KING AND TANNER CRAB RESEARCH IN ALASKA:

ANNUAL REPORT FOR

July 1, 1998 THROUGH June 30, 1999

Submitted Under Cooperative Agreement NA67FM0212 To

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
P.O. Box 21668
Juneau, Alaska 99802

Edited By

Gordon H. Kruse
ADF&G Project Coordinator

Regional Information Report No. 5J99-10
Alaska Department of Fish & Game
Division of Commercial Fisheries
P.O. Box 25526
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OVERVIEW OF KING AND TANNER CRAB RESEARCH

Dr. Gordon H. Kruse, ADF&G Project Coordinator

Alaska Department of Fish & Game, Division of Commercial Fisheries
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This is the final report for Cooperative Agreement NA67FM0212 between that National Oceanic and Atmospheric Administration (NOAA) and the Alaska Department of Fish and Game (ADF&G) during state fiscal year FY 99 that spans July 1, 1998 through June 30, 1999. Progress during this fiscal year was previously reported in two semi-annual reports (Kruse 1999a,b). The U.S. Congress funded this Cooperative Agreement in 1992 as a result of a $1.2 million annual budget initiative for federal and state crab research. ADF&G's portion was $237,500 during this sixth year of work.

The Cooperative Agreement supports research to address pivotal research questions associated with management strategies for king *(Paralithodes, Lithodes)*, Tanner *(Chionoecetes bairdi)* and snow crab *(C. opilio)* fisheries off Alaska. Research planning is an active, ongoing process involving many agency and university scientists, managers, and public. Research plans are discussed at annual meetings with crab researchers and industry. A questionnaire of crab researchers gathered additional input (Murphy et al. 1994). These collective efforts helped guide research under this cooperative agreement.

A long-term strategy for crab research was reported in the original statement of work (Kruse 1993a) and study plans (Kruse 1994, 1996). The strategy is to investigate crab stock structure, population estimation methods, biological productivity, and harvest strategies. Largely due to federal assistance, great strides have been made toward understanding crab population dynamics and management implications. Publications resulting from this Cooperative Agreement are listed in Appendix A. Copies of all reports are filed with NOAA. Additional copies are available on request from the lead author.

The need for crab research is highly motivated by large fluctuations in crab stocks and fisheries. The Gulf of Alaska (GOA), Aleutian Islands area (AI), and Bering Sea (BS) supported large commercial fisheries for crabs. Many stocks crashed in the 1980s, and many crab fisheries remain closed due to low abundance. Recently, Tanner crabs *(Chionoecetes bairdi)* in the Eastern Bering Sea declined sharply in abundance to depressed levels. The fishery was not opened in 1997 and 1998, and in March 1999 the stock was deemed overfished by the Secretary of Commerce. Assessments in 1999 have determined that two more crab stocks are overfished: Eastern Bering Sea snow crabs and St. Matthew Islands blue king crabs. On the other hand, after two years (1994 and 1995) of fishery closures the abundance of red king crabs *(Paralithodes camtschaticus)* in

Bristol Bay increased from depressed levels due to a relatively strong 1990 year class. These changes underscore the importance of understanding the interaction of crab stocks with fisheries and natural fluctuations. Poor success in maintaining productive fisheries over the long-term prompted a plea to re-evaluate fishery management strategies (Kruse 1993b). Since this cooperative agreement has been established, significant advances in fishery management have been implemented for Bristol Bay red king crabs and Eastern Bering Sea Tanner crabs. New strategies are currently being developed for the other stocks.

This seventh year of federal assistance continues progress on the long-term research plan. For FY 99, cooperative research was directed in the following four areas of study: (1) effects of wind chill on red king crabs (Appendix B); (2) hatching cues for red king crabs (Appendix C); (3) genetic stock identification (Appendix D); and (4) crab management strategies (Appendix E).

ACKNOWLEDGMENTS

Thanks are extended to Jie Zheng for reviewing a draft of this report.

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Appendix A. Publications under NOAA Cooperative Agreement

Submitted or Published in FY 99

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APPENDIX B: EFFECTS OF WIND CHILL ON RED KING CRABS

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Abstract

A laboratory study investigated the effects of cold windchill temperature on mortality, limb loss and activity (righting response) for sublegal-sized male red king crabs (*Paralithodes camtschaticus*). Responses of crabs were measured after five minute exposures to combinations of four temperatures (0, -5.0, -10 and –15 °C) and two wind speeds (8 and 16 m/s), plus a control group which received no aerial exposure. The sample size for all nine treatments was 15 crabs. Significant inverse relationships were found between windchill and crab mortality, and also between windchill and activity. Windchill did not induce limb loss. Management options are discussed.

Executive Summary

The commercial red king crab fishery in Alaska occurs during winter months. Sublegal-sized male and female crabs must be returned to the sea and may receive aerial exposure to harsh conditions during deck sorting. Other commercial pot and trawl fisheries also have bycatch of red king crabs that must be discarded. A laboratory experiment was conducted to measure the responses of crabs to a range of temperatures and wind speeds to quantify the effects of windchill. Sublegal-sized, male red king crabs were collected from Auke Bay, Alaska, and maintained in flowing seawater until experiments were completed. Treatment groups of 15 individually numbered crabs were subjected to a matrix of windchill values derived from combinations of four temperatures (0, -5.0, -10 and –15 °C) and two wind speeds (8 and 16 m/s) in a wind tunnel placed within a walk-in freezer. A control group received no aerial exposure. During exposures, all crabs had their positions restricted by an enclosure for a uniform exposure. Mortality and limb loss were recorded immediately after exposure, after one day, and again 7 days after exposure. The time required for crabs to right themselves after being placed on their dorsum was recorded prior to exposure, immediately afterward exposure and after 7 days. Mortality was restricted to the three harshest treatments. The maximum mortality was 40% at –22.7 windchill; however, all crabs in the two harshest exposures were moribund and non-responsive for the duration of the experiment, with only their gill bailers active; the crabs were functionally dead. Windchill did not induce limb autotomy in red king crabs. The effects of windchill on activity were demonstrable. A significant inverse relationship occurred between windchill exposure and time required for righting; however all treatments except for the two most severe had at least partial recovery within seven days post exposure. The effects of windchill on sublegal red king crabs are dramatic, and

undoubtedly results in decreased recruitment to adult stocks. Our laboratory measurements are conservative: crabs could not be handled as gently aboard processing vessels, and crabs that were non-responsive for 7 days *in situ* would be subjected to predation, parasitism or cannibalism. Management steps should be taken to restrict exposure of discarded crabs to debilitating windchill by regulating aerial exposure (e.g., use of wind blocks, heat lamps, or water sorting tables) or by regulating fishing effort during periods of extreme windchill.

Description of the Problem

A large percentage of crabs caught in the commercial crab fisheries of Alaska are less than minimum legal size (sublegals), are female, or belong to species not being harvested (bycatch) and must be returned to the sea (Zhou and Shirley, 1996). Other non-crab fisheries such as bottom trawl fisheries and cod pot fisheries also catch crabs that are bycatch and must be discarded. These bycatch crabs invariably receive aerial exposure during sorting and discarding procedures. As most crab fisheries and many of the trawl fisheries in Alaska occur during the winter months, there is a high probability that discarded crabs will receive exposure to cold air temperatures and high winds during their aerial exposure.

The fate of discarded crabs that were handled and received aerial exposure has been the topic of considerable recent concern. Red king crabs tolerate repeated handling and deck impacts with few short-term or long-term effects (Zhou and Shirley 1995, 1996). Tanner crabs autotomize injured limbs, but also have low mortality, even with repetitive handling and serious injuries (MacIntosh et al. 1996). In contrast, exposure to cold air temperatures has more detrimental effects than handling on Tanner crabs (Carls and O'Clair 1990, 1995).

The combination of cold temperatures and wind speed produces the phenomenon referred to as windchill (Court 1948); its effects on crabs have only recently been investigated (Shirley 1998). Average daily temperatures during the Bering Sea red king crab fishery from 1985 to 1993 was only slightly above freezing, with the average wind speeds being in excess of 18 knots (9.2 m/s) and average extremes exceeding –6 °C and 46 knots (24 m/s) (NOAA National Data Buoy Center; http://www.ndbc.noaa.gov/stations.shtml). Temperatures during the Tanner crab fishing seasons in the Bering Sea from 1974-1995 ranged from 10.8 to -21.7 °C (mean of –1.2 °C±5.7 SD) and wind speed varied from 0.7 to 21.1 m/s (mean of 8.1±3.3 SD) (S. Zhou, personal communication). Recent laboratory experiments attempting to mimic windchill exposures that discarded Tanner crabs might receive during the fishing season found dramatic responses (Shirley, 1998; G. Kruse, personal communication). Acute mortality occurred to almost all (100% in all but one treatment) crabs receiving exposure to temperatures of -10.4, and –17.1C synoptically with wind speeds of 7.7 or 15.9 m/s. The responses were dramatic; crabs exposed to the same temperatures without wind or –0.4 °C with wind had no mortality. Windchill also had significant effects upon limb loss and activity of Tanner crabs (Shirley 1998).

The current laboratory experiments measured the effects of windchill on the category of bycatch red king crabs, sublegal-sized males, most commonly caught in pot fisheries. The effects of windchill on red king crab autotomy, activity and mortality over a range of temperatures and wind speeds were determined. The results have relevance to understanding the effects of commercial fisheries on red king crabs and have management implications.

Objectives

The objectives of this study were to investigate the effects of cold windchill temperature (cold air temperature plus wind speed) on mortality, limb loss, and activity (measured as a righting response) for sublegal-sized male red king crabs (*Paralithodes camtschaticus*).

Approach

Detailed Description of Work

This was a laboratory experiment, with sublegal males (<178 mm carapace width) used as the test organisms. The author collected crabs with crab pots from Auke Bay, approximately 20 km N of Juneau, Alaska, during March and April, 1999. Crabs were kept in insulated containers until they were returned to the laboratory. Crabs were then maintained in flow-through sea water systems at the Juneau Center, School of Fisheries & Ocean Sciences (JCSFOS) and NMFS Auke Bay Laboratories (ABL) until completion of the experiment. Both seawater intakes are located at –30 m, and have little short-term variation in temperature or salinity, and provide hydrographic conditions similar to ambient field conditions. Crabs were individually numbered with plastic Floy tags attached to the basis of the third or fourth periopod with plastic cable ties. For all crabs, the carapace width and carapace length were measured to the nearest mm with Vernier calipers; carapace condition noted and the number of missing appendages recorded. Diseased crabs, crabs harboring rhizocephalan parasites, crabs in soft-shell (recently molted) condition and those missing multiple appendages were excluded from the experiment. Crabs were fed *ad libitum* a mixed diet of fish (herring) and squid from collection until the beginning of the windchill exposure experiments. No food was provided from initiation to termination of the experiments.

The experimental design included 9 groups of crabs (8 experimental treatments and a control) in order to seek threshold responses that might be useful for management to mitigate windchill effects. Responses of crabs were measured for a matrix of experimental temperatures of 0, -5.0, -10 and -15.0 °C with wind speeds of 8 and 16 m/s, plus a control group that did not receive aerial exposure.

For exposure, groups of 15 randomly selected (with a random number generator) crabs were moved adjacent to the cold room in containers while immersed in seawater. The

random selection of crabs was stratified to insure that no significant differences in average sizes of crab would occur among the treatments. Carapace widths of crabs used in the experiments ranged from 169.1 mm to 70.0 mm, with the same average size in all treatments.

Three crabs were quickly placed in small cage enclosures (43x33x23 cm with 2.5x3.8 cm mesh), then positioned in the wind tunnel and exposed for 5 minutes. The enclosures restricted movement of the crabs and insured a uniform exposure of the crabs. Crabs were arranged vertically such that no crab blocked wind from another. Crabs were placed centrally within the tunnel, facing into the wind, with their legs extending horizontally toward the sides of the tunnel. After exposure, the crabs were returned to seawater tanks and then returned to larger flow-through tanks in the wet laboratory.

The wood and plastic sheeting wind tunnel was 0.44 x 0.38 x 2.39 m (width x height x length) and was equipped with an electric-powered, squirrel cage blower with a 0.39 x 0.34 m (width x height) exit that provided air movement. Wind speed was measured prior to exposures with a hand-held electronic anemometer (Kestrel Pocket Wind meter) that was placed on the floor in the center of the wind tunnel, perpendicular to the wind. Temperature within the cold room was measured with an electronic thermometer (Thermistor Thermometer, Cole-Parmer) that averaged readings from 5 thermistor leads placed near the crab enclosures. Temperature readings were recorded every minute during exposures and treatment values represent means of all exposures.

The average temperatures attained in the exposures were quite close to the desired treatment temperatures. The desired experimental temperatures and the average temperatures of exposure were:

Desired	Exposure
0.0	0.0
-5.0	-5.1
-10.0	-10.0
-15.0	-15.3

The windchill for each treatment was calculated using the National Weather Service formula:

$$Temp_{windchill} = 0.045 * ((5.27 * \sqrt{Windspeed}) + 10.45 - (0.28 * Windspeed)) * (Temp_{air} - Temp_{initial}) + Temp_{initial}$$

where *Windspeed=mean wind speed (km/hr), $Temp_{air}$=ambient temperature (*°C), and *$Temp_{initial}$=initial temperature of body (*°C)

The initial temperature of a crab body was assumed to be the same as the water temperature from which it was removed (4.7 °C). This formula can be used to give an estimate of the windchill experienced by a crab. Although the formula was derived to give an index of human comfort level, the constants do not reflect properties of homeotherms but the rate of heat loss from a surface. In other words, the windchill is the interaction of wind and ambient temperature that results in the same rate of heat loss as a much lower temperature.

