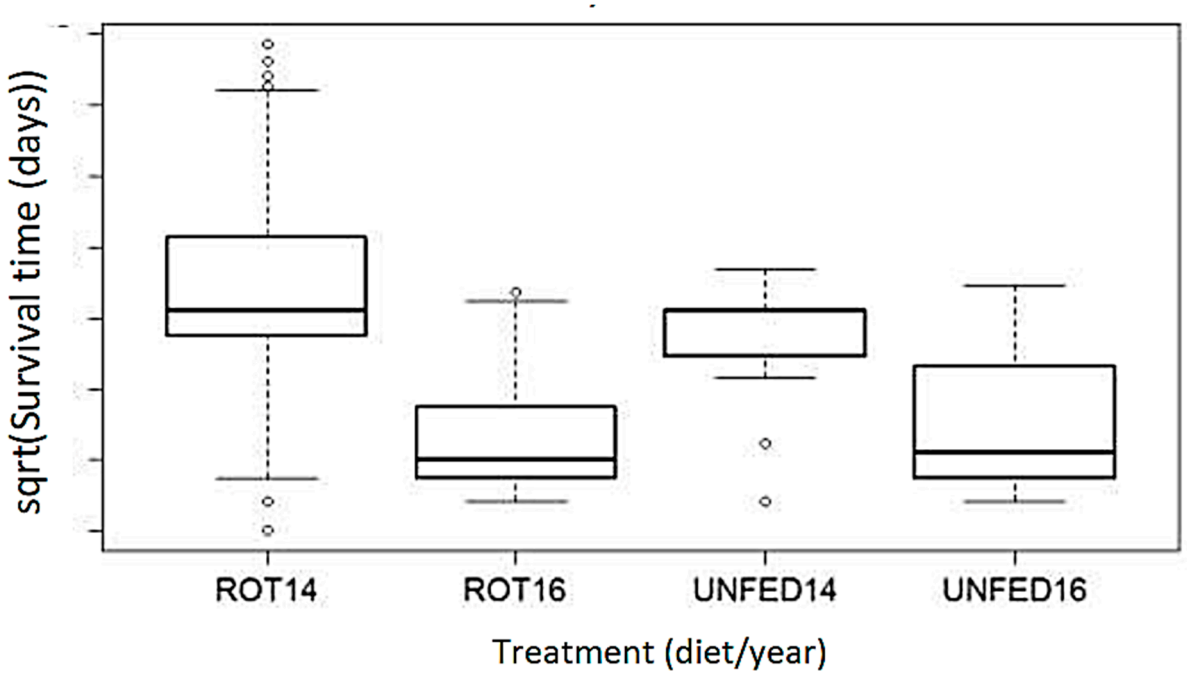

Figure 9. Boxplot of square root transformed survival time between diet type rotifer (ROT14 and ROT16) and unfed for each rearing system (LFSI in 2014 and LFSII in 2016) at 15 °C.

For LFS II, daily recorded larvae survival by diet and temperature was tested for homogeneity of variance with a Levene's test, histogram, and QQ plot. Levene's test did not show any violation in the homogeneity of variance by group = Group (Levene's test (center = median): $F = 1.2064$; df = 7, 526; $p = 0.2972$), even though the histogram and QQ plot showed a skewed distribution of larval survival proportion.

4. Discussion

This study is the first to analyze the effects of diet and temperature on larval stages of the red deep-sea crab, *C. quinquedens*, under laboratory conditions. Red deep-sea crab larvae were reared under differing diet and temperature regimes in two separate rearing system experiments. While some individuals did make it to the final zoeal stage, no animals developed to the megalopa stage which may be indicative of delayed development either due to the timing of prey availability or insufficient prey type. Additionally, the length of stage duration may also be indicative of delayed larval development due to diet, temperature, or rearing system. Similar barriers to larval rearing have been extensively reviewed for economically important mud crab species (genus *Scylla*) [32]. Crab larvae are usually considered carnivorous, however their survival in pelagic ecosystems may be tied to alternative prey resources and the timing at which these prey items are utilized [19,23,32,33]. Feeding behavior and nutritional requirements are different between the zoeal and megalopa stages of several crab species [23]. As such, inappropriate diet choice may decrease the survival of larval crabs as well as delay larval development by increasing stage duration [34,35].

