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Survival of blue king crab *Paralithodes platypus* Brandt, 1850, larvae in cultivation: effects of diet, temperature and rearing density

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Abstract

Blue king crab (*Paralithodes platypus*) larvae were cultivated to test the effects of diet, temperature and rearing density. Dietary treatments included no feeding (unfed), *Artemia* nauplii enriched with diatoms *Thalassiosira nordenskioeldii* (THAL), unenriched *Artemia* fed in addition to *Thalassiosira* (A+THAL) and a control diet of *Artemia* enriched with frozen *Isochrysis* paste (ISO 6). Trials were conducted at 6 °C, and a rearing density of 10 zoea L^{-1}, with six replicates per treatment. The ISO 6 diet was also tested at 3 °C (ISO 3) and 9 °C (ISO 9), and at densities of 20 (ISO 20) and 40 (ISO 40) zoea L^{-1}. Survival of zoea larvae fed the A+THAL diet (91.7%) was significantly higher than all others, whereas unfed zoea larvae died within 2 weeks. Temperature and rearing density had no significant effects on survival. Time required to reach stage C1 was significantly greater at 3 °C (109 days) than at 6 °C (70 days), but did not decrease further at 9 °C. After reaching the postlarval (glaucothoe) stage, half of the replicates in the ISO 20 and ISO 40 treatments were fed continuously, but survival did not differ significantly from unfed glaucothoe. We conclude that blue king crab larvae are not lecithotrophic and can be cultivated with high survival using the proper diet. These techniques can be used to produce large numbers of juvenile crab for laboratory research, or could be modified for use in stock-enhancement programmes.

Keywords: king crab, *Paralithodes platypus*, larvae, survival, diet, temperature, rearing density

Introduction

The blue king crab *Paralithodes platypus* Brandt, 1850, is a commercially valuable crustacean that occurs in isolated populations in the Bering Sea, Gulf of Alaska and southeast Alaska, as well as the western Pacific Ocean near Japan and Russia (Hoffman 1968). In 1997, commercial landings from the Eastern Bering Sea were 2361 mt, worth $12.0 million. The abundance of blue king crab populations near St Matthew Island and the Pribilof Islands declined precipitously in the late 1990s, and have been closed to fishing since 1999 and 2002 respectively (NPFMC 2002). The distribution of red king crab (*P. camtschaticus*) overlaps that of blue king crab in the Pribilof Islands, and population abundance has increased while that of blue king crab has decreased (Stevens, MacIntosh, Haaga, Armistead & Otto 2002). Red and blue king crabs are similar in size and life history, but exhibit quite different reproductive strategies. Adult female red king crab moult annually, mate and produce new egg clutches within a day or two after completion of hatching (Stevens & Swiney 2007), whereas large female blue king crab have a biennial spawning cycle (Jensen, Armstrong & Williams 1985). First-year spawners produce new eggs in late winter that develop for a year and hatch the following spring (Stevens 2006a; Stevens & Swiney 2007). Female blue king crab carry the empty egg cases for another year until they subsequently moult, mate and spawn in the spring 2 years after their previous moult (Somerton & MacIntosh 1985). It remains to be determined whether this biennial pattern is due to inherent physiological differences (Somerton & MacIntosh 1985)

or energetic restrictions caused by suboptimal conditions of temperature or food (Jensen *et al.* 1985).

Little is known about the early life history of blue king crab, whereas red king crab have been the focus of most research. Like red king crab, blue king crab develop through four zoeal and one postlarval stage (called a glaucothoe, equivalent to the megalops stage of brachyuran crabs) before metamorphosis to the first juvenile crab (C1) stage (Sato 1958; Hoffman 1968). Red king crab glaucothoe prefer to settle among structurally complex fauna (Stevens & Kittaka 1998; Stevens 2003). Juvenile (1–3 years old) blue king crabs have been found among shell hash in the Pribilof Islands (Armstrong, Palacios, Williams, Jensen & Pearson 1985) that is associated with fouling organisms. Juvenile stages of red and blue king crab overlap to an unknown degree, and may be highly competitive. Efforts to study the settlement behaviour and habitat preferences of these crabs are dependent on the need to develop cultivation methods to produce an adequate number of small crabs for laboratory research. Larvae of both species have been cultivated in the laboratory (Abrunhosa & Kittaka 1997; Stevens & Kittaka 1998; Stevens 2003) on a diet of *Artemia* with or without diatoms (Kittaka, Stevens, Teshima & Ishikawa 2002), but success with either species has been inconsistent. In the wild, the survival of newly hatched red king crab zoeas is associated with the availability of diatoms (*Thalassiosira* sp.) (Paul, Paul & Coyle 1989; Paul & Paul 1990).