One concern with the windchill estimate is that it is an estimate of the effective cooling rate that would be experienced from an exposed surface. The rate that king crabs lose heat due to this cooling is unknown, as the surface area is an important part of this estimate. The constants in the formula refer to the cooling rate experienced by a cylinder of water that is analogous to the temperature that would be read on a thermometer. King crabs may have a greater surface area (legs, spines) and although the rate of cooling would be the same, the greater surface area would result in a greater volume of heat loss. Thus a king crab might reach a lower body temperature at faster rate due to windchill than ambient temperature alone.

Observations of limb autotomy, mortality and righting response were made for each numbered crab immediately after crabs were returned to the wet laboratory, and then after one week, if the crab was still alive.

Righting responses of crabs were measured by placing the crabs on their dorsum and measuring with electronic stopwatches the time in seconds (to a maximum of 180 seconds) required for the crabs to right themselves. The righting response is a complex reflex requiring muscle coordination and balance and can be a sensitive measure of well-being of organisms (Shirley and Stickle 1982). Righting responses of individual crabs were measured prior to and after exposure to permit a comparison of the effects of exposure.

Project Management

All data collection from the windchill experiments were recorded by either Julie Cox, Graduate Research Assistant, Lynnette McNutt, Research Technician, University of Alaska Fairbanks, or by Dr. Thomas C. Shirley, Professor, University of Alaska Fairbanks. Cecil McNutt helped with logistics on numerous dates. Jonathan Warrenchuk, Graduate Research Assistant, assisted with statistical analyses, graphics and provided many insights. Dr. Shirley examined all crabs for disease and parasites. Mark Carls, National Marine Fisheries Service, provided some equipment. Dr. Adam Moles, NMFS, assisted with the wet laboratory setup. Crabs were collected between March 15 and April 17, 1999, and held in the laboratory for two weeks to determine if crabs had been stressed during collection and transport. All experimental exposures were completed by May 2, 1999.

Findings

Actual Accomplishments and Findings

Mortality. The mortality of red king crabs exposed to combinations of wind speed and temperature was inversely related to windchill values, i.e., mortality increased with decreasing windchill values (Figure 1).

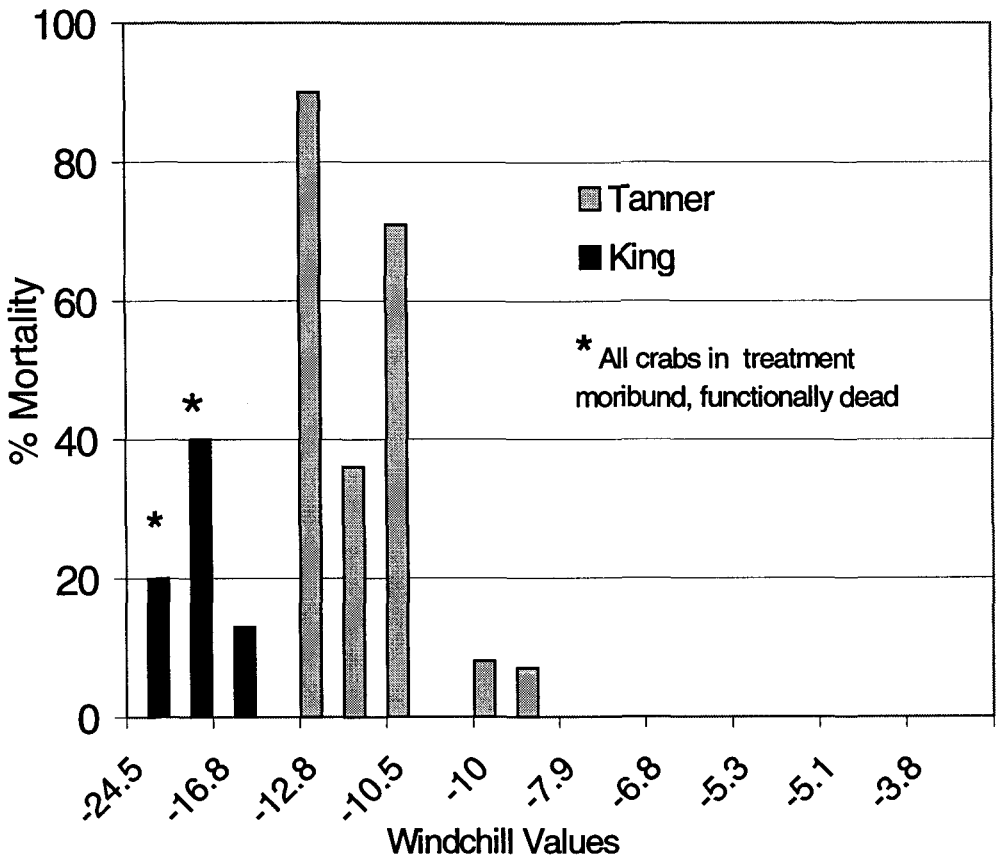

Figure 1. A comparison of mortality of red king crab and Tanner crabs to wind chill exposures

Mortality occurred in three combinations of wind speed and temperature (-15 °C x 16 m/s: -25.9 windchill or WC; -10 °C x 16 m/s: -22.9 WC; and –15 °C x 8 m/s: -17.4 WC) which resulted in the most severe windchill values (WC). The highest mortality was 40% at the intermediate exposure (-22.9 WC). All mortality was recorded immediately post exposure except for the least severe exposure (-17.4 WC) in which mortalities occurred after 24 hours. The more moderate wind chill values had no acute or delayed mortality for the 7 day duration of the experiment.

The mortality values are conservative, as all crabs were moribund and non-responsive for the duration of the experiment in the two most severe exposures. The only evidences of life were beating scathognathites (gill bailers). I have maintained crabs moribund for extended periods (up to a year) in the laboratory with no evidence of life other than beating scathognathites (unpublished observations). These crabs probably should be considered functionally dead, but do not meet the criteria of physiological mortality. Similarly, in the next two more moderate exposures (-17.4 WC and –15.6 WC) all crabs also were non responsive and moribund after 24 hours, but had marked recovery after 7 days. Some portion of these moribund crabs would have suffered mortality *in situ.*

Crabs served as their own individual controls for the righting response treatments. That is, measurements of righting times were made before exposure and these values subtracted from post treatment righting times. Variability in righting times between measurements thereby may result in slightly negative values for treatments receiving exposure to modest windchills (Figure 2). Treatments receiving exposure to the 4 most severe windchill values had no crabs capable of righting themselves immediately post exposure, while other treatments had a graded response in the time required for righting or showed no effect of the windchill exposure. Use of the percentage of crabs capable of righting was found to be more practical and introduce less variability for windchill exposure tests for Tanner crabs (Shirley 1998), but was not utilized for red king crabs.

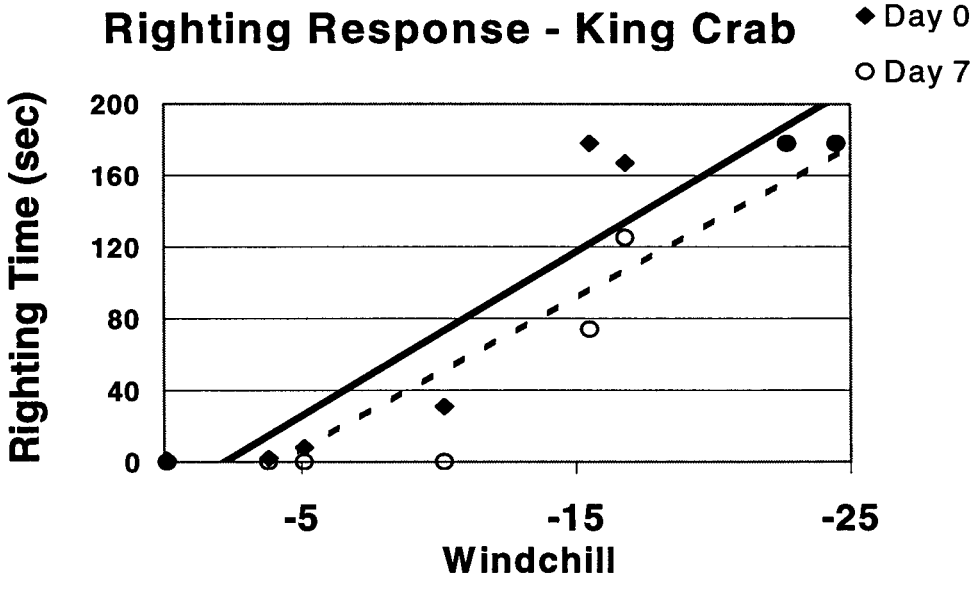

Figure 2. Righting time of red crabs immediately after exposure (solid line) and 7 days post exposure (dashed line).

It was most surprising that the recovery of the red king crabs after 7 days was evident at almost all exposures except the two most severe and the controls (Figure 2).

Discussion

Red king crabs had greater tolerance to windchill exposure than Tanner crabs. Red king crabs had lower mortality even when exposed to more severe windchill values than Tanner crabs. Red king crabs had no limb autotomy at any exposure, a stark contrast to the high rates of autotomy of Tanner crabs at less severe exposures (Shirley, 1998). Tanner crabs suffered 90% mortality at –12.8 WC and some mortality at all exposures more severe than –7.9 WC (Shirley, 1998); the highest mortality of red king crabs observed in this experiment was 40% at –16.8 WC. Red king crabs also demonstrated marked recovery in righting responses 7 days post exposure, a phenomenon not observed with Tanner crabs.

The greater tolerance of red king crabs may be related to the larger size of red king crabs in comparison to Tanner crabs. However, crab size was not a significant factor for survival in our windchill treatments, even though a large size range was used (70.0 mm CW to 169.1 mm CW). The small sample size (n=15) of crabs used within each treatment in this experiment did not permit a sufficiently large number of crabs of each size for robust evaluation of size. In an aerial cold air exposure experiment, body size was not a significant factor in lethality or reduced vigor for aerial exposure in ovigerous king crabs (Carls and O'Clair 1989) or Tanner crabs (Carls and O'Clair 1994)

Carls and O'Clair (1989) found temperature and aerial exposure time to cause death or reduced vigor in ovigerous king crabs in extreme conditions. Short exposure at lower temperatures caused the same effects as longer exposure at higher temperatures (Carls and O'Clair 1989). This response was clearly observed when exposure was defined as the product of exposure time and ambient temperature. This measurement of 'degree-hours' was inversely related to mortality and speed of righting response (Carls and O'Clair 1989). Mortality of red king crabs occurred at –3.2 °h but it did not exceed 50% until 19 days after exposure at –12.7°h (Carl's and O'Clair 1989). Righting response decreased at lower degree hours; 20% of the crabs ceased righting at –4.6°h and all crabs ceased righting at –12.7°h (Carls and O'Clair 1989). In contrast, windchill treatments of –15.6°C (or –1.299°h) produced no righting response in 33.3% of the crabs, and 100% no response was achieved at exposures of –22.9°C (or –1.908°h).

No autonomy was observed in crabs following exposure to windchill treatment. This contrasts with other laboratory experiments involving cold air exposure to king crabs and Tanner crabs (Carls and O'Clair 1989, Carls and O'Clair 1994). However, exposure conditions were harsher in both of these experiments and crabs were observed up to 32 days following treatment. Autotomy of king crabs occurred at exposures of –4 'degree-hours' and lower (Carls and O'Clair 1989). In contrast, when calculating 'degree-hours' from our windchill temperatures and 5 minute exposures, the

lowest degree-hours crabs were exposed to was –2.1 °h. Our exposures may not be severe enough to cause gross physical damage to the crabs. We question the utility of the 'degree-hour' calculations at more severe exposures.

Although autonomy did not occur at any treatment, death and functional death did. It is possible that –15 to –17 °C windchill is a threshold where crab fitness is affected. A sensitive regulatory mechanism could be severely impacted at these exposures so that recovery is minimal. At lower temperatures oxygen affinity in the blood becomes so high that delivery to the tissues is reduced (Mauro 1978). This is mitigated by a compensatory response of ventilation, so that scaphognathite beat remains the same at lower temperatures (Mauro 1978). Windchill exposure may interfere with the compensatory ventilation response, either by physical damage to the scaphognathities or lowered blood temperatures such that oxygen transfer cannot occur. However, respiratory effects alone cannot explain the immediate mortality associated with windchill.

Our estimates of mortality and limb loss in response to varying windchill exposure should be considered conservative. Crabs were often moribund immediately after exposure, so determinations of mortality could not be made with certainty until the following day. In the field, a moribund crab would most likely be subjected to movements by currents, consumed by predators or scavengers, or be exposed to possible infections or parasites. Also, the time frame of our observations was limited to one week post exposure. The extent of tissue or organ damage was not examined histologically and infections or organ-system failures would be expected to increase with time post exposure.

We handled crabs carefully after exposures to prevent autotomy; these handling procedures would not be practical at sea during commercial operations and greater limb loss might be expected, however red king crabs do not have a robust autotomy response. Discussions with ADF&G observers aboard commercial crabbing boats attest to greater limb loss of Tanner crabs and snow crabs during harvest operations.

Our study was limited to sublegal males because they represent the predominant bycatch that must be discarded by the commercial fishery. Sex of crabs was another variable which might have complicated interpretation of results, and the size of the crabs and wet lab rearing space precluded another set of experiments for females. We did not evaluate effects to ovigerous females, their brooded eggs, or to their future reproductive activities. Because of their smaller sizes, females would most likely have increased risk to windchill exposure. While embryos are somewhat protected from aerial exposure by the abdominal flap of females within the genus *Paralithodes*, increased susceptibility to mortality for the brooding females would affect larval recruitment, even if the larvae did not suffer direct mortality to windchill.

