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Diet and Water Source Effects on Larval Red King Crab Cultivation

Sara Persselin[1]
National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Kodiak Laboratory, Kodiak, Alaska, U.S.A.

Benjamin Daly
University of Alaska Fairbanks, School of Fisheries and Ocean Sciences, Seward, Alaska, U.S.A.

Abstract

King crab larval culture has expanded from small-scale research to hatchery and stock enhancement feasibility studies in Alaska. The goal of this project was to improve red king crab (*Paralithodes camtschaticus*) larval survival in culture by assessing diets and water sources in two separate experiments. Diet treatments included (1) newly hatched *Artemia* nauplii, and (2) newly hatched *Artemia* nauplii and the diatom *Thalassiosira nordenskioeldii*; both treatments were conducted at facilities in Kodiak and Seward, Alaska. The water source study was conducted at the Seward facility and treatments included (1) natural seawater from Resurrection Bay, and (2) artificial seawater made from (Instant Ocean®) sea salt.

At both facilities, mean survival to the glaucothoe stage was significantly higher and mean larval duration was significantly shorter for larvae fed the *Artemia*-diatom diet. Larval duration and survival to the glaucothoe stage were not significantly different between facilities. In Kodiak, larval survival and duration were carried through to the first juvenile stage (C1); all glaucothoe molted to C1 on the *Artemia*-diatom diet whereas only two glaucothoe molted to C1 on the *Artemia*-only diet by the termination of the experiment. In Seward, mean survival to glaucothoe and mean larval duration were not significantly different between larvae reared in artificial seawater and natural seawater. For higher yield

[1]Current address: P.O. Box 8936, Kodiak, Alaska 99615, U.S.A. Phone: +1 907 942-2812. kodiakocean@gmail.com.

in larval red king crab cultivation, a diet including *T. nordenskioeldii* is recommended while artificial seawater is likely unnecessary.

Introduction

Enhancement of red king crab (*Paralithodes camtschaticus*) populations has been promoted as a means for increasing the wild population in Alaska. After landings of red king crab peaked in the 1970s, catches of red king crab declined precipitously in the 1980s and have since remained depressed. Reasons for the decline and continued lack of recovery are still debated but may include egg predation (Kuris et al. 1991), disease, overfishing (Orensanz et al. 1998, Dew and McConnaughey 2005), climatic changes (Zheng and Kruse 2000), low survival through the juvenile life stage caused by predation (Blau 1986), drift of planktonic larvae from suitable habitat (Shirley and Shirley 1989), and insufficient food quantity or type (Paul et al. 1989). The successful establishment of red king crab in the Barents Sea in the 1960s provides evidence that red king crab populations can be enhanced by propagation (Kovatcheva et al. 2006). Whether this technique would work in the crab's endemic range in Alaska is unknown.

The collaborative Alaska King Crab Research, Rehabilitation and Biology (AKCRRAB) program was launched in 2006 to investigate the feasibility of king crab stock enhancement in Alaska. Success of small-scale larval culture techniques for red king crab and blue king crab (*Paralithodes platypus*) developed at the NOAA Alaska Fisheries Science Center, Kodiak Laboratory in Kodiak, Alaska (Persselin 2006, Stevens et al. 2008) formed the basis for developing large-scale diet and density studies for red and blue king crab larvae at the Alutiiq Pride Shellfish Hatchery (APSH) in Seward, Alaska. Initial results of this large-scale cultivation project, however, yielded poor overall survival (<1%) to the first juvenile instar; the combined effects of diet and density made it difficult to determine the source of the high mortality.

In the wild, female red king crabs carry their fertilized eggs for 11-12 months, releasing larvae over an average 32 day period in late winter to spring (Otto et al. 1990, Stevens and Swiney 2007). Larvae develop through four planktotrophic zoeal stages (Z1-Z4) and the nonfeeding glaucothoe stage before settling onto structurally complex habitat and molting into the first juvenile stage (C1) (Marukawa 1933). In the wild, newly hatched red king crab zoeae consume phytoplankton and zooplankton (Bright 1967). The diatoms *Thalassiosira* spp., present in the ocean during larval development, have been shown in the laboratory to support larval growth during the first zoeal stage (Paul et al. 1989, Paul and Paul 1990). At the Kodiak Lab, high survival (91.7%) to the first instar of *P. platypus* was achieved using a diet of newly hatched *Artemia franciscana* nauplii and the cold water diatom, *Thalassiosira nordenski-*

oeldii (Stevens et al. 2008). Although culture of larval *P. camtschaticus* and *P. brevipes* on a combination diet of *Artemia salina* nauplii and *Thalassiosira* spp. (dominated by *T. nordenskioeldii*) met with varied success (5.7%-87.2% survival in the zoeal stages), the results suggest that high survival on this diet is possible (Kittaka et al. 2002).