Our experiment was conducted as part of a research programme to improve knowledge of the reproduction and early life history of blue king crab, from fertilization through the first year of life. To ensure an adequate supply of young crab for research purposes, we investigated the best conditions for cultivation of the larvae from hatching to stage C1.

Materials and methods

Female blue king crabs with fertilized, eyed eggs were collected from the Pribilof Islands at two different times. Six ovigerous females, two old-shell females and one male crab were captured with a research-type 83–112 otter trawl near the Pribilof Islands, Alaska (between 56°54′N and 57°35′N, and between 169°18′W and 169°30′W), in July 2003 and shipped in chilled coolers to Kodiak where they were held in a chilled recirculating water system at 4 °C. Twelve ovigerous females were captured near the Pribilof Islands in September 2003 using side-loading crab pots, and shipped to Kodiak on 8 October. On 13 April 2004, three female crabs that had been releasing larvae for several days were placed in individual 120 L tubs in a water bath at ambient temperature (5.1 °C) and left overnight. The following morning, 240 newly hatched larvae were collected from each tub and mixed together, for a total of 720 larvae.

Cultivation of crab larvae

The experiment consisted of eight treatments grouped by diet, temperature and rearing density (Table 1). We did not use a completely crossed factorial design because of the large number of replicates and larvae required. Preliminary trials using red king crab larvae indicated that survival to stage C1 was not significantly different between diets using *Artemia* enriched with *Thalassiosira*, Algamac 3050 (Aquafauna Bio-Marine, Inc., Hawthorne, CA, USA), live *Isochrysis* sp. or *Isochrysis* paste (S. Persselin, unpubl. data). Therefore, we selected the *Isochrysis* paste as the control diet for this experiment based on ease of use. Each treatment comprised six replicates of 10 zoeas per beaker, except as noted. Treatments 1–4 consisted of larvae fed four different diets at 6 °C: (1) larvae receiving no food (unfed); (2) larvae receiving *Artemia* nauplii enriched with *Isochrysis* (ISO 6), considered the 'control' diet (see below for *Artemia* culture conditions); (3) larvae receiving *Artemia* nauplii enriched with *Thalassiosira nordenskioeldii* (THAL); and (4) larvae receiving unenriched *Artemia* nauplii plus live *T. nordenskioeldii* directly in the beaker (A+THAL). All beakers were fed daily with approximately 1750 *Artemia* nauplii/beaker (i.e., 2.2 nauplii mL^{-1}). Treatments 5 and 6 were also fed the ISO diet, but beakers were held at 3 °C (ISO 3) and

Table 1 Summary of culture conditions (treatments) for larvae of blue king crab (*Paralithodes platypus*)

Treatment	Temperature (°C)	Food	Density	Beakers	Total larvae
1	6	Unfed	10	6	60
2	6	ISO	10	6	60
3	6	THAL	10	6	60
4	6	A+THAL	10	6	60
5	3	ISO	10	6	60
6	9	ISO	10	6	60
7	6	ISO	20	6	120
8	6	ISO	40	6	240
Total				48	720

See text for a description of the treatments

9 °C (ISO 9) respectively. Treatments 7 and 8 were fed the ISO diet at 6 °C, but consisted of beakers with 20 (ISO 20) or 40 zoeas L^{-1} (ISO 40). All treatments were maintained on a 12:12 light:dark cycle at approximately 70 lx using indirect fluorescent lighting. Temperatures were recorded using electronic data loggers placed in adjacent beakers. All experiments conducted at 3 and 6 °C were held in separate constant-temperature rooms at mean temperatures [± 1 standard deviation (SD)] of 3.1 ± 0.1 and 6.3 ± 0.1 °C respectively. The 9 °C treatment beakers were held in one of two incubators that were exchanged every 3–4 days because seawater condensation on the chiller coils required them to be defrosted; the mean temperatures (± 1 SD) were 9.0 ± 0.4. Experiments were conducted by placing larvae inside a 150 mm length of 75-mm-diameter PVC tube, with a 675 μm polyethylene netting glued to the bottom. Each tube was set into a 1 L glass beaker filled with 800 mL of seawater that had been filtered to 5 μm and UV- sterilized. Larvae (in tubes) were transferred to clean beakers with fresh seawater daily before feeding. Feeding was terminated when all zoeas in the beaker had moulted to the glaucothoe stage, except for three randomly selected replicates in each of the ISO 20 and ISO 40 density treatments, which were fed continuously throughout the glaucothoe stage for comparison.