Our study only considered acute effects to sublegal male crabs. Considerations should also be given to productivity loss to the populations. That is, loss of a few appendages

to individual sublegal crabs might not result in their mortality, but would decrease growth and reproduction, thereby affecting productivity and recruitment rates to the population.

No significant problems were encountered in the experimental protocol. Crabs were often in a moribund state immediately after exposure, and determinations of mortality could not be made until the following day. Crabs that appeared deceased immediately after exposure were sometimes found to be alive and have activity the following day. Care in determinations of mortality is urged if future related research is undertaken.

Gear and techniques, which result in crabs being shielded from wind exposure or perhaps continuously immersed during the sorting process, should be developed and tested under field conditions. Alternately, management techniques, which involve monitoring meteorological conditions, might be modeled and experimentally tested.

Evaluation

All goals and objectives of the experiment were attained. All aspects of the project worked as designed.

Dissemination of Project Results

1. A summary of the research results will be presented at the Interagency Crab Workshop to be held in Anchorage, Alaska, in December, 1999. This is an excellent forum to discuss the research with federal and state management biologists and to discuss implications for management.

2. An oral presentation of the research results was made at the annual national meeting of The Crustacean Society held in Lafayette, Louisiana, May 26-29, 1999.

3. Seminars describing the research results were presented at Louisiana State University on May 25, 1999 and at Bodega Bay Marine Laboratory on July 22, 1999.

4. A manuscript describing the results of the research will be submitted to a peer-reviewed scientific journal, such as the Journal of Crustacean Biology, in spring 2000.

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APPENDIX C: HATCHING CUES FOR RED KING CRABS

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Abstract

A laboratory experiment sought to evaluate possible cues to hatching of red king crab larvae, in particular the possibility of a waterborne cue perhaps working in concert with increased photoperiod. Ovigerous red king crabs were cultured under four different experimental regimes: (1) ambient light conditions with ambient flow-through seawater; (2) ambient light conditions but in recirculating seawater; (3) extended photoperiod (20L:4D) receiving ambient seawater; and (4) under ambient photoperiod with ambient flow-through seawater with en masse (communal) culture. Individually numbered crabs were maintained in separate culture chambers except for those reared en masse. Successful hatching occurred among only a few females reared en masse. One female receiving flow through, ambient seawater began hatching small numbers of eggs on April 23 and continued through April 27, but molted on May 8 with many eggs still attached to her pleopods. Most crabs molted with eggs still attached to their pleopods.

Executive Summary

Red king crabs brood their eggs for approximately 11 months before releasing larvae approximately coincident with the spring phytoplankton bloom. Survival of the planktotrophic red king crab larvae has been postulated to be linked to the timing of larval release with the sharply pulsed spring phytoplankton bloom; larvae released early or late might be prey limited and have poor survival. An understanding of the mechanisms triggering hatching of red king crab eggs might provide insights into recruitment success and allow predictions of responses of red king crabs to variations in hydrographic conditions or decadal scale changes in oceanographic regimes. Laboratory experiments were conducted to test the efficacy of water-borne substances, perhaps originating with the spring phytoplankton bloom, and extended day lengths, as possible cues for hatching in red king crabs.

Hatching was not successful among crabs reared in individual containers in any experimental treatment, and occurred only minimally among crabs reared in group tanks. The lack of hatching was unexpected, as larvae have been hatched and successfully cultured in the same laboratory in numerous years for other experimental purposes. The lack of hatching was most probably linked to some alteration of the seawater system or a new filtration system, but may be representative of broader scale hatching failures *in situ.*

Description of the Problem

Timing of larval release can be crucial to survival of planktotrophic (feeding) larvae, particularly in high-latitude marine ecosystems, where the spring phytoplankton bloom is sharply pulsed. Diatoms in the initial successional sequence reproduce quickly, deplete the water column of nutrients, senesce, and rapidly sediment to the benthos. Primary production continues at a greatly reduced rate subsequent to the initial bloom unless wind events regenerate deeper nutrients and trigger secondary bloom periods. Herbivorous zooplankton that are dependent upon the bloom must have mechanisms to synchronize their larval release.

Red king crabs brood their eggs for approximately 11 months and larval release (hatching) is coincident with the spring phytoplankton bloom (Shirley and Shirley 1989; Shirley et al. 1990). In southeastern Alaska, ovigerous females either remain in nearshore environments or return to embayments as the time for larval release approaches (Stone et al. 1993). Hatching time is relatively synchronous within a population, with smaller females releasing larvae first and larger females hatching later (Shirley et al. 1990). The early zoeal stages of red king crab are herbivorous (Shirley and Shirley 1988, 1989) and are dependent upon planktonic diatoms for growth and survival. The highest densities of zoeal stages are found immediately beneath the chlorophyll maximum layer during daylight hours, with larvae descending to depths during the night (T. Shirley, UAF, unpublished observations).

Within thermal tolerance limits (approximately 0-12 °C for incubation temperatures of red king crabs), developmental rate of red king crab embryos increase with increasing temperature. Embryos cultured at higher temperatures attain advanced development earlier and become developmentally competent (a state of advanced embryonic development) to hatch sooner (T. Shirley, UAF, unpublished observations). However, hatching is delayed until almost the spring bloom period. Also, embryos competent to hatch from ovigerous red king crab females reared in Atlantic Ocean seawater did not hatch (T. Shirley, UAF, unpublished observations made at Duke University Marine Laboratory). Hatching of red king crabs appears to be triggered by environmental cues that are created by the spring bloom or synoptic with it.

Synchrony of larval release may be dependent upon a variety of environmental cues, including photoperiod, temperatures, salinity, exudates from phytoplankton, combinations of these variables, or a variety of other environmental or biotic signals (Morgan 1995). In the current experiments, hatching of red king crabs was postulated to be triggered by water-borne exudates of phytoplankton associated with the initial spring phytoplankton bloom, but may be initiated or mediated by photoperiod (daylength).

Objectives

The primary objective of this study was to investigate water-borne exudates associated with the initial spring phytoplankton bloom as cues for hatching for red king crabs

(*Paralithodes camtschaticus*). Because these cues might be initiated or mediated by photoperiod, an experimental treatment with extended daylength was incorporated.

Approach

Crabs were collected with baited crab pots from Auke Bay, Alaska, in March and April 1999. Crabs were transported in insulated containers to the laboratory, held in flow-through seawater tanks and fed fish and squid *ad libitum* until the experiments were initiated. The crabs were collected at approximately 40 m depth; the seawater intake for the laboratory is at -30 m in Auke Bay and is similar in salinity and temperature to ambient waters to which the crabs were exposed *in situ*. Carapace length (CL) and carapace width (CW) of crabs were measured with vernier calipers to the nearest 0.1 millimeter and limb loss recorded. A significant relationship exists between female crab size and hatching date (Shirley et al. 1990); therefore crabs of similar size and reproductive condition were used in all treatments. Crabs were screened for diseases and parasites such as rhizocephalans, e.g., *Briarosaccus callosus*, and any diseased crabs were excluded from the experiment (Hawkes et al. 1986; Shirley et al. 1986). Crabs were individually numbered with Floy tags attached to the basis of a walking leg with a plastic cable tie.

To examine the possibility of a water-borne substance triggering hatching and larval release, ovigerous crabs were held in tanks receiving two types of seawater. One water source was ambient seawater from a -30 m intake in Auke Bay; the other source was seawater collected prior to the spring bloom and king crab egg hatching. Crabs were cultured in individual chambers (27.8 liters), each receiving either the ambient seawater or pre-bloom seawater cooled to ambient seawater temperatures. The chambers containing pre-bloom water were aerated and had charcoal and fiberglass filters; each crab was exposed to only its own seawater, but the individual chambers were cooled by ambient seawater.

Crabs were placed in individual containers and assigned randomly (using a random number generator) to one of three treatments: (1) receiving flow-through ambient sea water under ambient photoperiod; (2) in recirculating sea water under ambient photoperiod; and (3) receiving flow-through sea water under extended light photoperiod (20L:4D, twenty hours of light, 4 hours of darkness) under full spectrum bulbs. Crabs brooding *in situ* receive only dim light, as the overlying water column normally attenuates much of the light intensity. All flow-through tanks containing crabs were either covered, or in the case of those receiving light, were enclosed within black plastic to decrease disturbance. Eight crabs were used in each treatment. Twenty additional females were cultured in groups (en masse, communal) of 4 to 8 crabs in flow-through seawater tanks, not in individual containers. All access to the crabs in the experimental darkrooms for cleaning or inspection occurred only during the light part of the diel cycle.

The carapace width of crabs used in the experiments ranged from 100.5 mm to 133.0 mm, with no significant difference in CW among the treatments. Fine mesh (<0.05mm)

bags were placed on the outflow of those containers receiving flow-through seawater to collect larvae that might have hatched. Water temperature and salinity of treatments were measured daily; temperature varied from 4.0 to 7.4 °C and salinity varied from 31 to 34 ppt. Containers were inspected for larvae daily. Crabs were not fed for the duration of the experiment to reduce fouling; red king crabs can persist for more than a year without food.

Project Management

All data collection from the hatching experiments were recorded by either Julie Cox, Graduate Research Assistant, Lynnette McNutt, Research Technician, University of Alaska Fairbanks, or by Dr. Thomas C. Shirley, Professor, University of Alaska Fairbanks. Dr. Shirley examined all crabs for disease and parasites. Dr. Adam Moles, NMFS, provided culture chambers and assisted with laboratory plumbing and setup.

Findings

Hatching occurred from a single female (124.8 mm CW, multiparous) receiving flow-through seawater under ambient light conditions. Hatching occurred daily from April 23-27, and again from May 1-6 for this female. The female molted on May 8 with many eggs still attached to her pleopods. No other hatching was observed in any experimental treatments. However, crabs reared en masse (communal) in flow-through tanks hatched continuously beginning April 22. The number of crabs participating in hatching, and the timing and amount of hatching for each crab could not be determined in these communal tanks, however most females were not participating in hatching and the number of larvae was greatly reduced from what had been observed in laboratory hatching in prior years.

The hatching cues experiment was terminated June 2 when crabs began to molt and fouling in tanks became problematic. The remaining female crabs were maintained in tanks to gather data on the fate of unhatched eggs and molt timing. No additional hatching was observed, although eggs were sloughed off or remained attached to pleopods at the time of molting.

Discussion

The lack of hatching by the majority of ovigerous females being cultured was unexpected. Large numbers of ovigerous red king crabs, Dungeness crabs and golden king crabs had hatched in the same lab in prior years for a variety of larval and reproductive experiments. Sand filters have been installed in the JCSFOS seawater system since the last culturing of red king crabs and may be a possible cause of the hatching anomalies. Water temperature and salinity were measured daily in each experiment and the range of temperatures (4.0 to 7.4 °C) and salinities (31 to 34 ppt) encountered throughout the experimental period were within ranges used with prior successful hatchings.

The hatching that did occur in the communal tanks fell within the range of prior laboratory hatching dates (Shirley et al. 1990) and near the time of peak appearance of stage I zoeal larvae in plankton collections from 1985 to 1989 (Shirley and Shirley 1990).

One attempt was made to test the possibility of the interaction of the sand filters. Ambient sea water collected from the NMFS Auke Bay docks without filtering and added to tanks did not stimulate hatching.

One unsettling concern is that the hatching failure may be representative *of in situ* hatching failure. Prior laboratory hatching dates agreed well with field collections of larvae in plankton tows (Shirley and Shirley 1990).

Evaluation

The experiment was an abject failure and contributes only more uncertainty to deciphering the complexity of the reproductive biology of red king crabs.

Dissemination Of Project Results

The lack of results precludes presentations or publications. However, the experiments will be discussed with university, state and federal crab biologists in an attempt to gain insights into possible causes for the hatching failures.

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APPENDIX D: CRAB GENETICS

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ABSTRACT

Stock delineation of commercially exploited populations of crabs in the eastern Bering Sea (EBS), Gulf of Alaska (GOA), and Southeast (SE) Alaska is a management and conservation concern for the State of Alaska. A pilot study using allozymes is complete for blue king crabs (*P. platypus*). A total of 43 loci were resolved and nine polymorphic loci were detected. Genetic differences between the two studied populations (St. Matthew Island, Pribilof Islands) were significant. Similar to red king crabs, *ALP** is largely driving these detected differences. A pilot study using allozymes for golden king crabs (*Lithodes aequispinus*) is underway. A total of 40 loci have been resolved for two populations (Bering Sea, Frederick Sound/Chatham St. in Southeast Alaska). To date we have detected polymorphisms at four loci. Preliminary allele frequencies are presented, and data collection is continuing on a third population, Adak Island.

Allozyme and mitochondrial DNA (mtDNA) data have been used successfully to identify stock components in a number of species although delineation of population substructure in crustaceans using allozyme methods have generally revealed low levels of genetic variation. The desire for increased resolution of red king crab (*Paralithodes camtschaticus*) population structure in Alaska has prompted development of microsatellites, a highly variable class of nuclear markers that hold much promise for stock delineation. Microsatellites are promising for providing variable markers to assess population substructure. Four microsatellite loci were evaluated for describing population structure in red king crabs. In general, the microsatellite data were concordant with allozyme data. Estimates of the parameter F_{ST} were statistically significant (greater than zero) for seven populations from three broad geographic regions (Southeast Alaska, Gulf of Alaska, and the Bering Sea). Microsatellites revealed significant intra-regional population structure not evident with allozyme markers. This result is consistent with the expectation that more variable markers have greater statistical power to detect population structure and suggests that microsatellites can play a role in genetic characterization of red king crab populations.

EXECUTIVE SUMMARY

- Stock delineation of commercially exploited crab populations in the Bering Sea, Gulf of Alaska, and Southeast Alaska is a management and conservation concern for the State of Alaska.