Fundamental parameters of larval culture such as temperature, water quality, and food availability can be more difficult to control in large rearing tanks than small systems where variables can be more precisely controlled and manipulated. In this small-scale study, we investigated diet and water source as factors contributing to poor survival in previous experiments and compared results for differences in survival at both the Kodiak Lab and University of Alaska Fairbanks Seward Marine Center, which is adjacent to APSH. Based on the success with blue king crab larvae, the *Artemia–T. nordenskioeldii* diet was selected for red king crab larvae at both facilities for this experiment (Stevens et al. 2008). Additional research suggests seawater contaminants such as hydrocarbons could have been a contributing factor in larval mortality at APSH (Duesterloh 2002). To address this, we compared larval survival using artificial and natural seawater. These controlled, small-scale experiments are a critical step in evaluating the feasibility of large-scale culture necessary for a stock enhancement program.

Materials and methods

Cultivation techniques were standardized between facilities. We used the same laboratory methods, fed the larvae identical diets from the same source of *Artemia* nauplii and *Thalassiosira nordenskioeldii*, held the larvae at the same temperatures, kept identical larval densities, and used larvae from the same broodstock cohort. The only difference between facilities was the additional comparison of natural and artificial seawater sources at the Seward Marine Center.

Eighteen ovigerous crabs were collected with commercial crab pots in Bristol Bay, Alaska, during November 2007. Six crabs were retained at the Kodiak Laboratory in flow-through, ambient seawater tanks with a temperature range of 2.4 to 7.7ºC (mean 4.05ºC, SE = 0.1). Twelve crabs were shipped to holding tanks at the Seward Marine Center and held in flow-through, ambient seawater with a temperature range of 3.4 to 8.0ºC (mean 5.15ºC, SE = 0.1). Crabs were fed to satiation with squid and herring twice per week at both locations.

Newly hatched larvae (less than 24 hours old) were collected on April 21, 2008, at the Kodiak Lab and on April 4, 2008, at Seward Marine Center from three female crabs that had been isolated in separate hatching bins. Four larvae from each female were placed in 1 L glass beakers for a total of 12 larvae per beaker. The project consisted of two experiments: (1) the effect of diet on larval survival and duration, and

Table 1. Treatments and study locations for diets and water sources investigated for effect on survival and duration of red king crab *Paralithodes camtschaticus* larvae.

Treatment name	Treatment definition	Location of study
ArtK	Natural seawater with newly hatched *Artemia* nauplii	Kodiak Lab
ThalK	Natural seawater with newly hatched *Artemia* nauplii + *Thalassiosira nordenskioeldii*	Kodiak Lab
ArtS	Natural seawater with newly hatched *Artemia* nauplii	Seward Marine Center
ThalS	Natural seawater with newly hatched *Artemia* nauplii + *Thalassiosira nordenskioeldii*	Seward Marine Center
ASW	Artificial seawater with a diet of newly hatched *Artemia* nauplii	Seward Marine Center

(2) the effect of natural vs. artificial seawater on larval survival and duration. The diet experiment, conducted at the Kodiak Lab and the Seward Marine Center, consisted of two treatments: (1) larvae receiving *Artemia* nauplii (ArtK, ArtS), and (2) larvae receiving *Artemia* nauplii plus live *T. nordenskioeldii* microalgae (ThalK, ThalS) (Table 1). The seawater experiment, conducted at the Seward Marine Center, consisted of two treatments: (1) larvae receiving *Artemia* nauplii in natural seawater (ArtS), and (2) larvae receiving *Artemia* nauplii in artificial seawater (ASW) (Table 1). All *Artemia* nauplii were newly hatched, collected at less than 24 hours after initial hydration. Treatments comprised five replicates of 12 zoeae per beaker held in a temperature-controlled room at 7ºC. Larvae were fed approximately 1750 *Artemia* nauplii per beaker (2.2 *Artemia* nauplii per ml) daily and were maintained on a 12 hour dark:12 hour light photoperiod cycle at 70 lux using indirect fluorescent lighting. Larvae were placed inside a 150 mm long 75 mm diameter PVC tube, with 675 µm nylon screen glued to the bottom (Persselin 2006, Stevens et al. 2008). For the diet experiment, each tube was set into a beaker filled with 800 ml of seawater that was filtered to 5 µm and UV-sterilized (ArtK, ArtS) or 800 ml of filtered and sterilized seawater containing 10,000 cells per ml of *T. nordenskioeldii* (ThalK, ThalS). For the seawater experiment each tube was set into a beaker filled with 800 ml of seawater that was filtered to 5 µm and UV-sterilized (ArtS) or 800 ml artificial seawater made using deionized water and Instant Ocean® sea salt (ASW). Salinity of natural and artificial seawater was 31 ppt. Tubes containing larvae were transferred to clean beakers with new seawater daily prior to feeding. Feeding was terminated when all zoeae in the beaker molted to the glaucothoe stage. Molts and mortalities were

recorded and removed daily. Final survival and duration to glaucothoe was determined when the last larva in each replicate had molted to glaucothoe (Seward Marine Center and Kodiak Lab). Final survival and duration to C1 (Kodiak Lab only) was determined when the last glaucothoe in each replicate had molted to C1 or died.

