Projecting Range Expansion of Invasive European Green Crabs (*Carcinus maenas)* to Alaska: Temperature and Salinity Tolerance of Larvae

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Research Report

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Prince William Sound Regional Citizens' Advisory Council
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EXECUTIVE SUMMARY

The European Green Crab (*Carcinus maenas*) is a global invader, successfully colonizing many world regions and having significant ecological and economic impacts. The Green Crab colonized western North America in the late 1980s, spreading primarily northward from the initial establishment in San Francisco Bay to several other bays in northern California, Oregon, and Washington. Initial analysis, based largely upon temperature tolerance of postlarval crabs, suggests Green Crabs will continue to spread and become established throughout much of Alaska. However, establishment of self-sustaining populations in Alaska may be restricted by environmental conditions for reproduction and larval development, instead of the broad tolerances of postlarval crabs. Using laboratory experiments, we tested conditions required for successful development of Green Crab larvae. We collected ovigerous Green Crabs from California and Maine, and cultured larval stages under various temperature and salinity conditions, measuring conditions necessary for survival and the length of time required for successful development (i.e., metamorphosis to postlarval crab stage). Our laboratory experiments indicate poor larval survivorship and development at temperatures below 10^{o}C and salinities below 20 ppt. Based upon temperature-specific development rates, several sites within Prince William Sound and elsewhere in Alaska appear warm enough to support self-sustaining Green Crab populations, even though larval tolerances are more restrictive than those for adult crabs. Coupled with northward natural dispersal and ship-mediated transfer in ballast water, our data indicate Alaska is at risk to invasion by Green Crabs. The extent to which biotic interactions (e.g., competition, predation, etc.) may affect colonization success and population sizes remain unresolved.

ACKNOWLEDGEMENTS

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INTRODUCTION

One of the greatest research challenges of invasion biology is to predict the introduction, establishment and spread of alien species outside their native range. As a result, invasions in marine ecosystems have been termed "ecological roulette" (Carlton & Geller 1993). Nevertheless, certain species have established invasive populations in several locations around the world, and these "repeat invaders" provide opportunities to assess factors that promote invasions. Such repeat invaders are typically characterized by life-stages that: interface with established transport systems; are ecologically adaptable; and have broad physiological tolerances. Once established, an invasive population often expands its range to the limits of its physiological tolerances set by climate and/or to biogeographic barriers created by ocean current systems. Human-mediated transport may facilitate range expansion at a faster rate than natural dispersal and may surpass natural barriers.

This study explores an approach to projecting the potential range expansion of a repeat invader – the European Green Crab (*Carcinus maenas*) - into Alaskan coastal waters from newly established populations in central California, Oregon and Washington. The ecological and economic impacts of invasive Green Crab populations have considerable intrinsic interest. Moreover, the Green Crab serves as a model for evaluating risk of invasive species spreading to Alaska by natural dispersal and further human-mediated transport along the West Coast of North America. Of particular concern is the potential for range expansion via organisms transported in ballast water of oil tankers traveling to Port Valdez from West Coast port systems (Los Angeles/Long Beach, San Francisco Bay, Puget Sound) that are highly invaded. Our earlier work (Hines & Ruiz 2000) showed that Prince William Sound is at risk of invasion because of the dense and diverse plankton in ballast water released by oil tanker traffic.

Carcinus maenas is native to Europe from Norway to Mauritania (Williams 1984) and has invaded several locations around the world (Carlton and Cohen 2003). Introduced populations have been established in Australia (Zeidler 1978, 1988; Rosenzweig 1984), South Africa (Le Roux et al. 1990) and on both coasts of North America (Cohen et al. 1995; Grosholz and Ruiz 1995; Glude 1955; Ropes 1968; Welch 1968). A new introduction was discovered in 1989 in San Francisco Bay on the West Coast of North America (Cohen et al. 1995, Fincham 1996) and rapidly expanded its range primarily northward to Washington State and possibly Vancouver Island, with only a small southerly range expansion in central California. Recently (1998-1999), the long-established population on the East Coast of North America suddenly expanded its range northward around Nova Scotia and into the Gulf of St. Lawrence (Audet et al. 2003, Carlton & Cohen 2003). Thus, natural dispersal of Green Crabs along the West Coast may allow this invasive population to spread to Alaska.

On both coasts of North America, this invasive crab has been shown to reduce the populations of native shellfish and other invertebrates, which are prey items for native crabs and shorebirds (Ropes 1968; Grosholz & Ruiz 1995; Grosholz et al. 2000; Walton 2003). By changing predator-prey dynamics, invasive green crabs have the ability to alter ecosystem function significantly, and to reduce populations of economically important species.

Cohen et al. (1995) concluded that temperature explained the general latitude limits of *Carcinus maenas* in the Atlantic, with equatorial limits characterized by average summer surface temperature limits of about 22°C, and polar limits by average winter ocean surface temperatures of –1°C to 0°C. However, we hypothesize that lower temperature limits of self-sustaining populations may be set by tolerances for reproduction and larval development instead of the very broad tolerances of adults. Larval tolerances of *C. maenas* have been well studied in Europe (Nagaraj 1993; Mohamedeen & Hartnoll 1989; Dawirs 1982; Dawirs 1985). However, all experiments to date have been performed using crabs from native populations. No experiments have been conducted on larval tolerance from the long-established invasive population on the East Coast of the North America or on the more recently invasive population on the West Coast. Furthermore, previous studies used small sample sizes, tested only a few temperatures, and rarely interpreted the results within an ecological framework. Using similar methods as the European studies, we investigated the salinity and temperature limits of *Carcinus* larvae from two invasive populations: Northern California and Maine. We used this information to project potential range expansion of *Carcinus* on the West Coast and to predict whether or not the crabs will complete development and survive in the environmental conditions of coastal Alaska.

Natural History, Life Cycle and Tolerances:

Green Crabs are found throughout a range of habitats and environmental conditions. They inhabit subtidal to intertidal zones of rocky coasts to soft-bottomed estuaries, embayments, and marshes (Crothers 1968; Grosholz & Ruiz 1995). Moreover, they have broad thermal and salinity tolerances. Green Crabs are found in areas with average temperatures of 22°C to -1°C (Cohen et al. 1995). A maximum temperature for survival of adult crabs caught in summer and acclimated to 22°C is about 35°C, but is lower for crabs caught in autumn or winter and for crabs acclimated to lower temperatures (Cuculescu et al. 1998). Low temperatures below 7-10°C inhibit adult feeding and growth (Ropes 1968; Berrill 1982), and trigger die offs and partial migration to deeper, warmer, more saline water (Broekhuysen 1936: Naylor 1962; Cothers 1968; Welch 1968). Adult crabs are generally found in salinities between 10 and 33 ppt, but can be found in fresh-water flooded intertidal zones with salinities down to 1.4ppt (Broekhuysen 1936; Crothers 1968; Perkins 1969).

The reproductive cycle of the Green Crab progresses predictably across temperature, but its timing varies with location. Copulation occurs when the female is soft after molting (Broekhuysen 1936). Copulation and molting occur only in warmer months in colder areas (Berrill 1982), but may occur throughout the year in warmer areas (Broekhuysen 1936; Naylor 1962). After copulation, an egg mass appears in 1-4.5 months (Broekhuysen 1936). Release of larvae appears to be restricted to the spring, summer, and fall, with development of eggs slowed or stopped in cold winter months (Broekhuysen 1936; Crothers 1967; Williams 1984). Breeding has been successful in salinities as low as 13 ppt (Dries & Adelung 1982, from Cohen et al. 1995).