- Allozyme and mitochondrial DNA (mtDNA) data have been used successfully to identify stock components in a number of species; however delineation of population substructure in crustaceans using allozyme methods has been problematic. In general, the genetic variation expressed in these loci has yielded useful markers for species identification and delineation of stocks on a regional basis. However, the finer resolution desired for definition of self-recruiting populations may require development of additional variable markers. The desire for increased resolution of red king crab (*Paralithodes camtschaticus*) population structure in Alaska has prompted development of microsatellites, a highly variable class of nuclear markers that hold much promise for stock delineation.
- Four microsatellite loci were evaluated for delineating population structure in red king crabs. Seven populations are sampled from three broad geographic regions (Southeast Alaska, Gulf of Alaska, and the Bering Sea). Allozyme data from four loci for the same populations are also reported for comparison. The results show that microsatellites, like allozymes, reveal statistically significant population structure in red king crabs. Estimates of the parameter F_{ST} are significantly greater than zero at both marker classes for all populations and for a majority of population pairs. This result suggests microsatellites may be used in place of allozymes when non-lethal sampling is required or when tissue sources are limited. Finally, microsatellites did reveal significant intra-regional population structure not evident with allozyme markers although allozymes revealed inter-regional variation not apparent with microsatellites. This result is consistent with the expectation that more variable markers have greater statistical power to detect population structure and suggests microsatellites can play a role in genetic characterization of red king crab populations on a smaller geographic scale than with allozymes.
- The development of allozyme protocol specific for collection of data from blue king crabs is complete. We have resolved 43 loci and detected polymorphisms in nine loci. Expression of allozyme loci in blue king crabs is similar to red king crabs with differing common alleles detected at five loci. Although the number of useful markers is low, the implication of fine scale stock differences is evident.
- A pilot study using allozymes is underway in golden king crabs. Data collection is underway on an additional collection, Adak Island. We are continuing to detect new alleles in this species. To date, we have resolved 40 loci and have detected polymorphisms in four loci.

Red King Crab Microsatellite Study

Approach

Introduction

Microsatellites are a class of nuclear DNA that exhibit co-dominant allelic variation and Mendelian inheritance (Wright and Bentzen, 1994). In addition, microsatellites are often highly variable and can be amplified from several tissue types by the polymerase chain reaction (PCR). These attributes make microsatellites particularly powerful for

identifying population structure on a micro-geographic scale and for studies using minute quantities of tissue and non-lethal sampling (Jarne and Lagoda 1996). Microsatellites are often used in studies when less variable markers, such as allozymes, fail to delineate putative populations. In the case of red king crabs, allozymes are useful for distinguishing among populations from broad geographic regions (e.g., Southeast Alaska versus Gulf of Alaska) but sometimes fail to reveal population structure within these regions (Seeb and Seeb 1990).

The purpose of this study is to evaluate microsatellites as a tool for delineating genetic population structure of red king crabs, particularly within geographic regions. The first phase of this study, marker development, is complete and was described in an earlier report (see ADF&G 1998b). This report describes the final phase, marker testing, and summarizes intra- and inter-population genetic diversity at four microsatellite loci in 7 red king crab populations from three regions – Southeast Alaska, Gulf of Alaska and the Bering Sea. Allozyme data from the same populations are reported for comparison.

Methods and Materials

Sample Collection, DNA Extraction, and Allozyme Analyses

Tissue samples were utilized from seven red king crab populations representing three broad geographic regions (Southeast Alaska, Gulf of Alaska, Bering Sea, Figure 1). Between 45 and 106 samples were genotyped per population (Table 1). Sampling locations, methods for tissue preparation, DNA extraction, and allozyme analyses were described in earlier reports (Seeb and Seeb 1990; Seeb et al. 1990; ADF&G 1998b).

Multilocus Genotyping using Microsatellites

Four microsatellite loci were co-amplified by polymerase chain reactions (PCR) in two multiplex sets. Multiplex A contained *Pca*4, *Pca*13, and *Pca*24. Multiplex B contained *Pca*14. A negative and positive control was amplified with each PCR group. The 10 µl PCR mixture contained 0.5U AmpliTaq (Perkin Elmer), 3.0 mM $MgCl_2$, 10X PCR Buffer II, 0.2 mM each dNTP, and 0.2-1 µl of genomic DNA. Primer concentrations ranged between 0.25 to 1.5 µM per reaction. Microsatellite loci were amplified in a Perkin Elmer 9600 thermocycler using the following profile. An initial melt at 94°C for 10 seconds was followed by the amplification cycle: (1) denature at 94°C for 30 seconds, (2) anneal at 60°C for 15 seconds, and (3) extension at 72°C for 30 seconds. We used 25 cycles for multiplex A, and 26 cycles for multiplex B. PCR products were loaded on a 4% denaturing polyacrylamide gel and run on an ABI 377 automated sequencer.

Allele size estimates were generated using the local Southern-sizing algorithm in the GeneScan Analysis (version 3.0) software (ABI 1998). These data were imported into Genotyper (version 2.1) where allele sizes were labeled (ABI, 1996). Allele labels were checked by two scorers; conflicts were resolved or the individual was excluded. This

data set was combined with the data sets collected by ADF&G and the University of Washington on an ABI 373 Sequencer (ADF&G 1998b). These three data sets were standardized by using allelic ladders and the histogram function in Genotyper (version 2.1) to establish allele bins.

Statistical Analysis and Marker Evaluation

Estimates of three genetic parameters were used to assess genetic variability and evaluate microsatellites for inferring population structure in red king crabs. First, estimates of expected heterozygosity (H_E) were calculated for each locus in each population using equation 8.4 of Nei (1987). The mean H_E was calculated as the sum of H_E across loci divided by the number of loci. Tests for conformity to Hardy-Weinberg expectation (HWE) were performed using a probability test in GENEPOP ver. 3.1b (Raymond and Rousset 1995). Statistical significance levels (α) for the probability tests were determined using sequential Bonferroni adjustments for simultaneous tests (Rice, 1989). Second, unbiased estimates of Wright's F_{ST} were computed using the statistic θ (Weir and Cockerham 1984). F_{ST} (θ) is a relative measure of population structure that quantifies the fraction of total genetic variation that is due to differences in allele frequency between populations. Values of θ were computed for each locus and for all loci in each population using FSTAT ver. 1.2 (Goudet 1995). The probability that each estimate was greater than or equal to zero was tested in FSTAT by permuting the data set (alleles among samples) 1,000 times. The sample variance and 95% confidence interval for each F_{ST} estimate was estimated by bootstrap sampling loci. Third, estimates of R_{ST} (Slatkin 1995) were computed using the statistic ρ (Goodman 1997). Like F_{ST}, R_{ST} is a relative measure of population structure but R_{ST} quantifies the difference in mean allele size (length) between populations (rather than allele frequency). Simulations suggest that R_{ST} is a more accurate measure of genetic population structure at microsatellite loci but empirical support is equivocal (Estoup et al. 1998; Olsen et al. 1998). Values of ρ were computed for each locus and for all loci in each population using R_{ST} Calc ver. 2.2 (Goodman 1997). The statistical significance and sampling variance for each estimate were computed as described for F_{ST}. Finally, estimates of H_E and F_{ST} were computed for four allozyme loci in each population for comparison with the microsatellite data. These four loci (*sAH**, *ALP**, *GPI-A1**, *PGDH**) were chosen because they were polymorphic and revealed population structure in red king crabs (ADF&G 1998b; Seeb and Seeb 1990).

F_{ST} estimates for 21 population pairs were computed for microsatellites and allozymes using the computer program ARLEQUIN ver. 1.0 (Schneider et al., 1996). These values were used as genetic distance estimates for multidimensional scaling (MDS, Kraznowski and Marriott, 1994) in the program NTSYS-pc ver. 1.8 (Rohlf 1993). Two MDS plots were generated to depict the estimated genetic relationships among populations based on the microsatellite and allozyme data.

Findings

Results

Expected Heterozygosity

The microsatellite loci exhibit exceptional polymorphism (Figure 2). For example, estimates of mean H_E range from 0.834 (Deadman Reach) to 0.907 (Uganik Passage, Pribilof Islands, Table 1). The single locus estimates of H_E among all populations are 0.918, 0.950 0.806, 0.871 for *Pca*4, *Pca*13, *Pca*14, *Pca*24. The estimates of H_E are lowest in the two populations from Southeast Alaska (Deadman Reach, Barlow Cove) with one exception (Pribilof Islands, *Pca*14, Table 1). Each locus exhibits statistically significant heterozygote deficiency in at least five of seven populations after adjusting the critical value for 28 simultaneous tests (initial $\alpha = 0.0018$).

The allozymes are less variable than the microsatellites (Figure 3). The estimates of mean H_E range from 0.183 (Barlow Cove) to 0.251 (Uganik Passage, Table 1). Consistent with the microsatellites, the two populations from Southeast Alaska exhibit the lowest mean H_E. In contrast to the microsatellites, the single locus estimates of H_E among all populations for allozymes show two levels of polymorphism: H_E = 0.008 - 0.020 for *GPI-A1** and *sAH**, and H_E = 0.363 - 0.509 for *ALP** and *PGDH**. In fact, *GPI-A1** is polymorphic in only one of the seven populations (Norton Sound). Only two instances of statistically significant heterozygote deficiency (Kachemak Bay - *ALP** and Uganik Passage – *ALP**) are apparent after adjusting the critical value for 28 simultaneous tests (initial $\alpha = 0.0018$).

F_{ST} and R_{ST}

The multilocus estimates of F_{ST} for all populations are 0.013 for microsatellites and 0.103 for allozymes (Figure 4). The multilocus estimate of R_{ST} for all populations for microsatellites is 0.012. The permutation test results indicate that each estimate is significantly greater than zero. Multilocus estimates of each parameter are also shown excluding the Norton Sound sample because allele frequency data suggest that this one population is clearly distinct from the others at allozyme loci *ALP** and *GPI-A1**. Without the Norton Sound sample the estimate of F_{ST} for allozymes decreases 2.5 times while F_{ST} and R_{ST} for microsatellites show little change (Figure 4). Similarly, the two measures of population structure for microsatellites, F_{ST} and R_{ST}, are not statistically different. R_{ST}, however, has a larger sample variance (wider confidence interval).

Estimates of F_{ST} show little variation across microsatellite loci (Figure 5). In contrast, the estimate of F_{ST} at allozyme locus *ALP** is much larger than for the other three allozyme loci. The estimate of F_{ST} at *ALP** decreases when the Norton Sound sample is excluded, but the value remains greater than for all other loci (Figure 5).

Multilocus estimates of F_{ST} vary widely among pairs of populations for both marker classes (Table 2). The magnitude of the estimates are similar with the exception of population pairs that include Norton Sound. Here the large allele frequency difference at *ALP** results in an exceptionally large multilocus F_{ST} for allozymes (e.g. BS2 and BS3 of Figure 6). The number of statistically significant F_{ST} estimates are similar for microsatellites (14) and allozymes (12) when the critical value is adjusted for 21 simultaneous test (initial • = 0.0024, Table 2). Of the F_{ST} estimates for the five intra-regional population pairs, two (Bristol Bay x Norton Sound and Pribilof Islands x Norton Sound) are significant for both marker classes and one (Deadman Reach x Barlow Cove) is significant for microsatellites only (Table 2). With the exception of Southeast Alaska, estimates of intra-regional F_{ST} are highest for the allozymes (Table 2, Figure 6). Multilocus estimates of F_{ST} and R_{ST} are similar for microsatellites. Nevertheless, only two R_{ST} estimates are statistically significant.

Two different pictures of the genetic relationship among populations emerge from the MDS plots (Figure 7). The microsatellites, and less so the allozymes, reveal a strong separation of Southeast Alaska populations from all other populations. The allozymes, and less so the microsatellites, support the unique character of the Norton Sound population suggested by the large allele frequency difference at *ALP**. It is possible that this exceptional locus and population are concealing the relationships revealed by the microsatellites.

Discussion

Expected Heterozygosity

The fact that intra-population genetic diversity, as measured by mean H_E, is greater for microsatellites than allozymes is consistent with the relatively high mutation rate common for many microsatellite loci (Jarne and Lagoda 1996). Similar results have been reported in a number of comparative studies in other organisms (e.g., Allendorf and Seeb, in press). What is interesting, however, is that four microsatellite loci exhibit statistically significant heterozygote deficiency in five or more populations. There are two common causes of this result. First, if the seven population samples actually represent admixtures of two or more randomly mating groups then a deficit of heterozygotes is expected and this is called a Wahlund effect (Hartl and Clarke 1989). This explanation is untenable, however, because similar results would be expected at other genetic markers, and this is not the case for the allozyme loci. The other common cause of a heterozygote deficit at microsatellite loci is a null allele(s) (Callen et al. 1993). A null allele does not amplify in PCR and therefore is not observed. Non-amplification is typically a result of a mutation at a priming site. Given that the data do not support a Wahlund effect, a null allele is the most likely source of heterozygote deficiencies at the microsatellite markers in this study. It should be mentioned, however, that it is unusual to observe a null allele at every microsatellite locus. A more reasonable expectation in this study would be one locus, assuming approximately 30%

of microsatellites exhibit null alleles (Callen et al. 1993). To further examine this, however, would require additional data and is beyond the scope of this study.

Although the microsatellites are more variable than the allozymes, they both reveal an interesting trend in relative H_E across populations. Namely that the two Southeast Alaska populations exhibit lower genetic diversity than populations from other regions. This suggests that the high mutation rate at the microsatellite loci is not masking the effect of other forces influencing genetic diversity in these populations.