San Francisco Bay strain *Artemia* cysts (Brine Shrimp Direct, Ogden) from the same batch were used at the Kodiak Lab and the Seward Marine Center to eliminate the effect of *Artemia* quality on the treatments. *Artemia* cysts were hatched daily in aerated seawater sterilized as above. Newly hatched (<24 hours old) *Artemia* nauplii were collected and rinsed in freshwater prior to feeding to larvae.

The chain-forming diatom *T. nordenskioeldii* (15-27 µm long), obtained from the Center for Culture of Marine Phytoplankton at Bigelow laboratory for Ocean Sciences (West Boothbay Harbor), has been under cultivation at the Kodiak Lab since 2002. A starter culture was shipped to the Seward Marine Center for cultivation. Diatoms were cultured under a 16 hour light:8 hour dark photoperiod cycle in a temperature-controlled room at 7ºC in seawater filtered to 5 µm, UV-sterilized, chlorinated with 6% sodium hypochlorite and dechlorinated with sodium thiosulfate. The seawater was enriched with f/2 algal culture formula (Kent Marine, Franklin), and sodium metasilicate.

Analysis of variance (ANOVA) and post-hoc comparisons (Tukey's HSD) were used to determine significance in larval survival and duration. Survival and duration to C1 at the Kodiak Laboratory was compared using Student's *t*-test. For the water source experiment, survival and duration to glaucothoe were compared between treatments using Student's *t*-test. Significance was determined with an alpha level of 0.05. Means with standard error (SE) are reported.

Results

Diet

Larval survival

For survival, the main effect of diet was significant (Table 2). Survival to glaucothoe was higher when *Thalassiosira nordenskioeldii* was included in the diet at both locations (Tukey's HSD, $p < 0.001$). When fed *Artemia* nauplii only, overall survival was 30.7% (SE = 5.3) with mean survival of 29.8% (SE = 6.8) at the Kodiak Laboratory (ArtK) and 31.7% (SE = 10.3) at the Seward Marine Center (ArtS) (Fig. 1A and B, Table 3). When *T. nordenskioeldii* was included in the diet, overall survival was 84.4% (SE = 5.6) with mean survival of 93.8% (SE = 4.1) at the Kodiak Lab (ThalK) and 75.0% (SE = 7.0) at the Seward Marine Center (ThalS) (Fig. 1A and B, Table 3). The main effect of site was not significant and there was no diet site interaction (Table 2).

Table 2. Analysis of variance (ANOVA) for survival and duration of red king crab *Paralithodes camtschaticus* larvae.

	Effect	SS	d.f.	MS	*F*	*p*-value
Survival	Diet	13540.8	1	13540.8	47.96	<0.001
	Site	335.3	1	335.3	1.19	0.293
	Diet × site	500.1	1	500.1	1.77	0.203
	Residual	4234.7	15	282.3		
Duration	Diet	786.14	1	786.14	50.49	<0.001
	Site	16.54	1	16.54	1.06	0.319
	Diet × site	96.36	1	96.36	6.19	0.025
	Residual	233.55	15	15.57		

SS = sum of squares; d.f = degrees of freedom; MS = mean square.

Table 3. Survival rates of red king crab *Paralithodes camtschaticus* larvae in each replicate of diet and seawater treatments conducted in Kodiak and Seward, Alaska.

		Replicate						
		A	B	C	D	E	Total	
Treatment[a]	Stage[b]	% survival	% survival	% survival	% survival	% survival	Mean %	SE[c] %
ArtK	G	33	25	8	50	33	29.8	6.8
ArtK	C1	[d]	[d]	[d]	8	8	3.2	2.0
ThalK	G	100	100	92	[g]	83	93.8	4.1
ThalK	C1	33	100	75	[g]	42	62.5	15.4
ArtS	G	42	8	25	67	17	31.7	10.3
ThalS	G	67	75	100	58	75	75.0	7.0
ASW	G	33	8	33	33	50	31.7	6.7

[a]Treatments: see table 1 for treatment definitions.
[b]Stages: G = glaucothoe; C1 = first juvenile.
[c]Standard error.
[d]No molts to C1.
[e]1 molt to C1 on day 79.
[f]1 molt to C1 on day 77.
[g]Replicate dropped due to unexplained mortality not observed in any of the other replicates.