The numbers of surviving larvae were counted once each week in all beakers, after which beakers were rinsed in fresh and distilled water before reuse. As larvae began moulting to glaucothoe or the first crab stage (C1), moults and mortalities were removed daily to determine the start and end time of moulting for each beaker. The number of days to the middle of the moulting period from Zoea IV to glaucothoe in each beaker was defined as the half-way point between the appearance of the first and last glaucothoe (Mid-G). Similarly, the half-way point between the appearance of the first and last stage C1 crab was defined as Mid-C. Final survival was determined when all surviving crabs had moulted to stage C1, and each treatment was considered to be completed when larvae in all six beakers finished moulting to stage C1.

Diatom and *Artemia* culture

The chain-forming diatom *T. nordenskioeldii* was obtained from the Center for Culture of Marine Phytoplankton (CCMP) at Bigelow Laboratory for Ocean Sciences (West Boothbay Harbor, ME, USA). The diatoms were cultured in seawater that was filtered to 5 μm, UV- sterilized, chlorinated with 6% sodium hypochlorite and dechlorinated with sodium thiosulphate. Culture water was enriched with f/2 medium, with the addition of sodium metasilicate. The diatoms were grown under a 16:8 L:D cycle in a temperature-controlled room at 3 °C. *Artemia* cysts (Brine Shrimp Direct, Ogden, UT, USA, San Francisco Bay strain) were hatched in 2 L plastic brine shrimp hatching cones (Florida Aqua Farms, Dade City, FL, USA) in aerated seawater sterilized as above. For the A+THAL diet, newly hatched (18–24 h old) *Artemia* nauplii were collected, rinsed in freshwater and fed to the larvae along with diatoms. For the THAL and ISO diets, the nauplii were first collected 30 h post hydration, rinsed with freshwater and returned to a hatching cone filled with either *T. nordenskioeldii* culture (THAL) or Instant Algae® *Isochrysis* paste (Brine Shrimp Direct, Ogden, UT, USA) at 0.5 mL L^{-1} (ISO). Nauplii were collected again 18 h later, rinsed with freshwater and fed to the zoeas.

Data analysis

Proportional survival data were subjected to angular transformation (Zar 1984). Homogeneity of variances was tested using Levene's test before the use of ANOVA. Survival to stage C1 was compared between treatments using ANOVA, and post hoc multiple comparisons of each treatment versus the 'control' ISO 6 diet were conducted with Dunnett's test. A two-sample t-test was used to compare differential survival between days 48 and 76 (approximate dates of Mid-G and Mid-C transition) of fed and unfed glaucothoe in the ISO 20 and ISO 40 treatments (after angular transformation). Analysis of variance was also used to compare the raw numbers of surviving crab in each of the three rearing density treatments that used the same diet and temperature (ISO 6, ISO 20 and ISO 40), and post hoc multiple comparisons were made using Tukey's HSD test. Time (days) to Mid-G and Mid-C were compared directly using ANOVA without transformation, and the mean values were compared with the ISO 6 diet with Dunnett's test. Values of $P < 0.05$ were considered to be significant. Mean values ± 1 SD are given where appropriate.

Results

The survival of blue king crab larvae from hatching to stage C1 varied widely and significantly