F_{ST} and R_{ST}

The results of this study show that microsatellites, like allozymes, do reveal statistically significant population structure in red king crabs. Nevertheless, the multilocus F_{ST} estimate for the allozymes is nearly 8 times greater than the F_{ST} estimate for the microsatellites (largely the result of a single locus, *ALP**, and a single population, Norton Sound). These results highlight the importance of individual loci, regardless of marker class, in describing population structure in some circumstances.

The number of statistically significant F_{ST} estimates among population pairs is similar for the two marker classes. There is one notable exception, however, at the intra-regional level. Microsatellites, and not allozymes, show statistically significant population structure in Southeast Alaska although both marker classes yielded similarly low estimates for F_{ST}. This result is consistent with the expectation that more variable markers have greater statistical power to detect population structure (Estoup et al. 1998). Interestingly, the two Southeast Alaska populations (Deadman Reach and Barlow Cove) are the two most geographically proximate populations in this study. The significant population structure revealed by the microsatellites (specifically *Pca*13, Figure 6) suggests that gene flow may be limited among red king crab aggregates in these small fjords.

Estimates of the parameter R_{ST} are generally similar to estimates of F_{ST} for all populations and for all population pairs. Nevertheless, only two R_{ST} estimates for population pairs are statistically significant. In addition, the multilocus R_{ST} estimate for all populations has a larger sampling variance (less precise) than the multilocus F_{ST} estimate. These results are consistent with other empirical studies (e.g. Estoup et al. 1998; Olsen et al. 1998) and suggest F_{ST} is a more appropriate measure of relative population structure for microsatellites in red king crabs. Further, simulation results reported by Slatkin (1995) suggest this relationship between R_{ST} and F_{ST} is indicative of relatively high gene flow or short divergence time among populations or both.

Summary

The purpose of this study is to evaluate microsatellites for delineating populations of red king crabs. The results are encouraging and suggest microsatellites can be a useful management tool for identification of exploited stocks. The fact that the microsatellites

and allozymes yield similar estimates of relative population structure is not unusual (Allendorf and Seeb, in press; Scribner et al. 1998). Indeed, this result suggests that microsatellites may be used in place of allozymes when non-lethal sampling is required or when tissue sources are limited. Finally, microsatellites did reveal significant intra-regional population structure not evident with the allozyme markers. This result suggests that microsatellites can play a role in genetic characterization of red king crab populations on a small geographic scale unattainable with allozymes.

Additional Work Needed

Two important issues should be addressed as a follow up to this study. First, we need to verify the presence of null alleles. This can be done directly by genotyping siblings from known single pair crosses of adults suspected of having a null allele. Unfortunately this requires many controlled matings which are very difficult to maintain. Alternatively, new microsatellite markers could be developed and applied to the same populations. This approach would only provide indirect verification but the results would be compelling since the probability of a null allele at every locus in a sample of 10 loci, for example, is very low. The second issue also supports the need for developing new microsatellites. The loci used in this study are large dinucleotide repeats and exhibit moderate to excessive "stutter" or shadow bands that can inhibit accurate allele scoring (Weber and Wong 1993). New tetranucleotide microsatellites would likely exhibit the same level of polymorphism but not the same stutter (Jarne and Lagoda 1996).

Investigations of Blue King Crab Allozymes

Approach

Introduction

Blue king crabs, *Paralithodes platypus*, are large anomuran decapods of the family Lithodidae and is a congener of the red king crab, *P. camtschaticus*. Blue king crabs occur discontinuously around the north Pacific rim from Hokkaido to southeastern Alaska and as far north as outer Kotzebue Sound (Somerton 1985). The species is not known from the Aleutian Islands. Both species have latitudinal ranges that, in the northeast Pacific, extend from the Bering Strait to southeast Alaska. Within their shared ranges in Alaska, red king crabs are nearly ubiquitous, but blue king crabs occur only in small isolated populations associated with either offshore islands in the eastern Bering Sea (EBS) or enclosed bays and fjords in the Gulf of Alaska. In the eastern Bering Sea, the largest populations occur in association with offshore islands (Pribilof Islands, St. Matthew Island; Otto 1990a) while small populations are found in Herendeen Bay, Nunivak Island, Seward Peninsula-King Island (Otto 1990a), the Diomede Islands (near the U.S.-Russia border), and St. Lawrence Island. In the Gulf of Alaska, blue king crabs are known in Olga Bay, Port Wells, Russell Fjord, Glacier Bay, Lynn Canal, and Endicott Arm (Somerton 1985). Although both species are found on soft and hard

bottom substrate in relatively shallow waters, each species is absent or rare in areas inhabited by the other (Somerton 1985).

Commercial fisheries for blue king crabs in Alaska have occurred primarily near the Pribilof Islands and St. Matthew Island in the Bering Sea. Catches in other areas have been sporadic, incidental to other commercial crab fisheries, or limited to small subsistence/personal use fisheries. The Pribilof Islands fishery was developed by the Japanese in the late 1960's (Otto 1990b). Development of this fishery was accelerated by declining abundance in Bristol Bay red king crabs and decreasing quotas negotiated through U.S.-Japan Bilateral Agreements (Otto 1990b). Landings were, in part, incidental to Tanner and snow crab fisheries until the development of a domestic fishery in 1974. Catches have ranged from <0.1 million pounds (1986/87) to 10.9 million pounds (1980/81). Due to low abundance, this fishery was closed from 1989-1993 (ADF&G 1998a), and will be closed for the 1999 season (D. Tracy, ADF&G Kodiak, Alaska, personal communication). The St. Matthew Island fishery developed in conjunction with that of the Norton Sound red king crab fishery. Although these stocks were known in the late 1940's, there was little commercial interest until 1977 (Otto 1990b). Catches in this fishery have ranged from 0.2 million pounds in 1979 to 9.4 million pounds in 1983 (ADF&G 1998a). Because of low stock abundance, this fishery will be closed for the 1999 season also (D. Tracy, ADF&G Kodiak, Alaska, personal communication).

Interest in examining the genetic basis of stock structure in blue king crabs was first expressed at the 1992 annual research meeting of the ADF&G and NMFS. Population collections of blue king crabs were initiated in 1993 during the Pribilof Islands commercial fishery. A small collection (N=56) from Pribilof Islands and a collection from St. Matthew Island (N=100) were obtained that year. Because of the remoteness and relatively small size (compared to red king crabs) of the EBS blue king crab fisheries, obtaining collections proved problematic. Additional samples were not obtained until 1996. Collections were obtained from both the Pribilof Islands and St. Matthew Island by ADF&G onboard observers and port samplers. These collections were used to begin protocol development and assessment of levels of genetic variation for this species using allozymes.

Methods and Materials

Sampling

Three collections of blue king crabs were analyzed for allozymes in this pilot study (Figure 1). Samples were collected by ADF&G personnel and observers from two sites in the eastern Bering Sea, St. Matthew Island (1993, N=100) and Pribilof Islands (1993, N=56; 1996, N=100). Individual crabs were sampled from commercial pot catches by the onboard fishery observers and from dockside commercial catches delivered to St. Paul Island. Crabs caught in pots in adjacent areas were pooled into a single collection. Sampling included multiple-year collections from Pribilof Islands to increase sample

sizes from this geographic location. Data from a total of 256 crabs were analyzed for this study (Table 3).

Tissues (muscle, heart, gill, and hepatopancreas) were dissected from each live individual crab, placed in a labeled tube chilled on wet ice, capped, and immediately frozen in liquid nitrogen. Within a few weeks, all samples were transported to the laboratory in liquid nitrogen and stored at –80°C until analysis.

Laboratory analysis

Protein extracts were prepared from the tissues and electrophoresed following the general procedures of Harris and Hopkinson (1976) and Aebersold et al. (1987), and the red king crab allozyme protocols of Seeb and Seeb (1990), Seeb et al. (1990), and ADF&G (1998b). We screened 59 allozyme loci. Activity reflecting 43 presumed loci were resolved with the following buffers (Table 4): (1) N-(3-aminopropyl)-morpholine, citrate (AC, pH 6.9) (Clayton and Tretiak 1972); (2) Tris, borate, citrate, lithium hydroxide (TBCL, pH 8.7) (Ridgway et al. 1970); (3) Tris, citrate (TC, pH 7.0) (Shaw and Prasad 1970); (4) Tris, citrate (TC, pH 8.0) (Selander et al. 1971); (5) Tris, borate, EDTA (TBE, pH 8.5) (Boyer et al., 1963); and (6) Tris, citrate, magnesium chloride (TCM, pH 7.5) (Shaklee and Samollow 1984). We used the enzyme nomenclature adopted by the American Fisheries Society (Shaklee et al. 1990). Red king crab controls were used during protocol development and population screening. Allelic designations were standardized to that of red king crabs.

Statistical analysis

We estimated allele frequencies, calculated average observed (H_o) and expected (H_e) heterozygosties, and tested conformation of genotype frequencies to Hardy-Weinberg expected frequencies with log-likelihood ratios (α=0.05; modified from Weir 1990) for each collection. Geographic and temporal heterogeneity among collections were evaluated with log-likelihood statistics (Sokal and Rohlf 1995). Inter-annual variation between two collections from the Pribilof Islands area was tested. Allele frequencies were used to generate distance matrices of Cavalli-Sforza and Edwards chord distances (Cavalli-Sforza and Edwards 1967). All computations were performed using *S-Plus* analytical software (MathSoft Inc. 1997). We used FSTAT (version 1.2; Goudet 1995) to estimate degree of population subdivision (F_{ST}; Wright's unbiased method of Weir and Cockerham 1984) and to test significance of F_{ST} values (1,000 permutations).

Findings

Results

Polymorphisms were observed in nine of the 43 loci assayed for genetic variation (Table 5, Figure 8). Nine polymorphic loci were: *ALAT*, ALP*, GPI-A*, MDH-A1*, MDH-A2*, MPI*, PEPA-1*, PGDH*,* and *SOD-3**. Monomorphic loci were: s*AAT-1*, mAAT-1*,*

ACP-1, ADA-1*, ADA-2*, sAH*, CK-A2*, EST-1*, EST-2* ,EST-3*, FBALD-1*, FBALD-2*, FH*, α-GALA-1, α-GALA-2*, β-GUS-1*, GAPDH*, G3PDH-1*, G3PDH-2*, GR-1*, GR-2*, IDHP-1*, IDHP-2*, LDH-2*, LDH-3*, MEP-1*, PEPB*, PEPD-1*, PGM-1*, PROT*, SOD-1*, SOD-2*, , TPI-1*,* and *TPI-2**. Of the nine polymorphic loci included in the analysis, just one locus, *ALP**, was variable at the 0.05 level or greater in at least one collection. Sample quality was very high across all tissues and collections analyzed.

Tests for Hardy-Weinberg fits were not significant, therefore we assumed our collections were from single panmictic populations. Observed (H_o and expected (H_e) heterozygosity ranged from 0.0086 and 0.0085, respectively, in Pribilof Islands (1996) to 0.0106 and 0.0113, respectively in St. Matthew Island (1993) (Table 5).

Temporal variation was examined for the multiple-year collections from Pribilof Islands. The 1993 and 1996 collections from Pribilof Islands were temporally stable (*P*=0.45), so these collections were pooled for subsequent analyses.

Various measures indicated a low level of population differentiation. F_{ST} values were low for most loci, however one locus, *ALP**, with an F_{ST} value of 0.0390 differed significantly from zero (*P*=0.0010). The overall F_{ST} value of 0.0260 for 9 polymorphic loci (*P*=0.0010) also differed significantly from zero. Geographic variation was examined between the St. Matthew Island and Pribilof Islands pooled collections. Again, *ALP**, showed significant heterogeneity (*P*=0.0003). The overall statistic in this comparison was also significant (*P*=0.0147). The genetic distance measure (Cavalli-Sforza and Edwards 1967) between blue king crab populations was 0.028.

Low frequency alleles detected by geographic area were: (1) St. Matthew Island, *ALAT*84, GPIA*0, MPI*97* and (2) Pribilof Islands, *MDH-A1*61, MDH-A2*85, PEPA-1*100, PGDH*100* and **104*, and *SOD-3*90*.

Discussion

Delineation of genetic structure, and thus identification of self-recruiting stocks, in natural populations cannot be successfully accomplished without the analysis of distinct alleles at defined loci (Allendorf et al. 1987; Utter et al. 1987). While allozyme studies have been used extensively to describe evolutionary relationships within genera of decapod crustaceans (e.g., Bert 1986; Busack 1989; Abdullah and Shukor 1993), these techniques have generally revealed very low levels of intraspecific genetic variation (Nelson and Hedgecock 1980; Smith et al. 1980, Seeb et al. 1990; Merkouris et al. 1998; ADF&G 1998b). Our findings are consistent with previous allozyme studies inferring that large, mobile species of crustaceans exhibit low levels of variation among populations as revealed by allozymes (e.g. red king crabs, Seeb et al. 1990 and ADF&G 1998b; Tanner and snow crabs, Davidson et al. 1985, Merkouris et al. 1998). However, we did detect significant differences between our two collections of blue king crabs.

Although the number of useful markers is low, the implication of fine scale stock differences is evident.

A comparison of our results to that of the red king crab data reveals similarly low overall levels of genetic variation. We detected lower levels of variation in blue king crab populations we surveyed than their congener. For example, of the 39 loci resolved in seventeen collections of red king crabs, 36% were polymorphic, and H_o ranged from 0.0114 to 0.0264 (ADF&G, 1998b). The genetic distance measures (Cavalli-Sforza and Edwards 1967) among red king crab populations (ADF&G 1998b) ranged from 0.013 (between Deadman Reach and Barlow Cove, Southeast Alaska) to 0.082 (between Deadman Reach and Chiniak Bay, NGOA). Two loci (*ALP**, *PGDH**) were variable at the 0.05 level or greater in at least one collection (ADF&G 1998b).