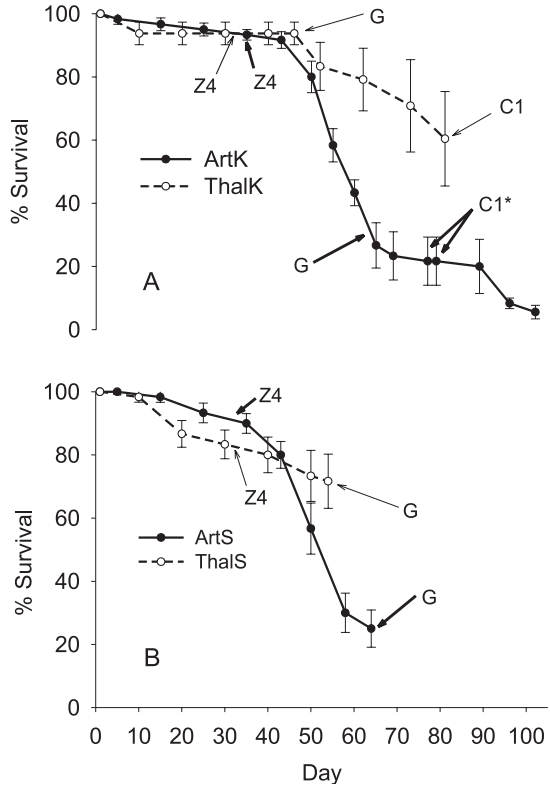

Figure 1. Mean percent survival of red king crab *Paralithodes camtschaticus* larvae in diet treatments in (A) Kodiak through C1 stage, and (B) Seward through glaucothoe stage. ArtK and ArtS are the newly hatched *Artemia* nauplii diets, and ThalK and ThalS are the newly hatched *Artemia* nauplii plus *Thalassiosira nordenskioeldii* diets. Arrows designate the mean day of molt with darker arrows for ArtK and ArtS and lighter arrows for ThalK and ThalS. Z4 is the molt to the zoea 4 stage, G is the molt to glaucothoe stage, C1 is the molt to the first juvenile crab stage, and C1* designates the two days when a molt occurred in the ArtK treatment.

Mean survival of all ArtK larvae to C1 was 3.2% (SE = 2.0), while mean survival of ThalK larvae to C1 was 62.5% (SE = 15.4) (Fig. 1A, Table 3). Survival to C1 was higher (d.f. = 7, $t = 4.31$, $p = 0.004$) when *T. nordenskioeldii* was included in the diet.

One of five replicates of the ThalK treatment experienced unexplained mortality on days 30 and 31. Survival dropped from 100% to 50% on day 30, and to 33% on day 31 at which point it remained constant for the rest

Table 4. Duration of red king crab *Paralithodes camtschaticus* larval development in each replicate of diet and seawater treatments conducted in Kodiak and Seward, Alaska.

		Replicate						
		A	B	C	D	E	Total	
Treatment[a]	Stage[b]	Days	Days	Days	Days	Days	Mean	SE[c]
ArtK	G	61	69	67	60	59	63.2	2.0
ArtK	C1	[d]	[d]	[d]	[e]	[f]	–	–
ThalK	G	46	46	45	[g]	46	45.6	0.3
ThalK	C1	74	74	74	[g]	81	75.8	1.8
ArtS	G	55	60	64	51	54	56.8	2.3
ThalS	G	47	48	48	54	45	48.4	1.5
ASW	G	51	52	57	49	45	50.8	2.0

[a]Treatments: see table 1 for treatment definitions.
[b]Stages: G = glaucothoe; C1 = first juvenile.
[c]Standard error.
[d]No molts to C1.
[e]1 molt to C1 on day 79.
[f]1 molt to C1 on day 77.
[g]Replicate dropped due to unexplained mortality not observed in any of the other replicates.

of the experiment. This abrupt increase in mortality was not observed in any of the other replicates and is attributed to causes other than the diet being tested. We did not include this replicate in the analysis.

Larval duration

For larval duration, the main effect of diet was significant. Larval duration to glaucothoe was shorter when *T. nordenskioeldii* was included in the diet (Tukey's HSD, $p < 0.001$). When fed *Artemia* nauplii only, overall duration was 60.0 days (SE = 1.3). At the Kodiak Lab (ArtK), the duration for all larvae to reach glaucothoe was 69 days; however, mean duration was 63.2 days (SE = 2.0) (Fig. 1A, Table 4). At the Seward Marine Center (ArtS), duration for all larvae to reach glaucothoe was 64 days, but mean duration was 56.8 days (SE = 2.3) (Fig. 1B, Table 4). When *T. nordenskioeldii* was included in the diet, overall duration was 47.1 days (SE = 1.3). At the Kodiak Lab (ThalK), duration for all larvae to reach glaucothoe was 46 days, with a mean duration of 45.6 days (SE = 0.3) (Fig. 1A, Table 4). At the Seward Marine Center (ThalS), duration for all larvae to reach glaucothoe was 53 days; however, mean duration was 48.4 days (SE = 1.5) (Fig. 1B, Table 4). The main effect of site was not significant, but there was a significant diet site interaction (Table 2). When fed *Artemia* nauplii only, the duration to reach glaucothoe was longer at the Kodiak

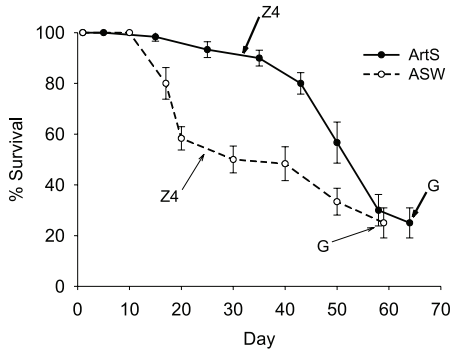

Figure 2. Mean percent survival of red king crab *Paralithodes camtschaticus* larvae in artificial and natural seawater treatments in Seward through the glaucothoe stage. ArtS is the natural seawater treatment with larvae fed a diet of newly hatched *Artemia* nauplii, and ASW is the artificial seawater treatment (Instant Ocean® Sea Salt) with larvae fed a diet of newly hatched *Artemia* nauplii. Arrows designate the mean day of molt with darker arrows for ArtS and lighter arrows for ASW. Z4 is the molt to the zoea 4 stage and G is the molt to glaucothoe stage.