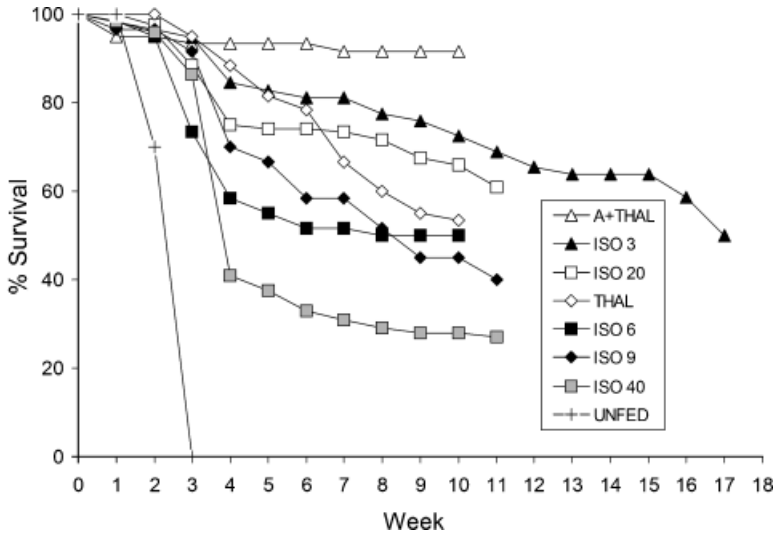

Figure 1 Per cent survival of blue king crab (*Paralithodes platypus*) larvae and postlarvae at weekly intervals, from hatching to stage C1, under different culture conditions. All points are means of six replicates. See text for a description of treatments.

Table 2 Survival of blue king crab (*Paralithodes platypus*) larvae from hatching to stage C1

	Beaker						Numbers			Percent	
Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**Sum**	**Mean**	**SD**	**Mean (%)**	**SD (%)**
THAL	7	0	4	8	5	7	31	5.2	2.9	51.7	29.3
A+THAL (a)	8	10	10	9	10	8	55	9.2	1.0	91.7	9.8
UNFED (b)	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0
ISO 20	**12**	4	**14**	12	19	**16**	77	12.8	5.1	64.2	25.4
ISO 40	5	10	3	**13**	**13**	**22**	66	11.0	6.8	27.5	17.0
ISO 3	9	5	8	3	8	1	34	5.7	3.2	57.9	30.9
ISO 6	10	2	9	6	0	2	29	4.8	4.1	48.3	41.2
ISO 9	5	7	2	9	2	0	25	4.2	3.4	41.7	34.3

Values are numbers of crab surviving in each replicate beaker, and sum, mean and SD of total survival. Mean and SD of per cent survival are also shown for each treatment. Best survival was on the A+THAL diet with 10 larvae L^{-1} at 6 °C. Groups that were significantly different from the control diet (ISO 6) by Dunnett's test are designated by letters (a and b). Bold numbers indicate beakers that were fed through the glaucothoe phase.
SD, standard deviation.

($F_{(7,40)} = 7.21$, $P < 0.0001$) among treatments (Fig. 1). Variances of the angular-transformed survival data were homogeneous (Levene's $F_{(2,15)} = 2.11$, $P = 0.0647$). The highest survival from hatching to stage C1 (91.7 ± 9.8%) occurred in the A+THAL treatment (at 6 °C, 10 zoeas per beaker), and was significantly greater than in any other treatment (Dunnett's test, Table 2). At the other end of the spectrum, all unfed zoea larvae died within 21 days (Fig. 1); this was the only treatment with survival significantly lower than the control (ISO 6) diet (Table 2). Survival in all other treatments was not significantly different from the control diet. Temperature had little effect, although the mean survival showed a decreasing trend among the ISO 3 (57.9 ± 30.9%), ISO 6 (48.3 ± 41.2%) and ISO 9 (41.7 ± 34.2%) treatments (Table 2). There was no difference in survival from stage G to stage C1 between fed and unfed glaucothoe in either the ISO 20 treatment ($t = 0.103$, $P = 0.928$, $n = 3$) or the ISO 40 treatment ($t = 1.982$, $P = 0.186$, $n = 3$) (Table 3).

At first glance, rearing density also appeared to have little effect, as proportional survival among the three density treatments was not significantly different (see ANOVA and Dunnett's test above), although it tended to be greater in the ISO 20 treatment (64.2 ± 25.4%) than in the ISO 6 (48.3 ± 41.2%) or ISO 40 treatments (27.5 ± 17.0%). However, the final numbers of surviving larvae in the ISO 20 and ISO 40 treatments (77 and 66 respectively) were similar (Table 2). The numbers of crab larvae in the ISO 40 treatment declined abruptly during week 4 to levels

similar to those in the ISO 20 treatment, and followed a similar trend thereafter (Fig. 2). Variances of raw data were homogeneous (Levene's $F_{(2,15)} = 0.83$, $P = 0.4535$) and so no transformation was applied; ANOVA showed that the mean numbers of surviving C1 crab were not significantly different ($F_{(2,15)} = 3.56$, $P = 0.0542$) between the ISO 20 (12.8 ± 5.1), ISO 40 (11.0 ± 6.8) and ISO 6 treatments (4.8 ± 4.1). These results imply that the experimental conditions of diet, *Artemia* concentration and temperature used could support an upper limit of about 13 zoeas per 800 mL beaker, or about 16.2 zoeas L^{-1}.