In contrast, 21% of the 43 loci resolved in blue king crabs were polymorphic, and H_o ranged from 0.0085 to 0.0113. However, in both species, the same locus (*ALP**) is largely driving the detected genetic differences among populations surveyed. The genetic distance measure (Cavalli-Sforza and Edwards 1967) of 0.028 was closest to the lower range of red king crab genetic distances. Just one locus (*ALP**) was variable at the 0.05 level or greater.

Other differences between the congeners were also evident. For example, blue king crabs express differing common alleles than that of red king crabs in five loci (*G3PDH-1*, G3PDH-2*, PEPA*, PGDH*, and SOD-2**). In addition, unique alleles are expressed by blue king crabs in five loci (*ALAT*, GPIA*, MDH-A1*, MPI**, and *PEPA*-1**).

In comparison to the relatively ubiquitous distribution of red king crabs, given the relatively discrete and isolated nature of blue king crab distributions in Alaska, we could logically hypothesize that blue king crabs would exhibit greater levels of genetic differentiation. Because blue king crabs have differing distributions, habitat requirements, and life history characteristics than red king crabs (see Somerton, 1985), the expectation follows that gene flow between populations is reduced. However, the within-species genetic distance measures do not make a strong case for this hypothesis. It is possible that gene flow is restricted, but that the differentiating forces of drift, selection, and mutation have not yet produced detectable genetic divergence at most loci examined to date.

Additional Work Needed

The differences detected between our two population collections of blue king crabs point to two possible areas of additional research: (1) development of more highly variable markers, such as nuclear DNA microsatellites, which could provide a suite of powerful loci to further examine population structure (e.g., red king crab microsatellites, this report). Red king crab microsatellite primers have been shown to amplify in blue king crab DNA (Carol Kerkvliet, ADF&G, Anchorage, Alaska, personal communication; Pam Jensen, University of Washington, Seattle, Washington, personal communication);

and (2) screening of additional populations of blue king crabs from outside the EBS area, such as the northern GOA and Southeast Alaska, may be warranted.

INVESTIGATIONS OF GENETIC VARIATION IN GOLDEN KING CRABS: A PROGRESS REPORT

Approach

Introduction, Methods and Materials

Four collections (Adak Island, Dutch Harbor, Bering Sea, and Frederick Sound/Chatham Strait, total N=410) of golden king crabs (*Lithodes aequispinus*) were obtained from 1994 to 1996 (Figure 1). Sampling methods followed those used for collections of blue king crabs for allozyme analysis.

Laboratory analysis methods follow those of Harris and Hopkinson (1976), Aebersold et al. (1987), Seeb and Seeb (1990), and Seeb et al. (1990), and this report (blue king crab protocol). Gene nomenclature follows Shaklee et al. (1990).

Allozyme data collection is complete for the Bering Sea (1996) and Southeast Alaska (Frederick Sound/Chatham Strait; 1996) collections. Preliminary allele frequencies are reported herein. A third population collection, Adak Island (1994) is currently being analyzed in the laboratory.

Results and Discussion

Polymorphisms have been observed in 10% of the 40 loci resolved to date. Four polymorphic loci are: *EST-3*, PEPA*, PEPD-1**, and *SOD-3** (Table 6). One locus, *EST-3**, is variable at the 0.05 level or greater. Sample quality is very high in all tissues and both population collections examined to date. We are continuing to detect new alleles in golden king crabs as we screen additional specimens from the Adak Island (1994) collection.

In our first semi-annual report for FY98, we indicated the allelic designations for golden king crabs would be standardized to that of red and blue king crabs. However, allozyme loci expression in golden king crabs differs markedly from that of red and blue king crabs, and standardizing across different genera is difficult. In comparing golden king crabs to the *Paralithodes* species, seven loci express differing common alleles: *AAT-1**, *CK-A2**, *G3PDH-1, G3PDH-2**, *MEP-1**, *PEPA-1**, and *SOD-2**. In addition, *EST-3** is highly polymorphic.

We anticipate completing collection of allozyme data for Adak Island (1994) samples by late fall 1999, with preliminary results available for the annual interagency crab research

meeting held in December, 1999. Pending results of analysis of these data, collection of allozyme data from the Dutch Harbor (1995) specimens may be warranted.

Additional Work Needed

Once all data are collected and analyzed, additional collections may be warranted if these markers appear promising for stock delineation of golden king crabs.

PROJECT MANAGEMENT

Lisa Seeb, Sue Merkouris, and Gordon Kruse conceptualized the red king crab microsatellite study. Development of red king crab microsatellite primers were performed by Pamela Jensen and Paul Bentzen, University of Washington Marine Molecular Biotechnology Laboratory, under contract to ADF&G. Carol Kerkvliet and Jeff Olsen did statistical analyses and report preparation laboratory data.

Sue Merkouris conducted allozyme protocol development and laboratory analyses of blue and golden king crabs. Sue Merkouris and Lisa Seeb conducted statistical analyses and report preparation.

PROJECT EVALUATION SUMMARY

Attainment of Project Goals and Objectives

Work was planned in the following areas for FY 99: (1) continue a study begun in FY 98 using DNA-level markers, primarily microsatellites, for stock discrimination among red king crab populations. In FY 99, we planned to examine up to 100 individuals from five population collections currently archived in –80° C freezers at ADF&G, (2) incorporate internal review comments on "A genetic investigation of hybridization between *Chionoecetes bairdi* and *C. opilio*" and submit for publication in a professional journal, (3) complete analysis of red king crab allozyme data and prepare a report (RIR), (4) complete analysis of blue and golden king crab allozyme data, and (5) contribute to a multi-authored RIR with recommendations for the management of Bering Sea Tanner crabs for use by the Alaska Board of Fisheries (BOF) and the North Pacific Fishery Management Council (NPFMC). These project goals have been largely attained during this fiscal year. DNA was extracted from 500 individuals for microsatellite analysis; 250 of these were completely genotyped during FY98. The final results of the *Chionoecetes* hybrid study have been presented at the invited symposium "The role of hybridization in the distribution, conservation, and management of aquatic species" at the 1999 American Fisheries Society meeting. The manuscript is being finalized for review and submission to a professional journal. Allozyme analysis of blue king crabs is complete and results are presented herein. Screening of allozyme variation in golden king crabs is underway; preliminary allele frequencies are presented in this report. Analysis of these data will be completed following additional population screenings. A summary of the findings of

Merkouris et al. (1998), specifically as they relate to EBS Tanner crabs, was included in an ADF&G report to the Alaska Board of Fisheries (AKBOF) as well as RIR No. 5J99-04: "Overview of population dynamics and recommended harvest strategy for Tanner crabs in the eastern Bering Sea" by J. Zheng and G. H. Kruse. This report was also attached to the draft for public review prepared by the NPFMC: "A rebuilding plan for the Bering Sea *C. bairdi* stock".

Modifications to Project Goals and Objectives

Few modifications were made to the project goals and objectives. A re-analysis combining two data sets from previously published red king allozyme studies has been delayed until completion and reporting of other (blue and golden king crabs) allozyme studies. Allozyme analyses of Dutch Harbor (1995) golden king crabs are on hold pending results of the first three population screenings. An additional task identified during FY98 was obtaining a new collection of *C. bairdi* from the Bristol Bay section of the EBS. A sample of 100 crabs was collected during the June 1999 EBS trawl survey. These samples will be used to further examine population structure of *C. bairdi* in the EBS.

Dissemination of project results

Final results for each of the studies will be disseminated as follows: (1) red king crab microsatellite study: preliminary data summarized in an ADF&G RIR (this report); final data may be submitted for publication in an appropriate professional journal; (2) red king crab allozyme data will be summarized in an ADF&G RIR upon completion; (3) *Chionoecetes* species hybrid study: publication in a professional journal; (4) blue king crab allozyme study: summarization in an ADF&G RIR or submitted as a short article in a professional journal; (5) golden king crab allozyme study: summarization in an ADF&G RIR or submitted as a short article in a professional journal; (6) Bering Sea *C. bairdi* allozyme results have been included in reports to the BOF and the NPFMC. Additionally, updated results will be disseminated at the December 1999 joint agency shellfish research meeting. Specific project results may also be presented at pertinent agency meetings.

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Table 1. Observed (H_O) and expected (H_E) heterozygosity for microsatellite and allozyme loci from seven red king crab populations. An asterisk denotes H_O values that depart significantly from Hardy-Weinberg expectation based on a probability test.

Locus		Deadman Reach	Barlow Cove	Kachemak Bay	Uganik Passage	Bristol Bay	Pribilof Islands	Norton Sound
Microsat.		n=95	n=95	n=45	n=94	n=106	n=100	n=100
*Pca*4	H_O	0.617*	0.580*	0.911	0.728*	0.750*	0.633*	0.436*
	H_E	0.879	0.877	0.938	0.945	0.932	0.929	0.926
*Pca*13	H_O	0.674*	0.702*	0.867	0.766*	0.833*	0.649*	0.629*
	H_E	0.932	0.930	0.956	0.962	0.965	0.949	0.953
*Pca*14	H_O	0.516*	0.568*	0.689	0.589*	0.583*	0.500*	0.444*
	H_E	0.758	0.794	0.807	0.837	0.832	0.793	0.823
*Pca*24	H_O	0.628	0.774	0.711*	0.702*	0.755*	0.687*	0.711*
	H_E	0.769	0.817	0.906	0.884	0.901	0.893	0.930
Avg.	H_E	0.834	0.855	0.902	0.907	0.907	0.891	0.908
Allozymes		n=100	n=100	n=45	n=100	n=75	n=100	n=94
*sAH**	H_O	0.022	0.000	0.000	0.041	0.027	0.000	0.054
	H_E	0.021	0.000	0.000	0.040	0.027	0.000	0.053
*ALP**	H_O	0.202	0.239	0.194*	0.298*	0.333	0.421	0.410
	H_E	0.244	0.228	0.419	0.448	0.404	0.442	0.357
*GPI-A1**	H_O	0.000	0.000	0.000	0.000	0.000	0.000	0.055
	H_E	0.000	0.000	0.000	0.000	0.000	0.000	0.054
*PGDH**	H_O	0.500	0.412	0.366	0.608	0.473	0.460	0.419
	H_E	0.499	0.504	0.519	0.515	0.510	0.494	0.524
Avg.	H_E	0.191	0.183	0.234	0.251	0.235	0.234	0.247

Table 2. Multilocus F_{ST} estimates for each population pair for each marker class and multilocus R_{ST} estimates for each population pair for microsatellites. An asterisk denotes estimates that are significantly greater than zero based on a permutation test.

Population	Deadman Reach	Barlow Cove	Kachemak Bay	Uganik Passage	Bristol Bay	Pribilof Islands
Microsatellites				F_{ST}		
Barlow Cove	0.0051*					
Kachemak Bay	0.0257*	0.0221*				
Uganik Passage	0.0219*	0.0164*	0.0015			
Bristol Bay	0.0239*	0.0185*	0.0008	0.0008		
Pribilof Islands	0.0296*	0.0219*	0.0041	0.0019	0.0019	
Norton Sound	0.0364*	0.0301*	0.0054	0.0063*	0.0036*	0.0078*
Microsatellites				R_{ST}		
Barlow Cove	0.0081					
Kachemak Bay	0.0173	0.0331				
Uganik Passage	0.0155	0.0279*	-0.0022			
Bristol Bay	0.0092	0.0160	-0.0041	0.0017		
Pribilof Islands	0.0167	0.0128	0.0069	0.0050	0.0063	
Norton Sound	0.0210*	0.0100	0.0127	0.0124	0.0076	-0.0036
Allozymes				F_{ST}		
Barlow Cove	0.0039					
Kachemak Bay	0.0229	0.0457*				
Uganik Passage	0.1081*	0.1156*	0.0039			
Bristol Bay	0.0217	0.0152	-0.0183	0.0328*		
Pribilof Islands	0.0412*	0.0547*	-0.0301	0.0179	0.0107	
Norton Sound	0.2961*	0.3047*	0.1467*	0.0653*	0.1816*	0.1509*

Table 3. Collection information for blue king crab samples analyzed in allozyme pilot study.