Lab (ArtK) (63.2 days, SE = 2.0) than at the Seward Marine Center (ArtS) (56.8 days, SE = 2.3) (Tukey's HSD, $p = 0.022$) (Table 2).

Duration for all ArtK larvae to reach C1 could not be calculated because only two glaucothoe molted to C1; one glaucothoe in one replicate on day 77 and one glaucothoe in a second replicate on day 79 (Fig. 1A, Table 4). All other glaucothoe had died by day 103. Duration for all ThalK larvae to reach C1 was 81 days, but mean duration was 75.8 days (SE = 1.8).

Water source

Mean survival to glaucothoe was 31.7% (SE = 10.3) in natural and 31.7% (SE = 6.7) artificial seawater (Fig. 2, Table 3). Duration for all larvae to reach glaucothoe was 64 days in natural seawater (ArtS) and 56 days in artificial seawater (ASW); however, mean duration was similar and took 56.8 days (SE = 2.3) in natural seawater and 50.8 days (SE = 2.0) in artificial seawater (d.f. = 8, $t = 1.98$, $p = 0.083$) (Fig. 2, Table 4).

Discussion

Effect of diet on larval survival and duration

A diet limited to newly hatched *Artemia* was inferior to a diet of newly hatched *Artemia* and *Thalassiosira nordenskioeldii* microalgae for rearing red king crab larvae. The addition of *T. nordenskioeldii* promoted higher survival and a shorter larval duration. By the termination of the Kodiak Lab experiment, all glaucothoe had molted to C1 when fed *Artemia* and *T. nordenskioeldii*, while only two glaucothoe had molted to C1 when fed *Artemia* only (Fig. 1A, Table 4). The low survival and prolonged duration on the *Artemia*-only diet would not support successful small- or large-scale larval rearing to the juvenile crab stage.

Artemia nauplii are commonly used in fish and crustacean larval culture due to their availability, ease of culture, and acceptability as a prey item by larvae. Newly hatched *Artemia* nauplii have commonly been used as a food source in culturing red king crab larvae in the laboratory but survival and development have been variable and generally suboptimal (Kurata 1960, Kittaka et al. 2002, Kovatcheva 2002, Epelbaum and Kovatcheva 2005, Kovatcheva 2006, Persselin 2006). *Artemia* have little, if any, of the highly unsaturated fatty acids (HUFAs) docosahexaenoic acid (DHA, 22:6*n*-3) or eicosapentaenoic acid (EPA, 20:5n-3) that are considered crucial for the normal development and survival of crustacean larvae (Levine and Sulkin 1984; McConaugha 1985; Navarro et al. 1991, 1993; Anger 2001; Evjemo et al. 2001; Kogane et al. 2007).

Interestingly, diets with and without *T. nordenskioeldii* had similarly high survival until after reaching the Z4 stage, at which point mortality increased in treatments without diatoms (Fig. 1). This suggests that development at the Z4 stage is highly sensitive to diet. At the Kodiak Lab, survival in the *Artemia*-only treatment plummeted at the same time the ThalK larvae molted to glaucothoe, suggesting that the ArtK Z4 stage larvae had passed a critical point for molting successfully to glaucothoe. Larval duration was also affected by the absence of diatoms. Development to glaucothoe was prolonged in the ArtK Z4 stage larvae and only two glaucothoe molted to C1. The ArtS Z4 stage larvae also experienced prolonged development to glaucothoe and a more extreme increase in mortality than the ThalS Z4 stage larvae. The extended larval duration and increased mortality in the *Artemia*-only treatments suggest that king crab larvae require additional nutrients for successful and timely molting to the C1 stage. Kittaka et al. (2002) observed that lipid accumulation during zoeal stages is critical for glaucothoe survival in king crab. Lipid accumulation also appears necessary for successful larval development in other crab species. Larval mud crab (*Scylla serrata*) had lower survival and a prolonged intermolt period to the C1 stage when fed only *Artemia,* while larvae fed *Artemia* enriched with EPA or DHA had shorter intermolt periods (Suprayudi et al. 2004).

EPA and DHA were significant in promoting successful development to the megalopa stage (comparable to glaucothoe) of the mud crab (*Eurypanopeus depressus*) and EPA was found to maintain the survival rate and DHA to accelerate the intermolt period in larval swimming crab (*Portunus trituberculatus*) (Levine and Sulkin 1984, Takeuchi et al. 1999). Though it is unknown what levels of EPA and DHA are optimal for red king crab larval development, previous fatty acid analysis of *T. nordenskioeldii* suggested that it would provide an adequate amount (Kittaka et al. 2002).