Green Crab larvae develop through four zoeal stages (ZI-ZIV) and one megalopa stage (M) (Rice & Ingle 1975), and then metamorphose into juvenile crabs (C1), which grow through molting to adult crab stages. Larvae are more sensitive to environmental conditions than adult crabs, and adults do not reproduce throughout the whole range of salinities and temperatures that non-reproductive adults inhabit. In lab experiments, the minimum salinity for successful larval

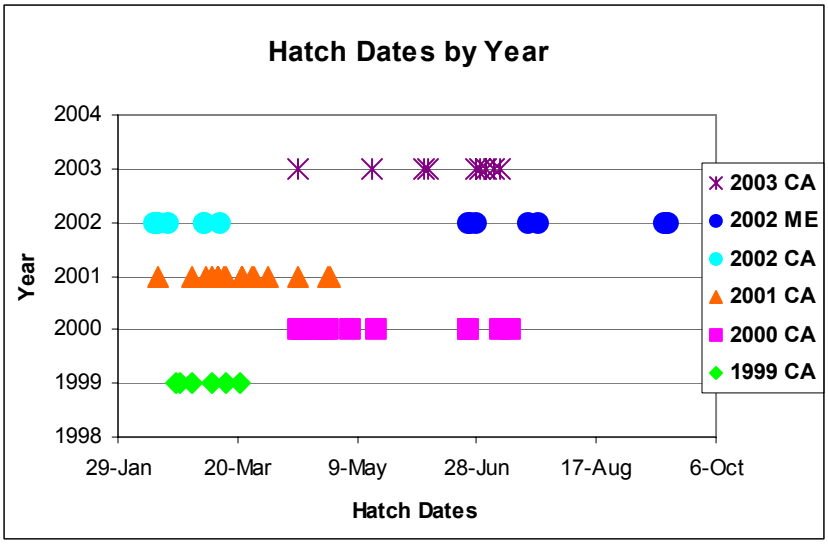
development was 20 ppt (Broekhuysen 1936). While most experiments have only tested larval development above 10°C, Dawirs (1985) reported that at 6°C zoea I does not develop to zoea II and that development to juvenile crab is only achieved at 12°C and above. Nagaraj (1993) reported successful larval development at 10°C and above.

METHODS

Collection and Care of Ovigerous Crabs:

Ovigerous female crabs were collected in the field using baited traps. Crabs were collected in California from Bodega Bay (1999-2001) and Tomales Bay (2002-2003) in the winter (January to March) and spring (April to June, 2002). Crabs were also collected in Maine from Casco Bay in summer (June to August 2002). Upon arrival at the Smithsonian Environmental Research Center, crabs were placed in sea water (30ppt) in individual aquaria in a cold room with a 12hrs light, 12hrs dark photoperiod. Ovigerous crabs were maintained at 15.0ºC in 1999 and 2000, both 8.0ºC and 15.0ºC in 2001, and 12.5ºC in 2002 and 2003. Dates were recorded for each brood of eggs hatched in the laboratory (Fig. 1). Upon hatching, larvae were removed from the aquaria and placed into experiments.

Figure 1: Hatch Dates of *Carcinus* larvae for all years.



Larvae:

Recently hatched, actively swimming larvae were captured with a plastic pipette and placed into glass or polystyrene culture dishes containing seawater of ambient temperature and salinity. Water for the experiments was obtained from the Rhode River (a subestuary of Chesapeake Bay), and adjusted to the proper salinity with artificial salt and filtered to 0.01 micron. An antibiotic, antifungal solution (100 mg/l each of Penicillin, Streptomycin, Chloramphenicol) was added to the water before employing it in the experiments. Groups of larvae were then stepped up or down to acclimate slowly (1ºC or 1ppt per hour**)** to their experimental salinity and/or temperature and placed in incubators with a 12L/12D photoperiod. Larvae were fed and their water changed every other day. Their diet consisted of algae (rotating cultures of *Nannochloropsis*, *Isochrysis* and *Tetraselmus*), rotifers (*Brachionis plicatilis*) and

newly hatched *Artemia* nauplii. The mixture of food types and sizes accommodated the nutritional needs of all larval stages. Treatment dishes were examined daily and data on mortality and molting were recorded. Molts and dead larvae were noted and removed from the treatment dishes.

During the first three years, larvae from Northern California were raised and monitored in groups of 10 individuals within a glass culture dish (100 ml), with each group of 10 larvae constituting a replicate. Mortality and development time to megalopae and juvenile crab stage were recorded. During the last 2 years, larvae were raised and monitored individually in polystyrene parts boxes (20 ml water per compartment), allowing us to record development and survivorship for each zoeal stage as individuals. In both cases, larvae that reached the megalopae stage were separated from the developing zoea and placed in larger containers (40 ml water), provided more food and given a piece of Nitex mesh upon which to settle. The water volume and food was again increased (80 ml, and pieces of shrimp or squid were added) for juvenile crabs.

Salinity Tolerance Experiments:

Full factorial experiments for temperature X salinity were conducted with larvae obtained from ovigerous crabs collected in Bodega Bay, CA (Table 1). In 1999, theses experiments included all combinations of four temperatures (5, 15, 25, 30 ^{o}C) and four salinities (5, 10, 20, 30 ppt). Experiments run in 2000 tested combinations of four low temperatures (5, 7.5, 10, 12.5 ^{o}C) and the two high salinities (20, 30 ppt). For each treatment combination, we tested three replicate groups of 10 larvae hatched from of each of 3-5 broods, such that there were 9-15 groups tested at each temperature X salinity combination.

Table 1: Salinity Experiments 1999 and 2000.

1999	Salinity			
Temp	**5**	**10**	**20**	**30**
5.0	X	X	X	X
15.0	X	X	X	X
25.0	X	X	X	X
30.0	X	X	X	X

2000	Salinity	
Temp	**20**	**30**
5.0	X	X
7.5	X	X
10.0	X	X
12.5	X	X

Constant Temperature Tolerance Experiments:

Temperature tolerance experiments were conducted seasonally over a five year period using larvae obtained from ovigerous female crabs collected from two invasive populations: California (all years) and Maine (year 2002 only) (Table 2). Experiments in the first year (1999) focused on salinity treatments and high and low extreme temperatures. The second year (2000) focused on lower temperatures and higher salinities. The third year (2001) tested not only the effect of exposure temperature on the larvae, but also the effect of acclimation temperature of the ovigerous females. In the fourth year (2002), larval development at relatively mid to high temperatures was compared between source populations of crabs from Northern California and Maine. In the fifth year (2003) California larvae were raised at cold temperatures to test for a potential lower threshold of development. For each treatment combination during 1999-2001, we tested three replicate groups of 10 larvae hatched from of each of 3-5 broods, such that there

were 9-15 groups tested at each temperature treatment. In 2002-2003, equivalent numbers of larvae and broods were tested, but the larvae were maintained individually in compartments instead of in groups of 10 larvae per bowl.

Table 2: Constant Temperature Experiments, all years.