Geographic Location	Region	Approximate Latitude/Longitude	Sample date(s)	N
St. Matthew Island	Bering Sea	60°15'27"N, 173°02'48"W	09/15/93	10
St. Matthew Island	Bering Sea	60°15'37"N, 173°01'32"W	09/15/93	10
St. Matthew Island	Bering Sea	60°18'43"N, 173°17'36"W	09/16/93	10
St. Matthew Island	Bering Sea	60°13'54"N, 173°14'13"W	09/16/93	8
St. Matthew Island	Bering Sea	60°13'49"N, 173°20'18"W	09/16/93	2
St. Matthew Island	Bering Sea	60°13'12"N, 173°17'19"W	09/17/93	9
St. Matthew Island	Bering Sea	60°14'12"N, 173°15'29"W	09/17/93	6
St. Matthew Island	Bering Sea	60°10'08"N, 173°13'47"W	09/17/93	5
St. Matthew Island	Bering Sea	60°10'11"N, 173°15'28"W	09/17/93	10
St. Matthew Island	Bering Sea	60°02'19"N, 173°09'11"W	09/18/93	10
St. Matthew Island	Bering Sea	59°57'58"N, 173°16'00"W	09/19/93	4
St. Matthew Island	Bering Sea	59°58'11"N, 173°17'31"W	09/19/93	5
St. Matthew Island	Bering Sea	60°03'43"N, 173°26'11"W	09/20/93	5
St. Matthew Island	Bering Sea	60°06'05"N, 173°24'29"W	09/21/93	6
			Total	**100**
Pribilof Islands	Bering Sea	57°54'59"N, 169° 19'15"W	9/22/93	10
Pribilof Islands	Bering Sea	57°53'45"N, 169° 18'15"W	9/22/93	10
Pribilof Islands	Bering Sea	57°54'36"N, 169° 18'48"W	9/22/93	10
Pribilof Islands	Bering Sea	57°56'45"N, 169° 18'12"W	9/22/93	10
Pribilof Islands	Bering Sea	57°57'59"N, 169° 18'43"W	9/22/93	10
Pribilof Islands	Bering Sea	57°52'44"N, 169° 18'58"W	9/22/93	6
			Total	**56**
Pribilof Islands	Bering Sea	57°17'54"N, 169° 50'54"W	11/01/96	19
Pribilof Islands	Bering Sea	57°16'52"N, 169° 49'14"W	11/02/96	4
Pribilof Islands	Bering Sea	57°24'00"N, 170° 11'44"W	11/02/96	3
Pribilof Islands	Bering Sea	56°46'53"N, 170° 00'00"W	11/07/96	11
Pribilof Islands	Bering Sea	57°03'00"N, 170° 15'35"W	11/07/96	3
Pribilof Islands	Bering Sea	56°49'01"N, 170° 01'09"W	11/09/96	10
Pribilof Islands	Bering Sea	57°13'58"N, 169° 47'32"W	11/09/96	2
Pribilof Islands	Bering Sea	57°18'45"N, 169° 50'47"W	11/10/96	5
Pribilof Islands	Bering Sea	56°48'36"N, 167° 57'42"W	11/15/96	6
Pribilof Islands	Bering Sea	56°50'36"N, 169° 54'45"W	11/15/96	9
Pribilof Islands	Bering Sea	56°19'21"N, 169° 52'44"W	11/15/96	3
Pribilof Islands	Bering Sea	57°06'42"N, 170° 29'59"W	11/16/96	4
Pribilof Islands	Bering Sea	56°41'04"N, 169° 16'14"W	11/17/96	5
Pribilof Islands	Bering Sea	56°42'59"N, 169° 26'48"W	11/18/96	6
Pribilof Islands	Bering Sea	57°24'32"N, 170° 10'15"W	11/19/96	8
Pribilof Islands	Bering Sea	56°54'52"N, 169° 56'53"W	11/25/96	2
			Total	**100**
			Grand Total	**256**

Table 4. Forty-three allozyme loci examined in this study. Enzyme nomenclature follows Shaklee et al. (1990). Included are Enzyme Commission number, locus abbreviation, tissues, and electrophoresis buffers. Tissue abbreviations are: (M) muscle, (H) heart, (G) gill, and (HP) hepatopancreas. Buffers are described in the text.

Enzyme or protein	Enzyme Number	Locus	Tissue(s)	Buffer(s)
Aspartate Aminotransferase	2.6.1.1	*sAAT-1*,mAAT-1**	M	AC6.9
Acid phosphatase	3.1.3.2	*ACP**	HP	TBCL
Adenosinedeaminase	3.5.4.4	*ADA-1*, ADA-2**	G	TBCL
Aconitate hydratase	4.2.1.3	*sAH**	H	TC8.0
Alanine aminotransferase	2.6.1.2	*ALAT**	M	TBCL
Alkaline phophatase	3.1.3.1	*ALP**	HP	TCM
Creatine kinase	2.7.3.2	*CK-A2**	H	TC8.0
Esterase	3.1.1.1	*EST-1*,EST-2*,EST-3**	G	TBE
Fructose-biphosphate aldolase	4.1.2.13	*FBALD-1*, FBALD-2**	M	AC6.9
Fumarate hydratase	4.2.1.2	*FH**	M	TBE/TBCL
β-N-acetylgalactosaminidase	3.2.1.53	*β-GALA-1*, β-GALA 2**	G	TBCL
β-Glucuronidase	3.2.1.31	*β-GUS-1**	HP	TBCL
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH**	M	TC7.0
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1, G3PDH-2**	M	AC6.9
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A**	M	TBCL
Glutathione reductase	1.6.4.2	*GR-1*, GR-2**	H	TBCL
Isocitrate dehydrogenase	1.1.1.42	*IDHP-1*, IDHP-2**	M	TC7.0
Lactate dehydrogenase	1.1.1.27	*LDH-2*, LDH-3**	M/H	TBCL
Malate dehydrogenase	1.1.1.37	*MDH-A1*, MDH-A2**	M	AC6.9
Malic enzyme	1.1.1.40	*MEP-1**	H	TC8.0/TBCL
Mannose-6-phophaste isomerase	5.3.1.8	*MPI**	M	TBE
Dipeptidase[1]	3.4.-.-	*PEPA-1**	M/H	TBCL
Tripeptide aminopeptidase	3.4.-.-	*PEPB**	M	TBCL
Proline dipeptidase	3.4.13.9	*PEPD-1**	G/M	TBCL
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M	TC7.0
Phosphoglucomutase	5.4.2.2	*PGM-1**	M	TC7.0
General (unidentified) protein		*PROT**	M	TBCL
Superoxide dimutase	1.15.1.1	*SOD-1*, SOD-3**	H,M	TBCL
		*SOD-2**	G	TBCL
Triose-phosphate isomerase	5.3.1.1	*TPI-1*, TPI-2**	HP	TCM

[1] Preferential stain is *PEPA-1** resolved with Glycyl-DL-Leucine.

Table 5. Estimated allele frequencies and observed and expected heterozygosities for blue king crabs.

		*ALAT**			*ALP**	
Population	N	*100	*84	N	*100	*129
Pribilof Islands 1993	50	1.0000	0.0000	44	0.1364	0.8636
Pribilof Islands 1996	100	1.0000	0.0000	95	0.1105	0.8895
Pribilof Islands pooled	150	1.0000	0.0000	139	0.1187	0.8813
St. Matthew Island 1993	100	0.9950	0.0050	92	0.2500	0.7500

		*GPIA**			*MDH-A1**	
Population	N	*100	*160	*0	N	*100	*61
Pribilof Islands 1993	50	0.9700	0.0300	0.0000	56	1.0000	0.0000
Pribilof Islands 1996	99	0.9798	0.0202	0.0000	100	0.9950	0.0050
Pribilof Islands pooled	149	0.9765	0.0235	0.0000	156	0.9968	0.0032
St. Matthew Island 1993	100	0.9850	0.0100	0.0050	100	1.0000	0.0000

		MDH-A2			*MPI**	
Population	N	*100	*85	N	*100	*97
Pribilof Islands 1993	56	0.9911	0.0089	50	1.0000	0.0000
Pribilof Islands 1996	100	1.0000	0.0000	100	1.0000	0.0000
Pribilof Islands pooled	156	0.9968	0.0032	150	1.0000	0.0000
St. Matthew Island 1993	100	1.0000	0.0000	100	0.9950	0.0050

		*PEPA-1**			*PGDH**		
Population	N	*100	*123	*169	N	*100	*104	*98
Pribilof Islands 1993	49	0.0000	0.9796	0.0204	50	0.0000	0.0000	1.0000
Pribilof Islands 1996	100	0.0050	0.9950	0.0400	100	0.0050	0.0050	0.9900
Pribilof Isands pooled	149	0.0034	0.9631	0.0336	150	0.0033	0.0033	0.9933
St. Matthew Island 1993	100	0.0000	0.9750	0.0250	100	0.0000	0.0000	1.0000

		*SOD-3**			H_o	H_e
Population	N	*100	*115	*90		
Pribilof Islands 1993	50	0.9900	0.0100	0.0000	0.0096	0.0087
Pribilof Islands 1996	100	0.9950	0.0000	0.0050	0.0086	0.0085
Pribilof Islands pooled	150	0.9933	0.0033	0.0033	0.0089	0.0085
St. Matthew Island 1993	98	0.9949	0.0051	0.0000	0.0106	0.0113

Table 6. Preliminary allele frequency and expected and observed heterozygosity estimates for two collections of golden king crabs.

		*EST-3**				*PEPA**		
Population	N	*100	*94	*104	N	*100	*67	*40
Bering Sea 1996	94	0.6755	0.2074	0.1170	97	0.9897	0.0000	0.0103
Southeast AK 1996	96	0.6406	0.2656	0.0938	100	0.9950	0.0050	0.0000

		*PEPD-1**		*sSOD-3**			
Population	N	*100	*131	N	*100	*129	*71
Bering Sea 1996	100	1.0000	0.0000	100	0.9750	0.0150	0.0100
Southeast AK 1996	100	0.9950	0.0050	100	0.9750	0.0250	0.0000

Population	H_e	H_e
Bering Sea 1996	0.0119	0.0140
Southeast AK 1996	0.0137	0.0145

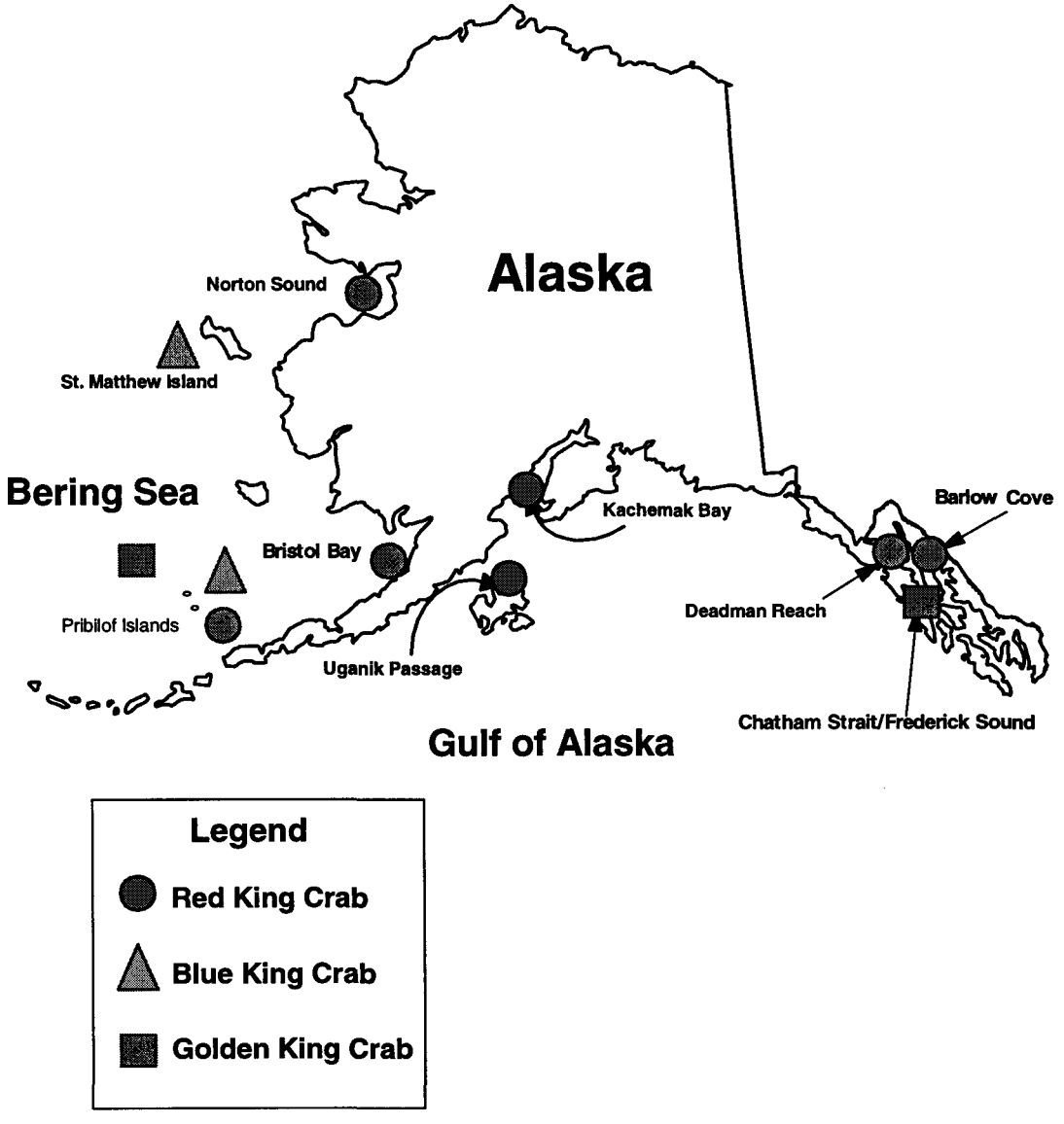

Figure 1. Sample collection sites of red king crabs analyzed in microsatellite study and blue and golden king crabs analyzed in allozyme studies.