Warm-water microalgae species such as *Isochrysis galbana* and *Nannochloropsis* spp. are typically used in aquaculture, including blue crab (*Callinectes sapidus*) larval culture, to provide essential fatty acids and other nutrients and to maintain water quality (Zmora et al. 2005). However, the cold-water species *T. nordenskioeldii* was chosen for this experiment because it is likely a component of the natural diet of red king crab larvae (Bright 1967, Shirley and Shirley 1989, Paul et al. 1989). Experiments conducted by Kittaka et al. (2002) indicated that combining *Thalassiosira* sp. with *Artemia* nauplii could provide increased survival to glaucothoe in red king crab; however, results were variable, ranging from 0% to 87.2%. Duration to the glaucothoe stage ranged from 27.9 to 35.0 days, with a mean of 29.7 days, at 8-10ºC (Kittaka et al. 2002). Larval duration on the combination diet was longer in our experiment likely due to the cooler culture temperature. With blue king crab larvae, the *T. nordenskioeldii–Artemia* nauplii diet produced high survival (91.7%) to the C1 stage (Stevens et al. 2008). Although the species are similar, the difference in survival may indicate different nutritional requirements. However, one ThalK replicate achieved 100% survival to C1, suggesting other factors may have contributed to mortality such as initial larval fitness or exposure to contaminants during culture. Survival in this replicate provides evidence that the addition of *T. nordenskioeldii* can promote optimal survival.

We also compared survival between facilities to test for location effects. Because larval survival was similar between the Kodiak Lab and the Seward Marine Center, seawater source was likely not the principal cause of the Seward Marine Center's low survival in 2007. Mortality may have been attributed to other factors such as fluctuating water flow and temperature, food presentation and nutritional quality, and larval rearing density. We noted a difference in larval duration between facilities in *Artemia*-only treatments despite attempts to duplicate temperature and light exposure and intensity at both locations. There were no differences in duration between facilities when *T. nordenskioeldii* was included in the diet. Slight differences in temperature and light parameters between facilities may have become significant over the longer duration of the *Artemia*-only treatments and contributed to the increased larval duration at the Kodiak Lab.

Effect of seawater source on larval survival and duration

Artificial seawater was not beneficial in terms of survival and larval duration when compared to natural seawater for rearing larvae to the glaucothoe stage. Zoeal survival declined much more rapidly in artificial seawater than natural seawater; however, the numbers of animals surviving to glaucothoe were similar. The cause of this difference in zoeal mortality rate is unknown and warrants further investigation should the use of artificial seawater become necessary.

Interest in using artificial seawater arose due to water quality concerns at the Seward Marine Center facility after initial cultivation attempts in 2007 resulted in lower than expected survival. A study conducted in 2001 found polyaromatic compound contamination in the Seward Marine Center seawater supply at levels reported to cause genetic damage to fish embryos (Rice et al. 2001, Duesterloh 2002). Artificial seawater is used for culture in facilities without access to natural seawater or to control seawater purity and has been used to successfully rear blue crab (*Callinectes sapidus*), red king crab, and other species (Cadman and Weinstein 1988, Konishi et al. 2002, Kovatcheva 2002, Xiao et al. 2003, Epelbaum and Kovatcheva 2005, Kovatcheva 2006, Zmora et al. 2005). Red king crab larvae previously reared in artificial seawater and on a diet of *Artemia* nauplii had an average larval duration of 39 days to the glaucothoe stage and an average survival to glaucothoe of 30.8% in 1 L beakers at 7-8ºC (Epelbaum and Kovatcheva 2005). Our results are consistent with these findings.

Conclusion

Although newly hatched *Artemia* nauplii provide an easily obtained and reared food source for red king crab larvae, they alone do not provide a diet adequate for producing high survival or timely development to the C1 stage. The addition of *Thalassiosira nordenskioeldii* increases survival and shortens larval duration likely due to essential fatty acids necessary for proper development. Further analysis of lipid content and fatty acid composition of *Artemia*, *T. nordenskioeldii,* and red king crab would help clarify the role of *T. nordenskioeldii* in larval development. A better understanding of these components would aid selection of the most feasible diet for large-scale culture. *Thalassiosira nordenskioeldii* is a slow-growing, cold-water species; a fast-growing, warm-water microalgae or a formulated enrichment product with a similar nutrient and fatty acid profile could be substituted to decrease costs in labor and time.

Artificial seawater does not increase larval survival when compared to natural seawater and our results suggest that inter-site variability in water quality was not the principal cause for reduced survival in previous Seward experiments. This finding is advantageous to large-scale

culture, as using natural seawater in a large-scale system eliminates the expense of artificial salts and the associated space, filtration, and labor requirements.

This research advances our understanding of larval culture parameters, informs the refinement of large-scale culture technique, and provides impetus for further research into improving the feasibility of large-scale larval production of king crab for stock rehabilitation.

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References

Anger, K. 2001. The biology of decapod crustacean larvae. Crustacean issues 14. A.A. Balkema, Lisse, p. 420.

Blau, S.F. 1986. Recent declines of the red king crab (*Paralithodes camtschatica*) populations and reproductive conditions around the Kodiak archipelago, Alaska. In: G.S. Jamieson and N. Bourne (eds.), North Pacific workshop on stock assessment and management of invertebrates. Can. Spec. Publ. Fish. Aquat. Sci. 92:360-369.