	Population		Acclimation Temperature (ºC)			Constant Temperature (ºC) Treatments (Salinity = 30)											
Year	**CA**	**ME**	**8.0**	**12.5**	**15.0**	**4.0**	**5.0**	**6.0**	**7.0**	**7.5**	**10.0**	**12.5**	**15.0**	**17.5**	**20.0**	**22.5**	**25.0**	**30.0**
1999	X				X		X						X				X	X
2000	X		X		X		X			X	X	X						
2001	X		X		X		X			X	X	X	X					
2002	X			X						X	X	X	X	X	X	X	X	X
2002		X		X							X	X	X	X	X	X	X	
2003	X			X		X	X	X	X				X					

Variable Temperature Tolerance Experiments:

In 2002, we conducted variable temperature experiments with larvae from the Maine crabs (Table 3) that were run concurrently with the constant temperature experiments. Dishes of larvae were moved from one constant temperature incubator to another to change the temperature after 7-, 14-, and 21-day intervals of larval development. Starting temperatures differed by increments of 2.5^{o}C over the range from 10-17.5^{o}C, which was the mid-range of successful development as determined in constant temperature tests. At the 3 time intervals, temperatures were either increased or decreased by increments of 2.5^{o}C. Control treatments were also run, in which larvae remained at constant temperature (10, 12.5, 15, 17.5 ^{o}C) throughout the experiment.

Table 3: 2002 Maine Variable Temperature Experiments.

Start Temp	End Temp	Day Moved		Start Temp	End Temp	Day Moved
10.0	10.0	control		15.0	15.0	control
10.0	12.5	7		15.0	12.5	7
10.0	12.5	14		15.0	12.5	14
10.0	12.5	21		15.0	12.5	21
12.5	12.5	control		15.0	17.5	7
12.5	10.0	7		15.0	17.5	14
12.5	10.0	14		15.0	17.5	21
12.5	10.0	21		17.5	17.5	control
12.5	15.0	7		17.5	15.0	7
12.5	15.0	14		17.5	15.0	14
12.5	15.0	21		17.5	15.0	21

Analyses:

Survivorship and development time were the two measures used in these experiments to quantify larval tolerance. Survivorship was calculated as the proportion of total larvae surviving to a particular stage (Zoea I to IV, Megalopae, Juvenile Crab). Mean survivorship to a given

stage at a given temperature X salinity treatment was compared between ovigerous females (mothers) for each year. Development time was calculated as the number of days it took a larva to reach a particular stage. Mean development time at each temperature was compared between mothers as well as between temperatures for each mother. We tested the fit of two models for development time as a function of temperature: a Day-Degree Model and Dawir's Model (see below).

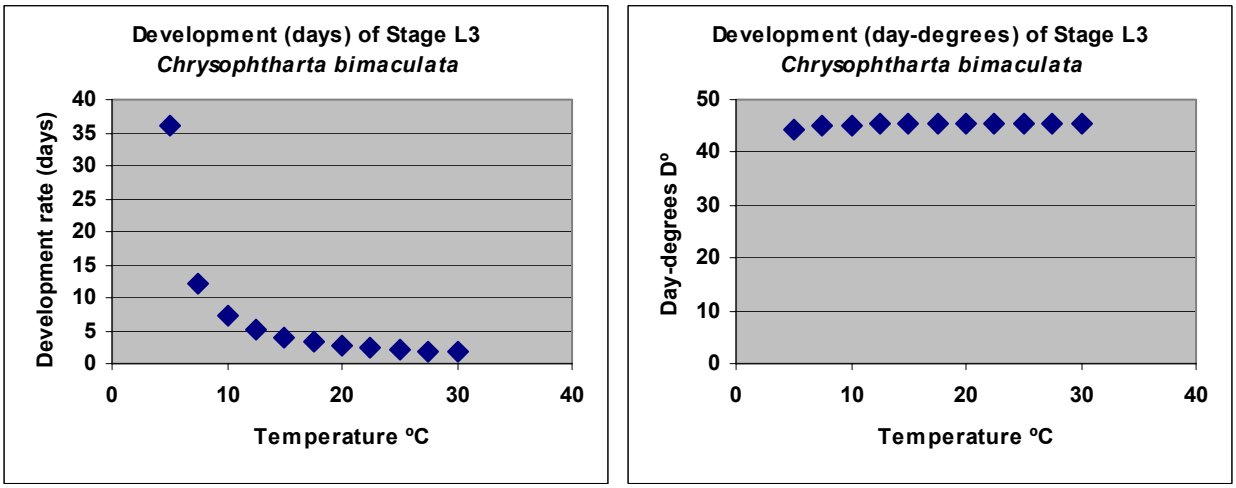
Thermal Requirement - Day-Degrees Model

Physiological time is a concept that has been used primarily in insect developmental ecology and agricultural pest management (Baskerville & Emin 1969) and has been employed recently in fisheries studies (Wilson & Barnett 1983). The concept considers that development of an organism proceeds only between upper and lower threshold temperatures, and that within these thresholds the total thermal requirement for development to a particular stage is independent of temperature (see Fig. 2). The time-at-temperature combination above its lower thermal threshold can be expressed in day-degrees (Dº).

*Day-degrees = days at stage * (temperature – lower threshold temperature)*

For example, if an organism with a lower threshold of 9º spends 5 days at 10º and then 5 days at 15º, its total accumulation is 35 Dº [= (5 days * (10º-9º))+(5 days * (15º-9º))] (Begon 1986). This method of determining the total thermal requirement for an organism provides a robust general model for predicting development rate in any temperature regime.

Figure 2: from Clarke, 1998. Development rate in days (left) and Day-Degree Estimate (right) for the Immature Stage L3 of the Tasmanian Eucalyptus Leaf Beetle, Chrysophtharta bimaculata (Olivier) (Coleoptera: Chrysomelidae).



Dawirs' Model

Dawirs (1985) developed a regression model specific to *Carcinus maenas* to predict development in the wild based on constant temperature experiments in the lab. Using data from four temperatures (12, 12.5, 18, 25 ºC), he determined development time to each zoeal stage and computed the regression equation: $\ln D = a - b \ln T$ where D is development duration (days), T is water temperature (ºC), a is a constant and b is the regression coefficient. To test the model,

he then kept larvae at a regime that mimicked field temperatures. Using the above equation, Dawirs predicted within 2 days the development time for each larval stage during this simulation experiment.

Projection of Thermal Requirements to Field Temperatures

We compared the thermal requirements for Green Crab larval development in the laboratory to field temperature conditions estimated from NOAA buoy data for Sea Surface Temperature at 11 sites along the Alaskan coast from Ketchikan to Unalaska Island.

RESULTS

Salinity Tolerance Experiments:

No larvae survived beyond first zoea (ZI) in salinities of 5 ppt or 10 ppt at any temperatures, or at any of the test salinities at 30ºC (Table 5). Larvae raised at 20 ppt survived to megalopae at 15 and 25ºC; however, larval development to juvenile crab was only achieved at 15ºC. Zoeae had poor survival (only 7%) survived at 5ºC and 30 ppt salinity and did not develop. When those few larvae that did survive at 5ºC were moved to 15ºC after 10 weeks, they subsequently resumed development to megalopae and juvenile crabs at the warmer temperature, indicating that their arrested development at low temperature was reversible. Development proceeded to megalopae (M) and juvenile crab (C1) stages at higher test salinities (20 and 30 ppt). Survivorship was significantly higher for larvae raised at 30 ppt than at 20 ppt (Fig. 3). However, larvae raised at 20 ppt developed faster than larvae raised at 30 ppt (Fig. 4). Unfortunately, overall survivorship was very low in 2000 (possibly due to a later collecting date of ovigerous crabs compared to 1999) making salinity results for that year inconclusive.