Table 3 Differential survival of fed and unfed blue king crab glaucothoe from stage G (day 48) to C1 (day 76) in the ISO 20 and ISO 40 treatments

Treatment	ISO 20		ISO 40	
Diet	Fed	Unfed	Fed	Unfed
Replicate 1	92.3%	80.0%	100.0%	62.5%
Replicate 2	87.5%	70.6%	100.0%	71.4%
Replicate 3	88.9%	100.0%	100.0%	100.0%
Mean	89.4%	85.4%	100.0%	72.0%
Treatment mean	87.5%		90.4%	
t-statistic	0.1027		1.9821	
P (two tailed)	0.9276		0.1860	

See text for a description of the treatments.

Time to the midpoint of moulting to glaucothoe (Mid-G) differed significantly between treatments (ANOVA, $F_{(6,33)} = 238.6$, $P < 0.0001$) and variances were homogeneous (Levene's $F_{(6,33)} = 2.16$, $P = 0.072$). Larvae raised at 3 °C (ISO 3) required significantly more time (mean 71.8 ± 2.2 days) to reach Mid-G than did the control (ISO 6) treatment (44.7 ± 2.0 days; Table 3), whereas all other treatments were similar to the control, requiring an average of 44.9 ± 1.7 days (Fig. 3). Time to Mid-G for larvae raised at 9 °C (ISO 9; 44.9 ± 1.7 days) was not

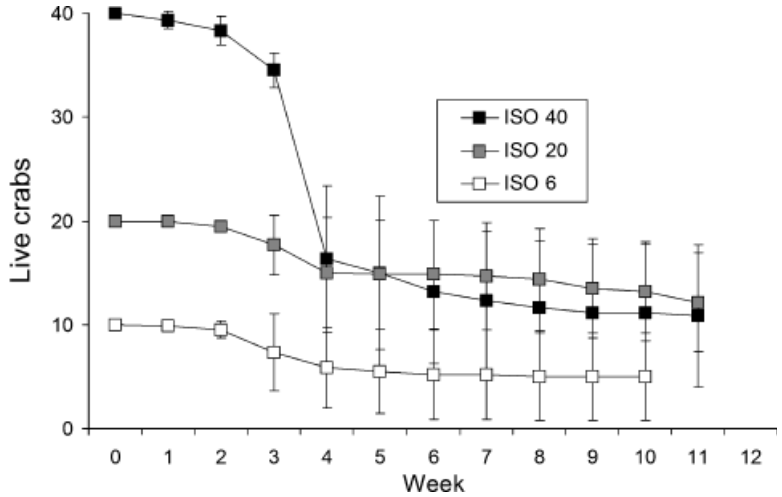

Figure 2 Mean number of surviving blue king crab (*Paralithodes platypus*) larvae and postlarvae at weekly intervals, from hatching to stage C1 in the three density treatments. All were fed the ISO diet at 6 °C. ISO 6 treatment started with 10 larvae per beaker, whereas the ISO 20 and ISO 40 treatments started with 20 or 40 larvae per beaker respectively.

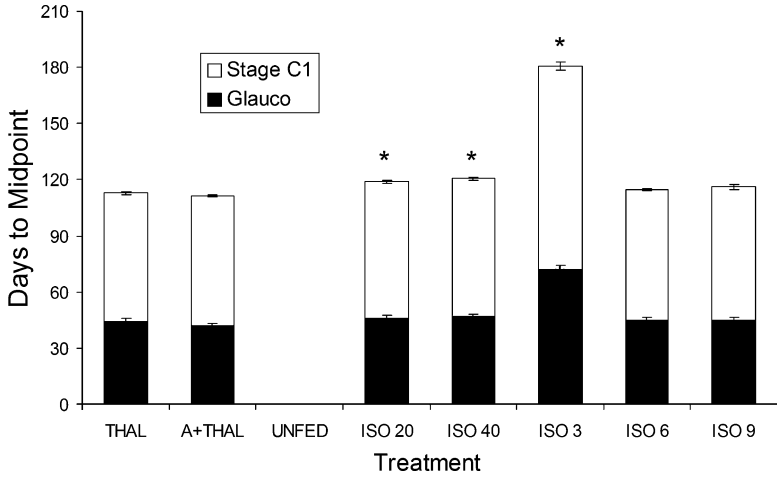

Figure 3 Mean number of days from hatching to the midpoint of moulting to glaucothoe (black) and stage C1 (white) for larvae of blue king crab (*Paralithodes platypus*). *Above the bar indicate those that were significantly different from the control (ISO 6) treatment. Error bars are ± 1 SD.