Our results indicate delayed development may have occurred, as nearly all treatments had most larvae still in the zoea I stage even after 20+ days of survival. The developmental time of red deep-sea crabs has been categorized in the past with stage durations ranging from 5 to 14 days between subsequent stages [13]. Therefore, given optimal growing conditions (i.e., prey choice) and/or food availability, *C. quinquedens* larvae should have progressed through developmental stages within the time frame of our study. Growth to juvenile crab occurred at 18–21 °C with newly hatched *Artemia* nauplii provided as a prey item [13]. Similarly, *Chaceon granulatus* was reared successfully to the juvenile stage

(after 81–92 d) on *Artemia* nauplii, where the average stage duration between zoeal I and IV ranged between 8.5 and 12 d, with an average of 46 days spent within the megalopa stage [14]. Survival between stages also varied, where out of 100 individuals 40 made it to the zoeal II stage, 38 to zoeal III, 36 to zoeal IV, 28 individuals to the megalopa stage, and only 2 to juvenile crabs [14].

The importance of diet, including type, size, and timing, in the larval development of several other Brachyuran crab species has also been explored [19–22,35–42]. Previous studies showed that limited access to prey items (4 to 6 h/day) did not significantly reduce survival of zoea I larvae, but did increase the stage duration for two brachyuran crab species, *Cancer pagurus* and *Chasmagnathus granulata* [40]. Additionally, when food limitation continued throughout larval development, survival to metamorphosis was strongly decreased. More recently, the effect of diet during the larval development of shallow-water brachyuran crab species *Menippe nodifrons* and *Callinectes danae* was investigated [42]. Larvae from these species were fed with three microalgae species, a rotifer species, and *Artemia* nauplii, where larvae of *C. danae* did not reach zoeal III stage on any diet, whereas *M. nodifrons* larvae were fed enriched rotifers until stage III, after which *Artemia* nauplii were supplied and they reached the megalopa stage [42]. Both the rotifer and *Artemia*/rotifer diets in our LFSI and LFSII treatments had larvae that developed to zoeal stage III and IV, whereas the unfed and algae-only diets had high mortality and delayed larval development. Growth, development, and survival of larval Brachyuran crabs *Mithraculus sculptus* and *Mithraculus forceps* were evaluated for the aquaculture potential of these ornamental crab species [41]. Starved unfed larvae did not develop past the zoea I stage, while growth rates/development time did not differ between species fed either *Artemia* or enriched *Artemia* diets, where mean larval duration was between 8 and 10 d and survivorship to the megalopa stage was over 92% [41].

Developmental delays in larval crustaceans may also be due to differences in essential fatty acid concentrations and the utilization of other biochemical compounds within a particular prey item [34,43–45]. Mud crab *(Scylla serrata)* larvae survival was strongly affected by essential fatty acid (EFA) concentrations between *Artemia* and enriched rotifer diets, where EFA deficiencies were observed in larvae fed unenriched *Artemia* [45]. Larvae with these deficiencies had lower survival, longer intermolt periods, and had a narrower carapace when they reached the first crab stage [45]. In the LFSII system, red deep-sea crab larvae were fed with *Artemia* enriched with DHA Selco (Artemia International), which is rich in highly unsaturated fatty acids. This likely helped larvae in the LFSII system to progress to later zoeal stages. Nghia et al. [44] investigated the effects of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) levels in rotifer and *Artemia* diets on the survival and development of mud crab larvae, *Scylla paramamosain*, where survival rate between diet was not statistically significant. However, the DHA/EPA ratio within mud crab larvae prey was a key factor in larval development, where a ~50% HUFA and 0.6 DHA/EPA concentration led to severely delayed/stunted development and metamorphosis failure [43]. In contrast, larvae fed diets with ~30% HUFA and a high DHA/EPA ratio (4) were the best for zoeal development and showed improved metamorphosis rates [43].

In addition to prey type/biochemical composition, discrepancies between larvae and prey size may cause limited larval development and survival [16]. This occurred during our *Artemia* trial in the LFSI system, where prey items were too big for ingestion by zoea 1 larvae. Increased intake of Artemia as a prey item occurred for mud crab larvae with larval growth, where ingestion of Artemia was low at early zoeal stages (I, II, III), but increased at later stages (IV, V) [46]. However, the small size of rotifers (*Brachionus* spp. 220–240 µm) likely makes them easy prey for early zoeal stages to capture when compared with larger sized *Artemia* prey (460–500 µm) [46]. Similarly, Genodepa et al. [47] found that suitable particle size for Scylla larvae during stages I, III, and V were <150 µm, 150–250 µm, 250–600 µm, and 400–600 µm, respectively.