Table 5: Results of Salinity Experiments 1999 and 2000.

1999	Salinity			
Temp	**5**	**10**	**20**	**30**
5.0	NS	NS	NS	**Z**
15.0	NS	NS	**C**	**C**
25.0	NS	NS	**M**	**C**
30.0	NS	NS	NS	NS

2000	Salinity	
Temp	**20**	**30**
5.0	NS	**Z**
7.5	NS	**Z**
10.0	NS	**C**
12.5	NS	**C**

Legend:
NS = no survivors
Z = survived to zoea II or higher
M = survived to megalopae
C = survived to juvenile crab

Figure 3: 1999 CA Survivorship to Megalopae and Juvenile Crab

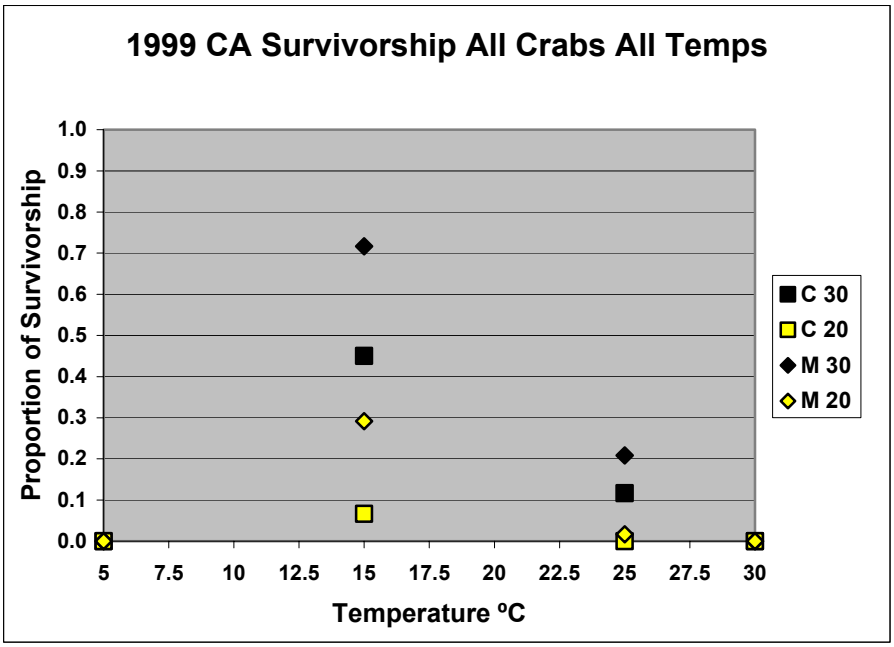
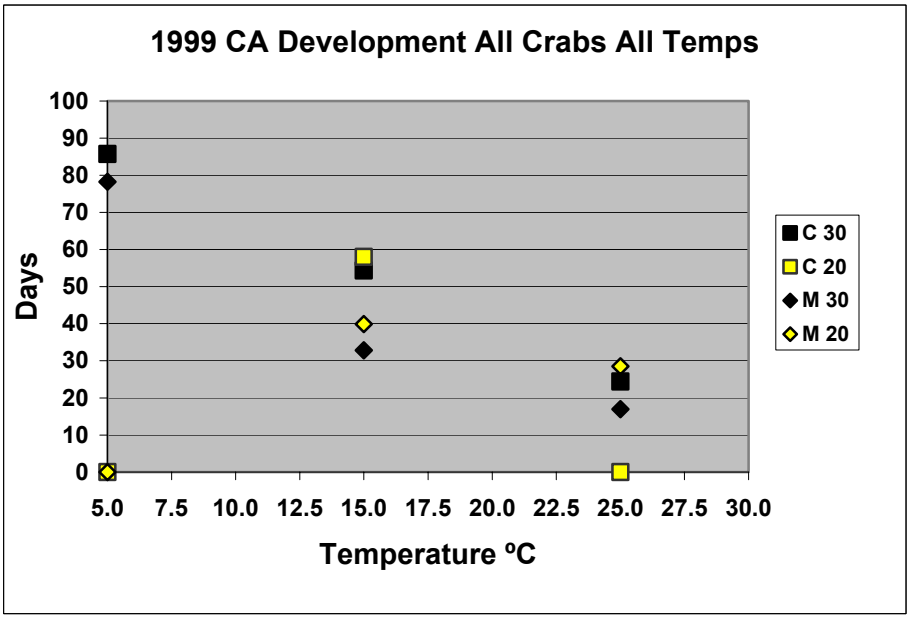


Figure 4: 1999 CA Development Time to Megalopae – Temperature and Salinity



Survivorship versus Temperature:

For both population sources of crabs, no development to juvenile crab occurred below 10°C, and survivorship to C1 approached 0% at 10°C. Survivorship of larvae exhibited a bell-shaped function of temperature (Figs. 5, 6). For California crabs, maximum survival of 65% for megalopae and 40% for C1 occurred at 15-17.5ºC (Fig. 5). Survivorship to C1 dropped below 10% at low temperatures of 10ºC and high temperatures of 22.5ºC. For Maine crabs, the survivorship response curve was more irregular (Fig. 6). Maine megalopae did not survive at

10ºC, and the range of temperatures for best survival (about 40%) of megalopae was shifted a bit higher to 15-20ºC; however, overall survival to C1 was generally low (<10%) except for a peak of 25% at 20ºC.

Figure 5: 2002 CA Survivorship to Megalopae and Juvenile Crab.

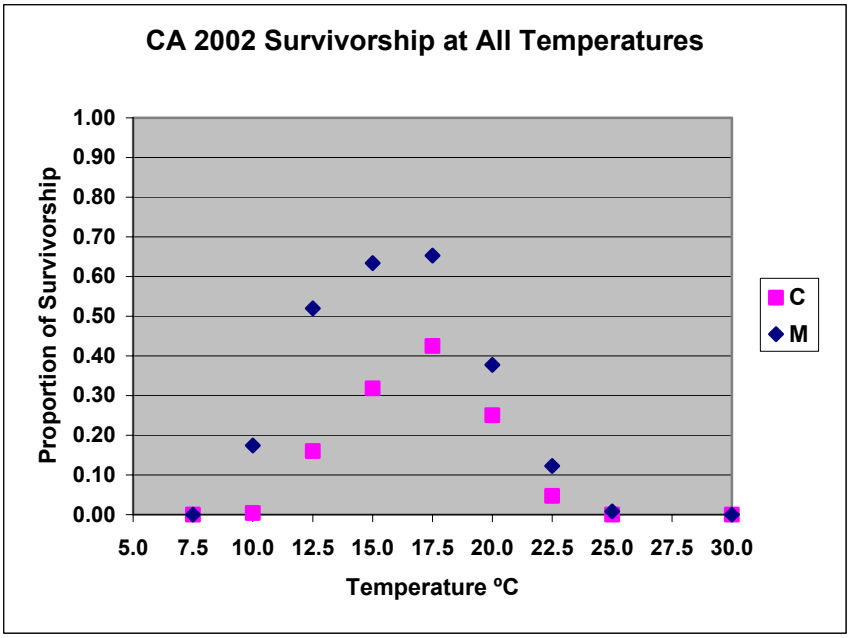
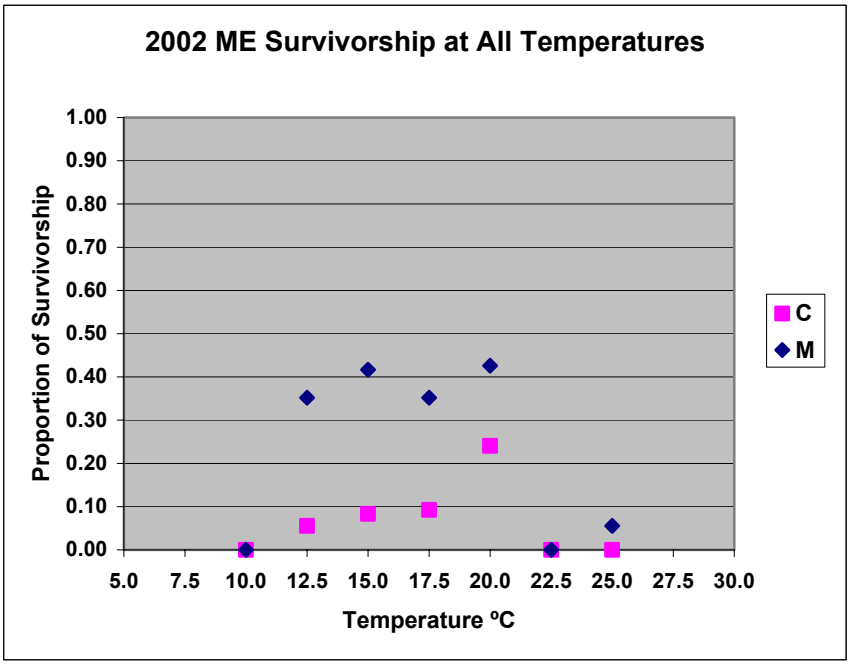