Table 4 Length of developmental stages (in days) for blue king crab (*Paralithodes platypus*) larvae

Means	THAL	A+THAL	ISO 20	ISO 40	ISO 3	ISO 6	ISO 9	Average	SD
H to first G	41.2	40.3	43.2	44.3	68.5	43.0	43.0	42.5	1.5
First G – last G	5.8	4.0	6.2	5.8	6.7	3.4	3.8	4.8	1.2
last G – first C	19.6	22.8	20.2	19.8	31.7	22.2	22.2	21.1	1.4
First C – last C	3.2	4.2	6.2	6.8	4.7	4.3	3.6	4.7	1.5
H to last C1	70.4	71.0	76.0	76.8	111.2	72.0	73.0	73.2	2.6
Mid-G	44.1	42.3	46.3	47.3	71.8	44.7	44.9	44.9	1.7
Mid-C	68.8	69.1	72.7	73.4	108.8	69.9	71.3	70.9	1.9
SD									
Mid-G	1.8	0.9	1.2	1.2	2.2	2.0	1.7		
Mid-C	0.8	0.7	0.8	0.9	2.3	0.6	1.6		

Average values were calculated across all treatments excluding the ISO 3 treatment. 'H to last C1' is a summation of all previous stages; Mid-G and Mid-C are midpoints of moulting as defined in the text. The last two rows are SD for time to glaucothoe and C1 stages.
SD, standard deviation; H, Hatching; G, Glaucothoe; C, first stage crab.

significantly different from that for those raised at 6 °C (ISO 6) or the overall mean. Time to the midpoint of moulting to stage C1 (Mid-C) also differed significantly between treatments (ANOVA, $F_{(6,26)} = 692.7$, $P < 0.0001$) and variances were homogeneous (Levene's $F_{(6,26)} = 1.79$, $P = 0.1408$). Time to Mid-C was significantly greater in the ISO 3 (108.8 ± 2.3 days), ISO 20 (72.7 ± 0.8 days) and ISO 40 (73.4 ± 0.9 days) treatments than in the control (ISO 6, 69.9 ± 0.6 days) (Table 4).

Discussion

In our experiment, extremely high survival (92%) of blue king crab larvae from hatching to first juvenile crab stage was obtained using a diet of unenriched *Artemia* and culture water supplemented with *Thalassiosira*, at a temperature of 6 °C. Much lower survival occurred when *Artemia* were enriched with *Thalassiosira* before feeding them to crab zoeas. Unfed zoeas died within a few weeks, demonstrating that blue king crab zoeas are, like those of red king crab, not lecithotrophic, whereas lecithotrophic larvae are typical of crabs in the genus Lithodes such as the golden king crab *Lithodes aequispinus* (Shirley & Zhou 1997) and the southern king crab *L. santolla* (Lovrich, Thatje, Calcagno, Anger & Kaffenberger 2003). Based on these results, we infer that crab larvae obtain nutrients directly from the diatoms that they do not obtain when the diatoms are first consumed by *Artemia*. Nutritionally, phytoplankton can supply vitamins, protein, carbohydrates, fatty acids and pigments that may not be available in sufficient quantities in unenriched *Artemia*. The presence of diatoms in the culture water may also provide other benefits such as removal of nitrogenous wastes, the addition of oxygen and helping to balance pH. Previous experiments with larval king crabs have indicated that *Thalassiosira* in the culture water controls water quality and microflora (Kittaka *et al.* 2002). Survival using the control diet (*Artemia* enriched with frozen *Isochrysis* paste) decreased with increasing temperature, from 3 to 9 °C, although not by a significant amount. However, development time from hatching to stage C1 was identical (mean 71 days) at 6 and 9 °C, and yet it was lengthened considerably (109 days) at 3 °C. Thus, increased survival at 3 °C is offset by a much longer development time, with associated labour costs and a higher risk of failure. Survival during the glaucothoe stage did not differ between fed versus unfed glaucothoe, supporting the conclusion that blue king crab glaucothoe do not need to be fed, and probably do not eat. These results are consistent with those for red king crab, which also do not feed during the glaucothoe stage (Nakanishi 1988) due to temporary degeneration of their mouthparts and midgut gland (Abrunhosa & Kittaka 1997).