In addition to the variety of diet effects on larval development, temperature has also been documented to impact the reproduction and development of Brachyuran crabs [37,48–51] Larval stages of brachyuran crabs are the most sensitive to water temperature, and therefore a critical part of crab rearing is assessing how different temperatures affect the growth, survival, and development of larval crabs [51]. In the laboratory, temperature affected the survival and development for *Scylla serrata* larvae where zoeal stage I was unable to molt to stage II at 17 °C, although normal development until stage V was found to occur at 20–35 °C [48]. In the Dungeness crab, *Cancer magister*, increased mortality was observed for all larval stages at 20 °C, where 100% mortality occurred at zoeal stage V [38]. Additionally, stage duration varied inversely with temperature, where differences in duration between 10 and 15 °C were greater than those between 15 and 20 °C. For another commercially important crab species, *Portunus pelagicus*, larval rearing experiments conducted at 30 °C and an ambient range 24–28 °C showed survival to megalopa in a 14 d stage at 30 °C but 100% mortality after day 7 in ambient temperatures. Additionally, survivorship was greater in *P. pelagicus* larvae at 25 °C and the developmental period was inversely related to temperature [52] It is possible that our temperature regimes were too low for the accelerated growth of larval red deep-sea crabs and, in combination with a poor diet, caused delayed development for most individuals [26].

5. Conclusions

These results provide information on the potential viability of the red deep-sea crab, *C. quinquedens*, as a commercial aquaculture candidate and indicate delayed larval development due to food choice/limitation/size. Although our red deep-sea crab larvae did not advance to the megalopa stage, we showed that rearing method, temperature, and diet all contribute to the success or failure of larval development in an aquaculture facility. These data provide understanding of potentially improper rearing diets for the red deep-sea crab, *C. quinquedens*, and whether it is economically viable as an aquaculture species in a modern aquaculture setting. Further research should include refining and improving larval rearing systems, perhaps under food saturation, to better allow for the growth of larvae to the juvenile settlement stage as well as testing other food sources/combinations that are necessary to improve larvae production and development. Larval rearing/development for economically important crab species is still a barrier across taxa [35,41,42]. Therefore, reporting both positive and negative results of larval rearing studies will hopefully benefit those engaged in hatchery research/operations to avoid techniques that are not successful and move toward more viable larval rearing methods.

Author Contributions: Conceptualization, B.S. and G.O.; Methodology, N.M.P.-P.; Formal analysis, N.M.P.-P.; Investigation, N.M.P.-P.; Resources, B.S.; Data curation, N.M.P.-P., B.S. and S.L.S.; Writing—original draft, N.M.P.-P.; Writing—review & editing, M.P. and G.O.; Supervision, G.O.; Project administration, G.O.; Funding acquisition, S.L.S. and G.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data and other information can be obtained from the primary author and the corresponding author.

Acknowledgments: This project was funded by the National Oceanic and Atmospheric Administration (NOAA), the Living Marine Resources Cooperative Science Center (LMRCSC) Grant# NA11SEC4810002, and the NOAA Educational Partnership Program (EPP)/Minority Serving Institutions (MSI) Graduate Research and Training Scholarship Program (GRTSP). Special thanks to Matthew Poach for mentoring the primary author of this manuscript during a graduate internship at the NOAA James J. Howard Marine Sciences Laboratory in Sandy Hook, NJ, as well as all the NOAA James J. Howard Laboratory personnel and students for their support and assistance. Special thanks to Ehren Habeck, Kristen Habeck, DeMond Timmons, and Grant Blank for their technical assistance and guide for every aspect of the experimental setup—the electrical, plumbing, scientific mechanisms, etc. Special thanks to undergraduate students Julianne Murphy and Juliana Tran and

high school students Hayley Ehrlich, Charles Lee, and Remi Goddu for their help in the long sampling hours during weekdays and weekends, looking at larvae survival, preparing diets, feeding, and data collection. We would like to thank Ali Parsaeimehr for his assistance with the references section.

Conflicts of Interest: The authors declare no conflict of interest.

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