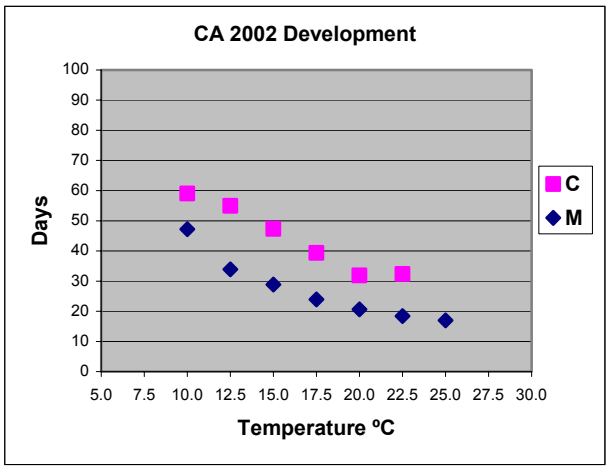
Figure 6: 2002 ME Survivorship to Megalopae and Juvenile Crab.

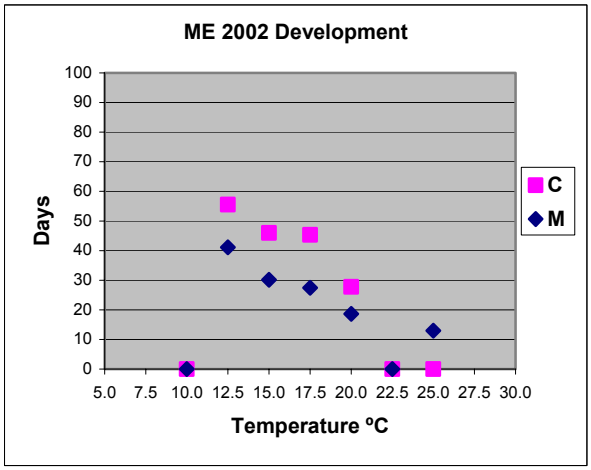


Development Time as a Function of Temperature:

For both population sources of crabs, development time at constant temperatures from 10-25ºC decreased with increasing temperature (Figs. 7, 8). For California crabs, development time to C1 required about 60 days at 10°C and approached 30 days at 22°C (Fig. 7). The Maine population required about 56 days to reach C1 at 12.5ºC, but development to megalopae did not proceed at 10ºC (Fig. 8).

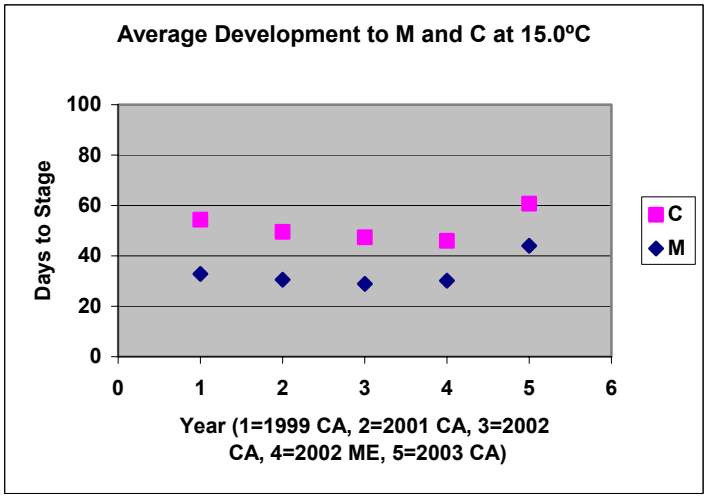
Figure 7 (left): 2002 CA Development Time to Megalopae and Juvenile Crab.
Figure 8 (right): 2002 ME Development Time to Megalopae and Juvenile Crab.





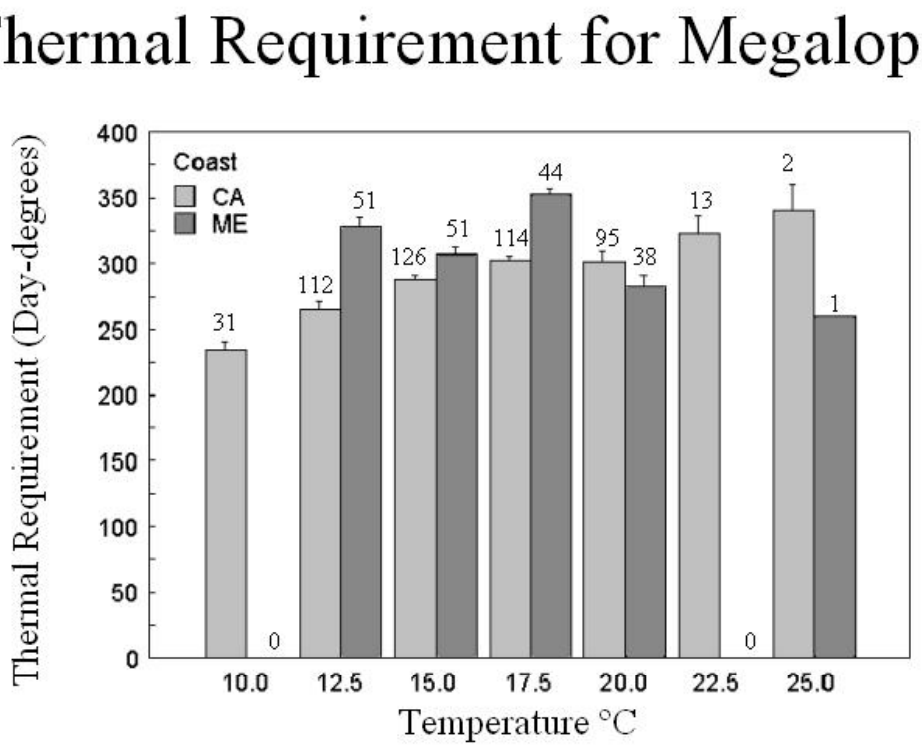
Across both population sources, development time at the "optimal" constant temperature of 15°C varied among years from 29-43 days for megalopae and 47-62 days for C1 (Fig. 9).