Our best treatment yielded survival exceeding that obtained by Kittaka *et al.* (2002), who cultivated larvae of red and Hanasaki king crab (*Paralithodes brevipes*) in large batches (1000–10 000) in 100–200 L containers, at densities of 10–50 L^{-1}, using unenriched *Artemia* plus *Thalassiosira*, or *Artemia* fed with *Thalassiosira* enhanced with tuna oil, squid oil, *Spirulina* or brewer's yeast. For those fed with unenriched *Artemia* plus *Thalassiosira*, i.e, the same diet used in our experiment, the best survival to stage C1 was 35% for red king crab (at a density of 25 larvae L^{-1}) and 53% for Hanasaki crab (at 10 larvae L^{-1}). The ef-

fects of rearing density on the survival of blue king crab were similar to those for Hanasaki crab, in which survival was significantly greater at densities below 25 larvae L^{-1} than at higher densities. Survival of *P. brevipes* to stage C1 was the greatest (12%) when fed with *Artemia* enriched with diatoms enhanced with tuna oil, as opposed to other enhancements. Kittaka *et al.* (2002) analysed both *Artemia* and *Thalassiosira* for their content of highly unsaturated fatty acids (HUFA), especially eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). The levels of EPA were similar (about 4000 $\mu g\, g^{-1}$) in both *Artemia* and *Thalassiosira*, and increased only slightly due to enhancement with tuna oil. The levels of DHA, however, were low ($< 400\ \mu g\, g^{-1}$) for both diets, but increased significantly (to about 12 000 $\mu g\, g^{-1}$) in *Thalassiosira* enhanced with tuna oil. Docosahexaenoic acid levels in the *Artemia* after feeding on enriched diatoms were not analysed, however. Preliminary experiments conducted in our laboratory showed that EPA levels in *Artemia* nauplii from two sources were about 12% of total fatty acids (TFA), and DHA was not detected (K. Reppond, NMFS Kodiak Laboratory, unpubl. data). Enrichment of *Artemia* with *Thalassiosira*, *Chroomonas* or *Isochrysis* sp. did not increase EPA or DHA levels, but enrichment with Algamac 3050 (a commercial aquaculture supplement) increased DHA levels to 18.4% of TFA.

Enrichment of fatty acid levels, especially DHA, may be critical for good survival of crab larvae raised on *Artemia*. Studies on the mud crab *Scylla serrata* indicate that EPA levels of 0.7–0.9% total dry weight and DHA levels of 0.5–0.7% provided the highest survival, shortest intermoult period and greatest carapace width at stage C1 (Suprayudi, Takeuchi & Hamasaki 2004), whereas lower or higher levels resulted in decreased values of these parameters. Although these values are not directly comparable to units based on per cent of TFA, they are similar to the levels reported by Kittaka *et al.* (2002) for enriched *Artemia*.

Our experimental conditions were adequate to produce numbers of larvae (several hundred) useful for laboratory experimentation, and could be repeated at this level. However, cultivation in larger volumes will be necessary to produce more larvae for future research and will require different laboratory apparatus, including a flow-through water system with built-in filtration, temperature control and a different feeding regime. These techniques have since been adapted to produce larger quantities of small crab for experimental stock-enhancement research. Japanese fishing cooperatives have conducted a large-scale enhancement project with *P. brevipes* for over a decade, releasing 200–500 000 early stage crabs annually (Stevens 2006b). The success rate of this endeavour is unknown, however, due to lack of monitoring efforts. More intensive efforts have been made to enhance populations of swimming crabs, *Portunus trituberculatus*, in the Japan inland sea, but there are little data to suggest that these efforts have produced improved fisheries (Secor, Hines & Place 2002). Studies on Chesapeake Bay blue crab *Callinectes sapidus* cultivation (Zmora, Findiesen, Stubblefield, Frenkel & Zohar 2005) and enhancement (Davis, Young-Williams, Hines & Zohar 2005) are also underway and show promise for the future.

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