Bright, D. 1967. Life histories of the king crab, *Paralithodes camtschatica*, and Tanner crab, *Chionoecetes bairdi*, in Cook Inlet, Alaska. Ph.D. thesis, University of Southern California, Los Angeles. 265 pp.

Cadman, L., and M.P. Weinstein. 1988. Effects of temperature and salinity on the growth of laboratory-reared juvenile blue crabs *Callinectes sapidus* Rathbun. J. Exp. Mar. Biol. Ecol. 121:193-207. doi:10.1016/0022-0981(88)90088-3

Dew, C.B., and R.A. McConnaughey. 2005. Did trawling on the broodstock contribute to the collapse of Alaska's king crab? Ecol. Appl. 15:919-941. doi:10.1890/03-5231

Duesterloh, S. 2002. The role of copepods in the distribution of hydrocarbons: An experimental approach. Ph.D. thesis, University of Alaska Fairbanks. 182 pp.

Epelbaum, A.B., and N.P. Kovatcheva. 2005. Daily food intakes and optimal food concentrations for red king crab (*Paralithodes camtschaticus*) larvae fed *Artemia* nauplii under laboratory conditions. Aquacult. Nutr. 11:455-461. doi:10.1111/j.1365-2095.2005.00374.x

Evjemo, J.O., T.L. Danielsen, and Y. Olsen. 2001. Losses of lipid, protein and n-3 fatty acids in enriched *Artemia franciscana* starved at different temperatures. Aquaculture 193:65-80. doi:10.1016/S0044-8486(00)00470-1

Kittaka, J., B.G. Stevens, S. Teshima, and M. Ishikawa. 2002. Larval culture of the king crabs *Paralithodes camtschaticus* and *P. brevipes*. In: A.J. Paul, E.G. Dawe, R. Elner, G.S. Jamieson, G.H. Kruse, R.S. Otto, B. Sainte-Marie, T.C. Shirley, and D. Woodby (eds.), Crabs in cold water regions: Biology, management, and economics. Alaska Sea Grant College Program, University of Alaska Fairbanks, pp. 189-209.

Kogane, T., S. Dan, and K. Hamasaki. 2007. Improvement of larval rearing technique for mass seed production of snow crab *Chionoecetes opilio*. Fish. Sci. 73:851-861. doi:10.1111/j.1444-2906.2007.01406.x

Konishi, K., T. Matsumoto, and R. Tsujimoto. 2002. The complete larval development of *Chionoecetes japonicus* under laboratory conditions. In: A.J. Paul, E.G. Dawe, R. Elner, G.S. Jamieson, G.H. Kruse, R.S. Otto, B. Sainte-Marie, T.C. Shirley, and D. Woodby (eds.), Crabs in cold water regions: Biology, management, and economics. Alaska Sea Grant College Program, University of Alaska Fairbanks, pp.135-146.

Kovatcheva, N. 2002. Observations on rearing red king crab (*Paralithodes camtschaticus*) zoea and glaucothoe in a recycling water system. In: A.J. Paul, E.G. Dawe, R. Elner, G.S. Jamieson, G.H. Kruse, R.S. Otto, B. Sainte-Marie, T.C. Shirley, and D. Woodby (eds.), Crabs in cold water regions: Biology, management, and economics. Alaska Sea Grant College Program, University of Alaska Fairbanks, pp. 273-282.

Kovatcheva, N. 2006. Red king crab (*Paralithodes camtschaticus*) reproduction and cultivation in artificial conditions in Russia. In: B.G. Stevens (ed.), Alaska crab stock enhancement and rehabilitation: Workshop proceedings. Alaska Sea Grant College Program, University of Alaska Fairbanks, pp. 15-22.

Kovatcheva, N., A. Epelbaum, A. Kalinin, R. Borisov, and R. Lebedev. 2006. Early life history stages of the red king crab *Paralithodes camtschaticus* (Tilesius, 1815). VNIRO Publishing, Moscow. 115 pp.

Kurata, H. 1960. Studies on the larva and post-larva of *Paralithodes camtschatica,* II. Feeding habits of the zoea. Bull. Hokk. Reg. Fish. Res. Lab. 21:1-8.

Kuris, A.M., S.F. Blau, A.J. Paul, J.D. Shields, and D.E. Wickham. 1991. Infestation by brood symbionts and their impact on egg mortality in the red king crab, *Paralithodes camtschatica*, in Alaska: Geographic and temporal variation. Can. J. Fish. Aquat. Sci. 48:559-568. doi:10.1139/f91-071

Levine, D.M., and S.D. Sulkin. 1984. Nutritional significance of long-chain polyunsaturated fatty acids to the zoeal development of the brachyuran crab, *Eurypanopeus depressus* (Smith). J. Exp. Mar. Biol. Ecol. 81:211-223. doi:10.1016/0022-0981(84)90141-2

Marukawa, H. 1933. Biological and fishery research on Japanese king crab, *Paralithodes camtschatica* (Tilesius). J. Imp. Fish. Exp. St. (Tokyo) 37:1-152.