Figure 9: Development Time - all years - 15ºC.



Development times in variable temperature experiments were similar to those in the constant temperature experiments, and the effect of varying time interval at the start temperature was not significant (Fig. 10). However, rate of development to juvenile crab was faster with more time spent at higher temperature.

Figure 10: 2002 CA and ME Constant Temperature Experiment – Dº vs Temperature.



Day-Degrees Model:

To estimate total Thermal Requirement for development, we applied the day-degrees model to the 2002 CA and ME data first using 5ºC as a conservative estimate of the lower temperature threshold for development (Fig. 10). For the constant temperature experiments with both populations, the Thermal Requirement estimates varied about 275 D^o (range 230-350 D^o) but differed significantly among groups ($P<0.05$, one-way ANOVA). This significant variation does not conform to the model's prediction. To further test the model, we conducted a series of variable temperature experiments with the Maine larvae (Fig. 11). The estimates of Thermal Requirement in the variable temperature experiments (2002 ME data; Fig. 12) indicated a mean of about 320 D^o (range of means: 310-350 D^o) that did not differ significantly among treatments ($P>0.05$, one-way ANOVA), which conformed to the model's prediction of constancy.

To test the sensitivity of the model to variation in the estimate of the lower temperature threshold, we recalculated Thermal Requirement using thresholds of 7ºC and 3ºC. Estimates of Thermal Requirement varied significantly among groups for both the 7ºC and 3ºC thresholds, and did not approach constancy until a low threshold of less than 3ºC was used. This failure to meet the assumption indicates that the Day-Degree Model does not adequately describe temperature dependence of larval development in *Carcinus maenas*.

Figure 11: 2002 ME Variable Temperature Experiment

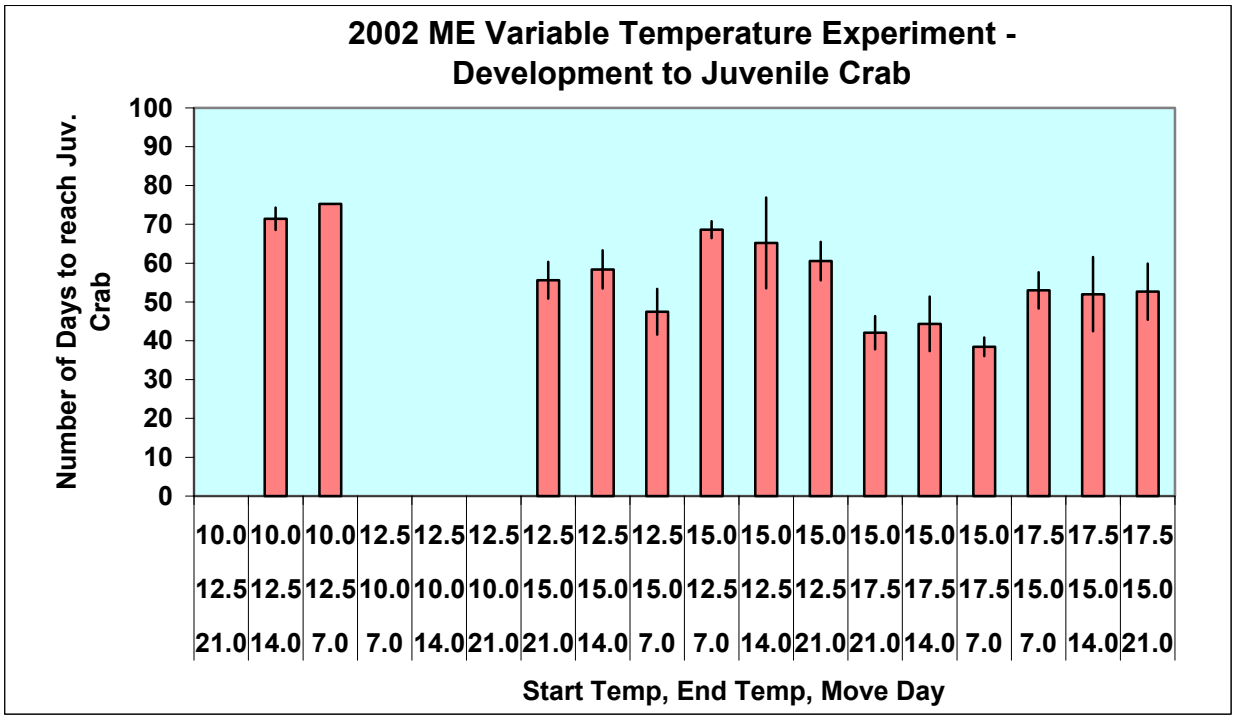
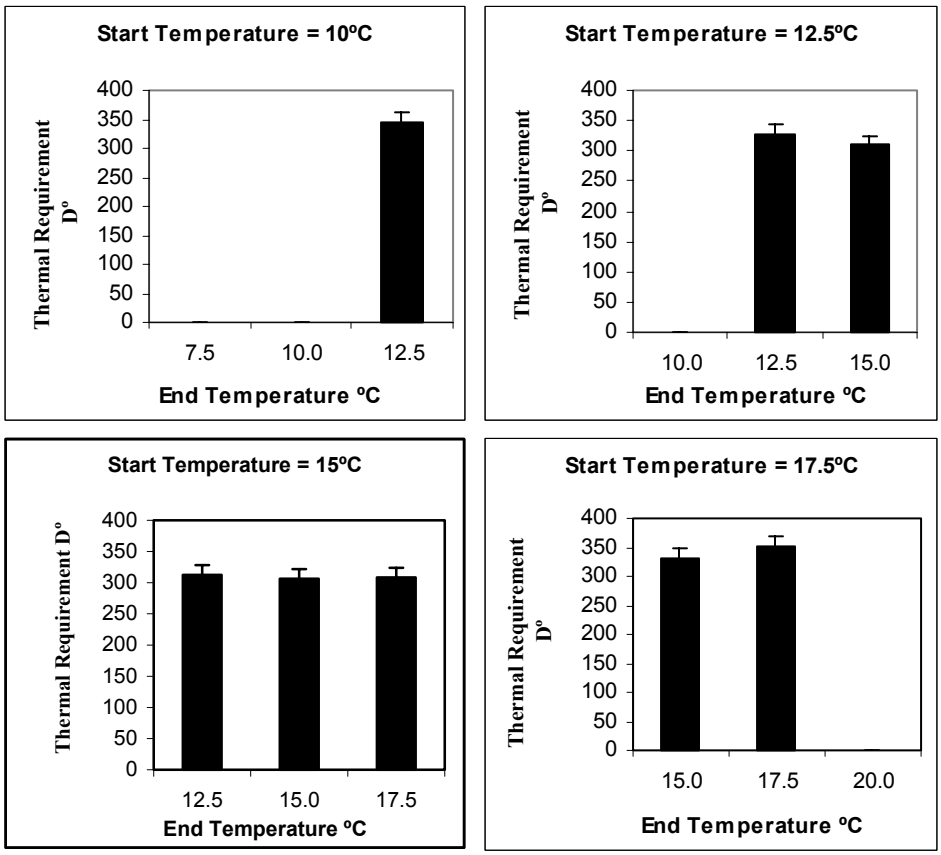


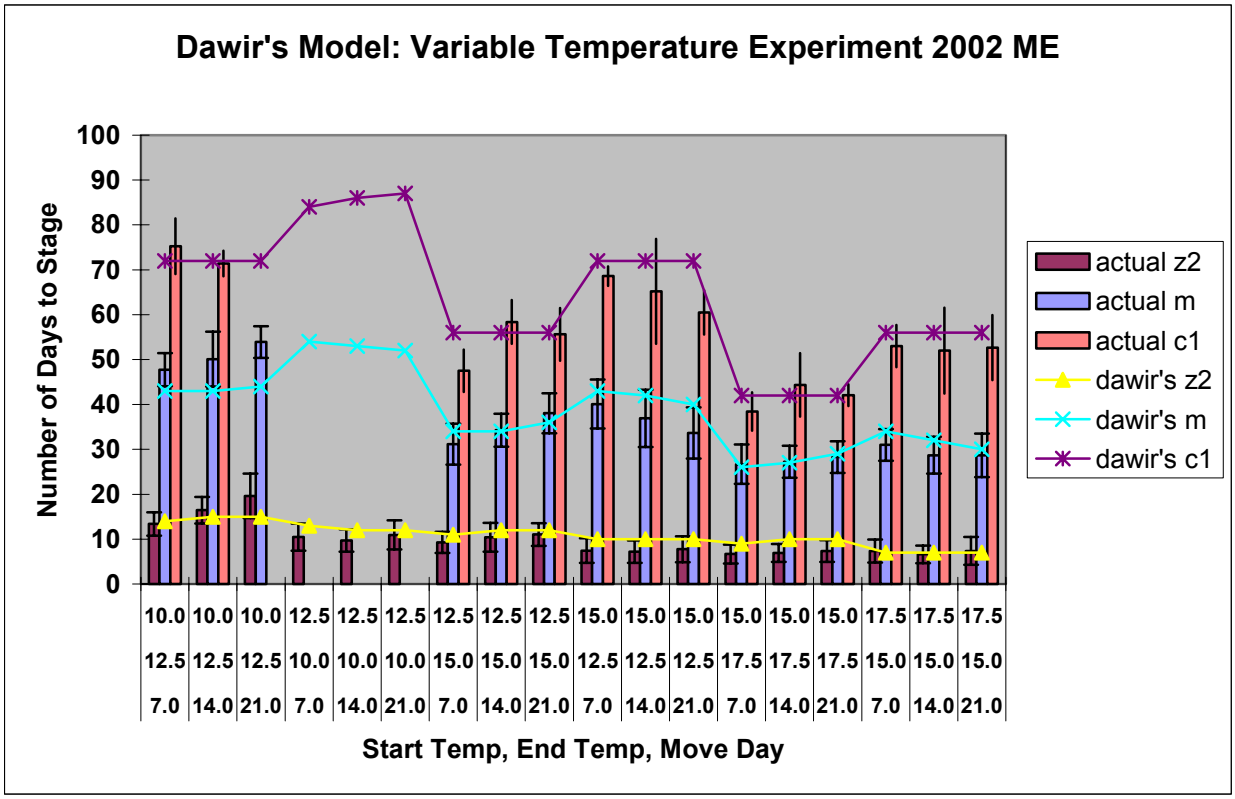
Figure 12: 2002 ME Variable Temperature Experiment - Dº vs Temperature



Dawir's Model:

We fit Dawir's model ($ln\ D = a - b\ ln\ T$) to the ME 2002 data on development rates at constant temperatures and used the model to predict results of our variable temperature experiements. Predictions did not differ significantly (P>0.05, one-way ANOVA) from experimental results of all variable temperature treatments (Fig. 13), indicating that Dawir's model accurately describes the temperature requirements for *Carcinus maenas*.

Figure 13: Dawir's Model comparison of predictions and actual 2002 ME Variable Temp Data.



Projection of Larval Thermal Requirements to Alaska Field Sites:

We compared the model predictions for development time to temperature data at various locations in Alaska. At 10°C, Dawir's model predicts that Green Crab larval development to first crab (C1) stage would require about 70 days. This estimate is conservative, as it is projected from the 2002 ME data (Fig. 8); whereas more extensive experiments using the CA population indicate that larval development at 10°C requires about 60 days (Fig. 7). Using NOAA buoy data for Sea Surface Temperature (SST), we estimated the number of days that the sea surface temperature is above 10ºC at 11 sites along the Alaskan coast from Ketchikan to Unalaska Island (Fig. 14). Seven of the 11 sites met the projected criterion for *Carcinus* larval development (60 days of sea temperature above 10°C – purple line on the graph) (Fig. 14). These locations extended from Southeast Alaska (e.g, Sitka) to the Aleutians (Sand Point) and included Prince William Sound (e.g., Cordova, Valdez), as well as Seward and Kodiak (Fig. 15).

Figure 14: Sea Surface Temperature from Nearshore AK Buoys.