McConaugha, J.R. 1985. Nutrition and larval growth. In: A.M. Wenner (ed.), Larval growth. Crustacean issues 2. A.A. Balkema, Rotterdam, pp. 127-154.

Navarro, J.C., F. Amat, and J.R. Sargent. 1991. A study of the variations in lipid levels, lipid class composition and fatty acid composition in the first stages of *Artemia* sp. Mar. Biol. 111:461-465. doi:10.1007/BF01319419

Navarro, J.C., R.S. Batty, M.V. Bell, and J.R. Sargent. 1993. Effects of two *Artemia* diets with different contents of polyunsaturated fatty acids on the lipid-composition of larvae of Atlantic herring (*Clupea harengus*). J. Fish Biol. 43:503-515.

Orensanz, J.M.L., J. Armstrong, D. Armstrong, and R. Hilborn. 1998. Crustacean resources are vulnerable to serial depletion: The multifaceted decline of crab and shrimp fisheries in the greater Gulf of Alaska. Rev. Fish Biol. Fish. 8:117-176. doi:10.1023/A:1008891412756

Otto, R.S., R.A. MacIntosh, and P.A. Cummiskey. 1990. Fecundity and other reproductive parameters of female red king crab (*Paralithodes camtschatica*) in Bristol Bay and Norton Sound, Alaska. In: Proceedings of the International Symposium on King and Tanner Crabs. Alaska Sea Grant College Program, University of Alaska Fairbanks, pp. 65-90.

Paul, A.J., and J.M. Paul. 1990. Growth of stage I king crab larvae of *Paralithodes camtschatica* (Tilesius) (Decapoda: Lithodidae) in natural communities. J. Crustac. Biol. 10:175-183. doi:10.2307/1548479

Paul, A.J., J.M. Paul, and K.O. Coyle. 1989. Energy sources for first-feeding zoeae of king crab *Paralithodes camtschatica* (Tilesius) (Decapoda, Lithodidae). J. Exp. Mar. Biol. Ecol. 130:55-69. doi:10.1016/0022-0981(89)90018-X

Persselin, S. 2006. Cultivation of king crab larvae at the Kodiak Fisheries Research Center, Kodiak, Alaska. In: B.G. Stevens (ed.), Alaska crab stock enhancement and rehabilitation: Workshop proceedings. Alaska Sea Grant College Program, University of Alaska Fairbanks, pp. 9-14.

Rice, S.D., R.E. Thomas, M.G. Carls, R.A. Heintz, A.C. Wertheimer, M.L. Murphy, J.W. Short, and A. Moles. 2001. Impacts to pink salmon following the *Exxon Valdez* oil spill: Persistence, toxicity, sensitivity, and controversy. Rev. Fish. Sci. 9:165-211. doi:10.1080/20016491101744

Shirley, S.M., and T.C. Shirley. 1989. Interannual variability in density, timing, and survival of Alaskan red king crab *Paralithodes camtschatica* larvae. Mar. Ecol. Prog. Ser. 54:51-59. doi:10.3354/meps054051

Stevens, B.G., and K.M. Swiney. 2007. Hatch timing, incubation period, and reproductive cycle for primiparous and multiparous red king crab *Paralithodes camtschaticus* (Tilesius, 1815). J. Crustac. Biol. 27:37-48. doi:10.1651/S-2663.1

Stevens, B.G., S. Persselin, and J. Matweyou. 2008. Survival of blue king crab *Paralithodes platypus* Brandt, 1850, larvae in cultivation: Effects of diet, temperature, and rearing density. Aquacult. Res. 39:390-397. doi:10.1111/j.1365-2109.2007.01798.x

Suprayudi, M.A., T. Takeuchi, and K. Hamasaki. 2004. Essential fatty acids for larval mud crab *Scylla serrata*: Implications of lack of ability to bioconvert C18 unsaturated fatty acids to highly unsaturated fatty acids. Aquaculture 231:403-416. doi:10.1016/S0044-8486(03)00542-8

Takeuchi, T., N. Satoh, S. Sekiya, T. Shimizu, and T. Watanabe. 1999. The effect of dietary EPA and DHA on the molting rate of larval swimming crab *Portunus trituberculatus*. Nippon Suisan Gakk. 65:998-1004.

Xiao, G., L. Pan, Z. Luan, H. Wang, and G. Wang. 2003. Impact of water quality adjustment technology on *Eriocheir sinensis* breeding in Sanila. Mar. Sci. 27:1-4.

Zheng, J., and G.H. Kruse. 2000. Recruitment patterns of Alaskan crabs in relation to decadal shifts in climate and physical oceanography. ICES J. Mar. Sci. 57:438-451. doi:10.1006/jmsc.1999.0521

Zmora, O., A. Findiesen, J. Stubblefield, V. Frenkel, and Y. Zohar. 2005. Large-scale juvenile production of the blue crab *Callinectes sapidus.* Aquaculture 244:129-139. doi:10.1016/j.aquaculture.2004.11.012