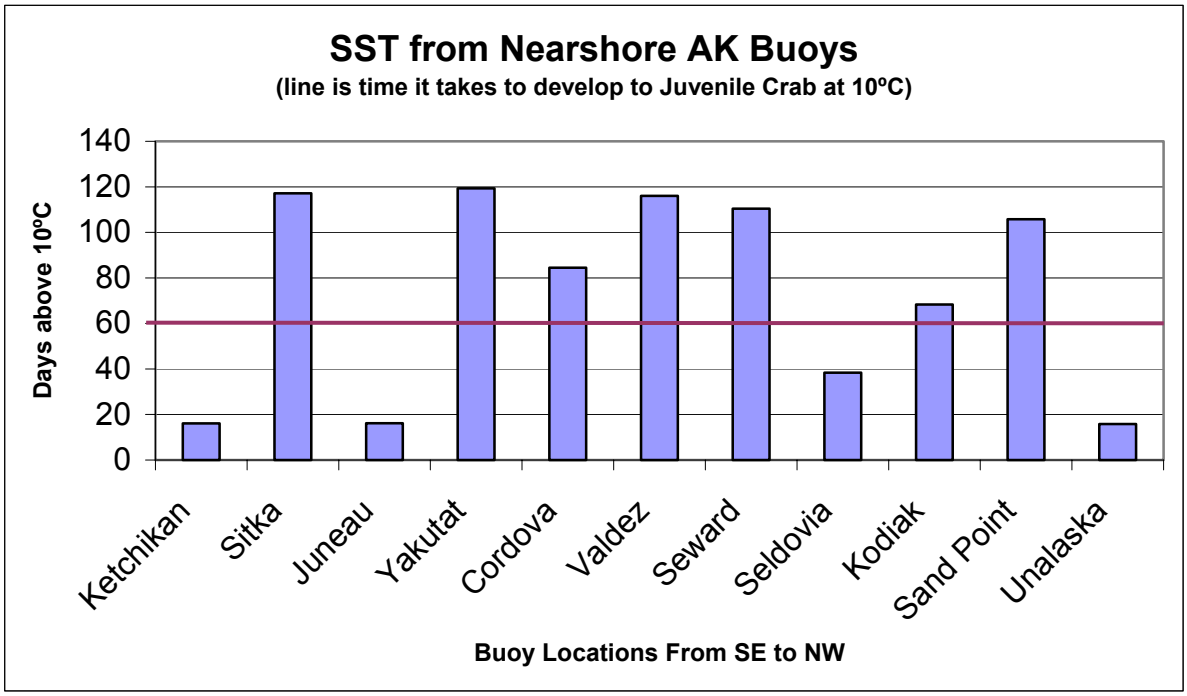
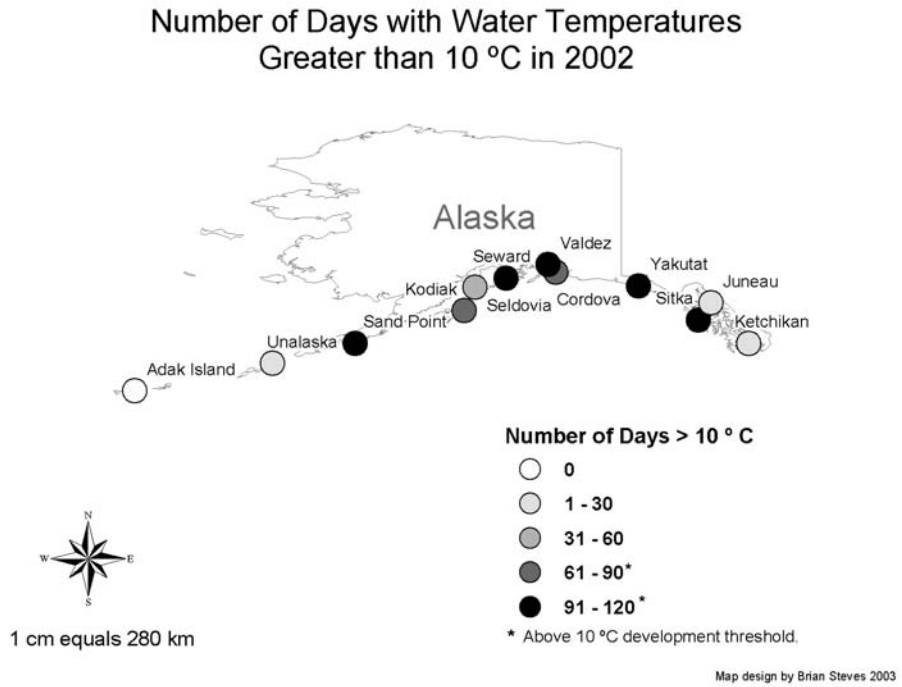


Figure 15: Number of Days with Water Temperatures Greater than 10ºC in 2002.



DISCUSSION

During episodic events repeated over 200 years, the European Green Crab (*Carcinus maenas*) has invaded at least 4 major regions of the world (East and West Coasts of North America, South Africa, Australia) and its closely related congener *C. aestuarii* (native to the Mediterranean Sea) has invaded Japan (Carlton & Cohen 2003). Newly established populations rapidly expanded their geographic ranges, often in a polar direction. Since its discovery in 1989, the invasive population on the West Coast of North America has rapidly spread from San Francisco Bay to Washington State, with individuals found as far north as British Columbia within 14 years of initial establishment. While warm El Niño years may have facilitated the rapid range expansion (D. Armstrong, School of Fisheries, Univ. Washington, Seattle, pers. comm.; S. Yamada, http://www.seagrantnews.org/news/aliens_010213/20010213_aliens_yamada.html), the coast-wise spread thus far appears to have resulted from natural larval dispersal by ocean currents. However, it is also evident that Green Crab larvae occur in port systems, such as San Francisco Bay, where up-take in ballast water of oil tankers could transport the population to Alaska (Hines & Ruiz 2000).

Although Cohen et al. (1995) concluded that the range of *Carcinus* is only limited broadly by temperatures of –1 to 22°C for adults, our detailed laboratory experiments indicate that self-sustaining, reproductive populations are probably more narrowly limited by temperatures needed for larval development. Larval survivorship dropped precipitously below 12.5°C, and tolerance varied between source populations, with California larvae surviving down to 10°C, while Maine crabs required water that was 2°C warmer. NOAA field data indicates that many coastal locations in Alaska have a sufficient thermal dose (days above 10°C) to complete development to juvenile crab stage, although larval development time increases markedly at colder temperatures. Slower development exposes larvae to predators and other potentially adverse conditions for longer periods; however, it also may increase the time and distance of larval transport. Moreover, several Alaskan sites appear to be warm enough for larval development to be completed in about 45 days in summer, a duration that corresponds to estimated larval periods of many native crab species. Thus, laboratory experiments reported here allow us to project that summer field temperature conditions could allow larval Green Crabs to survive and develop to juvenile crabs in numerous port locations along the Alaskan Coast, whether founded by natural dispersal or human-mediated transport.

Green Crab larval development and population growth varies among coastal habitats. Our laboratory experiments indicate that larval development proceeds at salinities above 20 ppt, suggesting that areas subject to high river discharge and ice/snow melt in warm summer months may prevent larval survival. For example, Port Valdez appears to have sufficiently warm conditions for development, but its summer surface salinities are typically well below larval tolerances (Hines & Ruiz 2000). However, many other sites within Prince William Sound appear to have temperature-salinity combinations suitable for Green Crab development, and several shallow embayments likely experience daytime summer temperatures greater than those recorded by NOAA buoy data (in deeper water).

The future geographic range of Green Crabs along the West Coast remains difficult to predict. Following introduction on the East Coast, Green Crabs spread slowly northward over 100 years to cause major damage to bivalve fisheries in upper New England in warm years, and

in cold years they remained rare from Maine to Nova Scotia (Ropes 1968). After many decades of apparent range stability, in 1999-1998 they expanded around Nova Scotia into the Gulf of St. Lawrence and are expanding up the estuary (Audet et al. 2003, Carlton & Cohen 2003). This sudden range expansion may have been a chance new founding by current eddies, or perhaps due to global warming or ballast water release. Importantly, Green Crabs have also exhibited a recent and rapid range expansion in Australia, colonizing the island state of Tasmania after nearly 100 years on the mainland (Thresher et al. 2003). They also exhibit wide fluctuations in abundance in northern Europe that appears to be driven by interannual variation in temperature. Thus, historical experience elsewhere indicates it is far too early to conclude when and where such an invasion may occur even though our data indicate environmental conditions in Alaska are suitable for colonization by Green Crabs.

Although several Alaskan sites can support successful larval development of Green Crabs and are vulnerable to invasion, based simply on salinity and temperature, the extent to which population establishment, abundance, and spread may be modified by biological interactions remains unclear. Habitat use of Green Crabs along the East Coast of North America and Europe appears to be broader than along the West Coast, where populations have been limited to intertidal zones and marsh areas of protected bays and outer estuaries (Grosholz and Ruiz 1996; P.S. McDonald, http://students.washington.edu/psean/html/CV_web.htm). Although it seems unlikely that habitat characteristics will limit invasion success in Alaska, given the success in many other global regions and habitat types, competition with native crab species and predation by larger crabs, fish and birds may have a much greater effect on local Green Crab populations, as appears to be the case in regions of eastern North America (deRivera et al., submitted).

The Green Crab serves as a good model for assessing risk of spread and geographic range of invasive species. Our analysis indicates that analysis of larval development is an important component of such assessments, and that temperature is a particularly important factor to consider. Because larvae develop in variable temperatures, careful analysis of thermal requirements (including temperature-time regressions) provides predictive insight to project the potential geographic range of invasive populations.

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