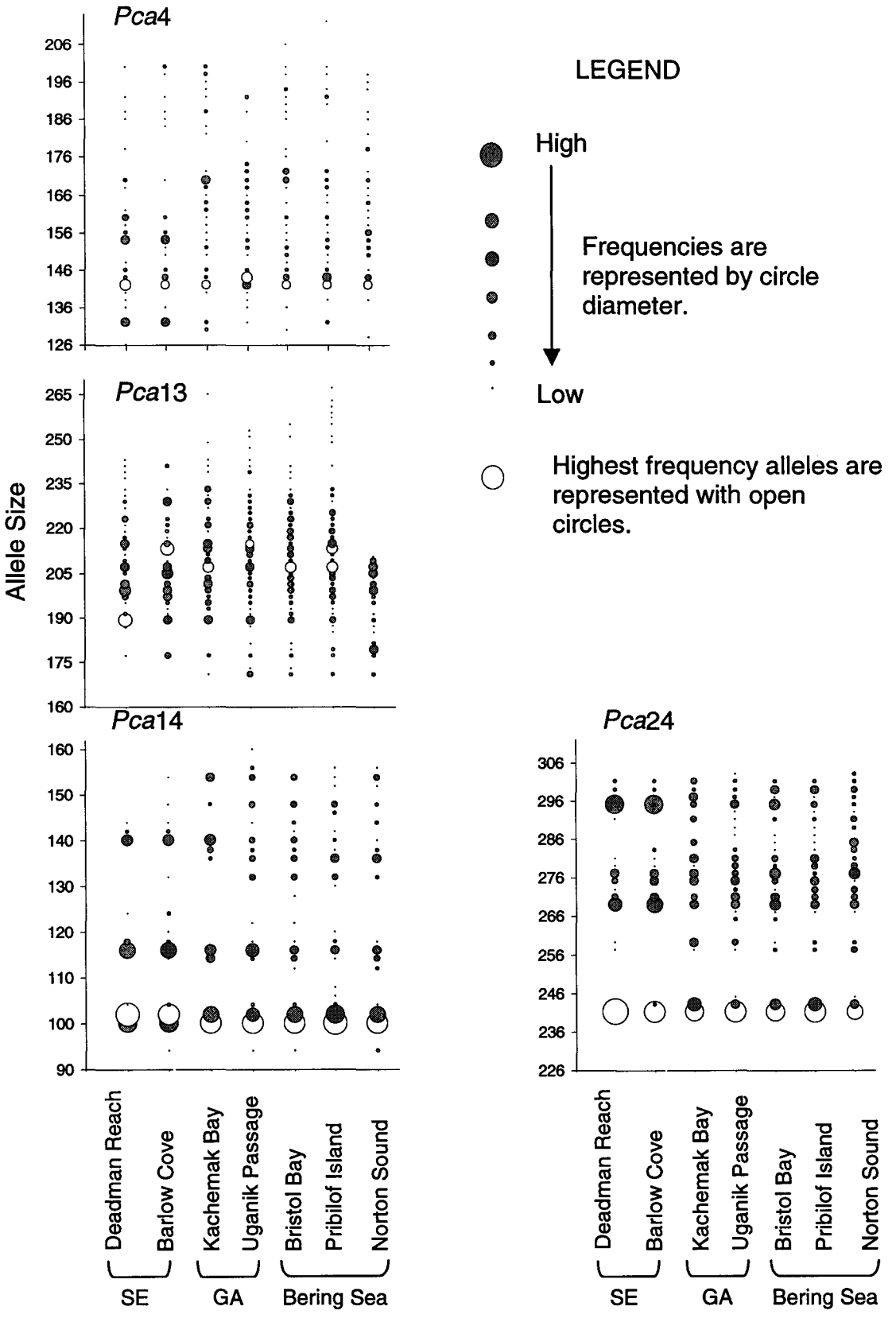

Figure 2. Allele frequency distribution at four microsatellite loci (*Pca4*, *Pca13*, *Pca14*, and *Pca24*) from seven Alaskan red king crab populations.

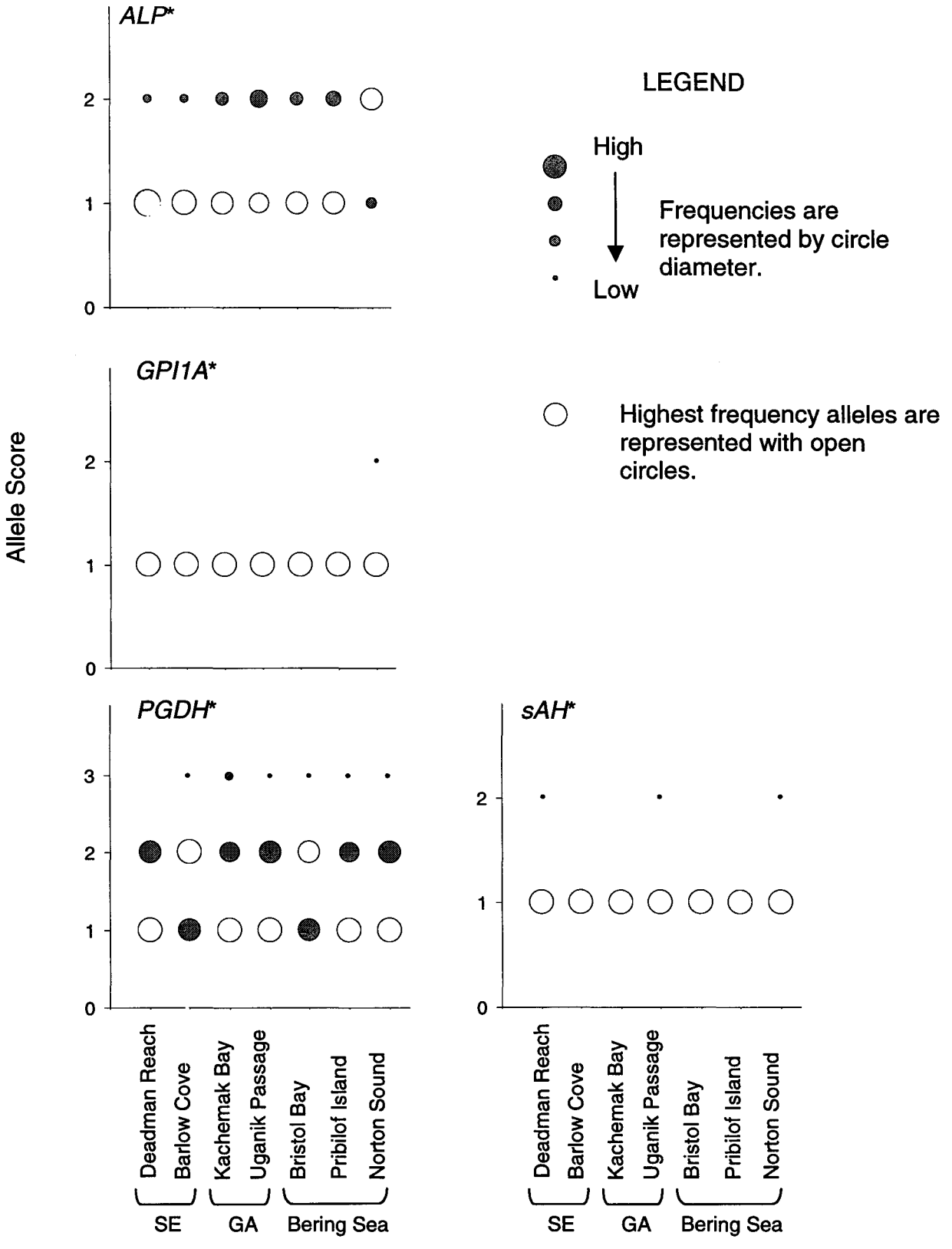

Figure 3. Allele frequency distribution at four allozyme loci (*ALP**, *GP1A**, *PGDH**, and *sAH**) from seven Alaskan red king crab populations.

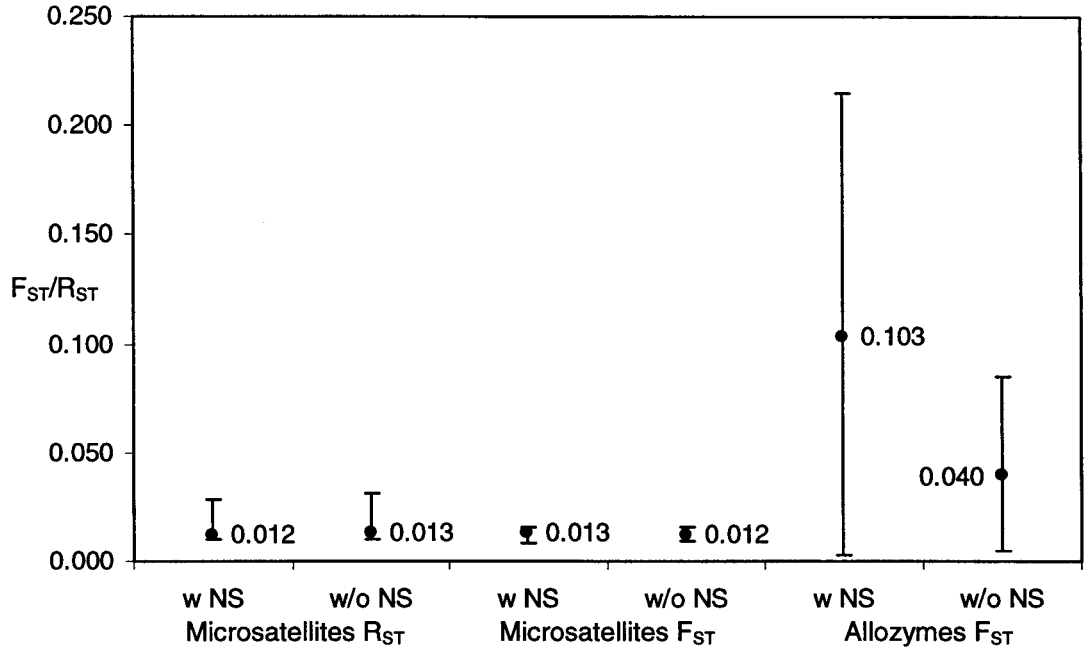

Figure 4. Multilocus F_{ST} estimates for each marker class and multilocus R_{ST} estimates for microsatellites. Estimates were computed with and without the Norton Sound sample. Error bars indicate 95% confidence intervals.

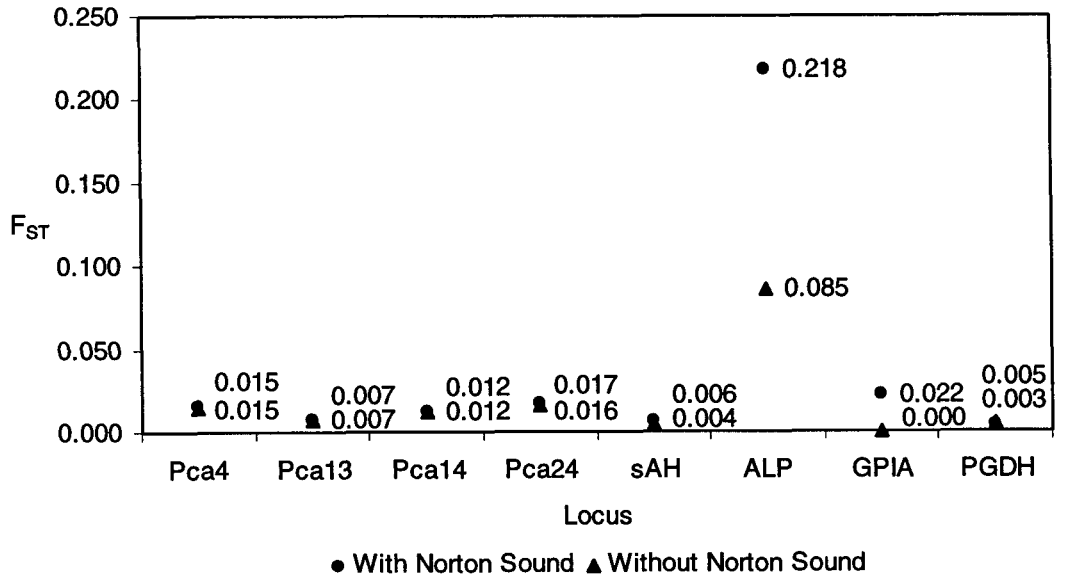

Figure 5. Single locus F_{ST} estimates for red king crabs with and without the Norton Sound sample.

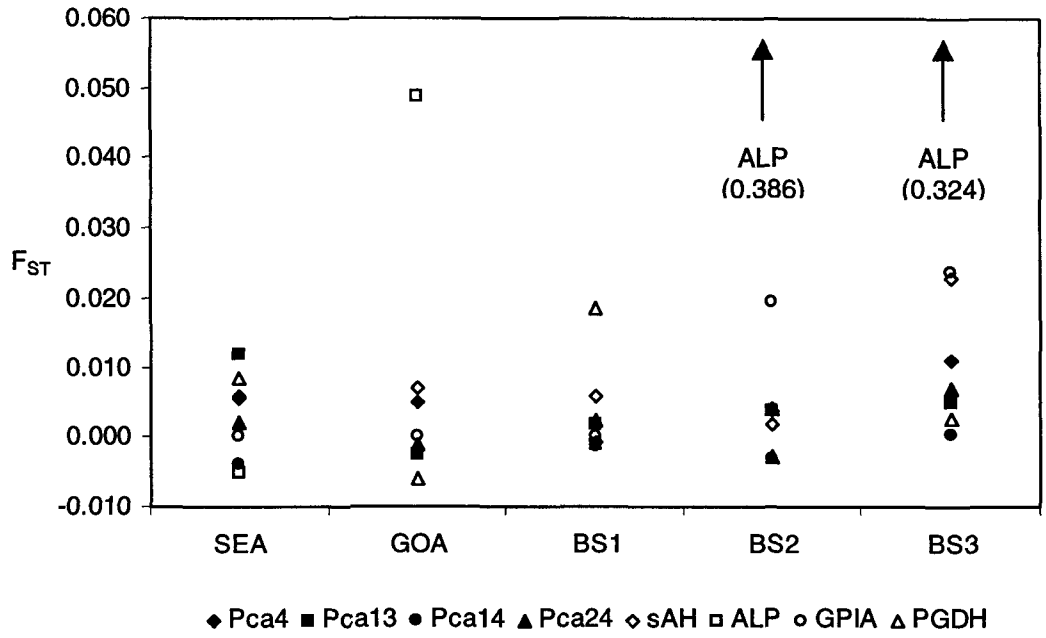

Figure 6. Single locus F_{ST} estimates for intra-regional population pairs: SEA (Deadman Reach x Barlow Cove), GOA (Kachemak Bay x Uganik Passage), BS1 (Bristol Bay x Pribilof Islands), BS2 (Bristol Bay x Norton Sound), BS3 (Pribilof Islands x Norton Sound).

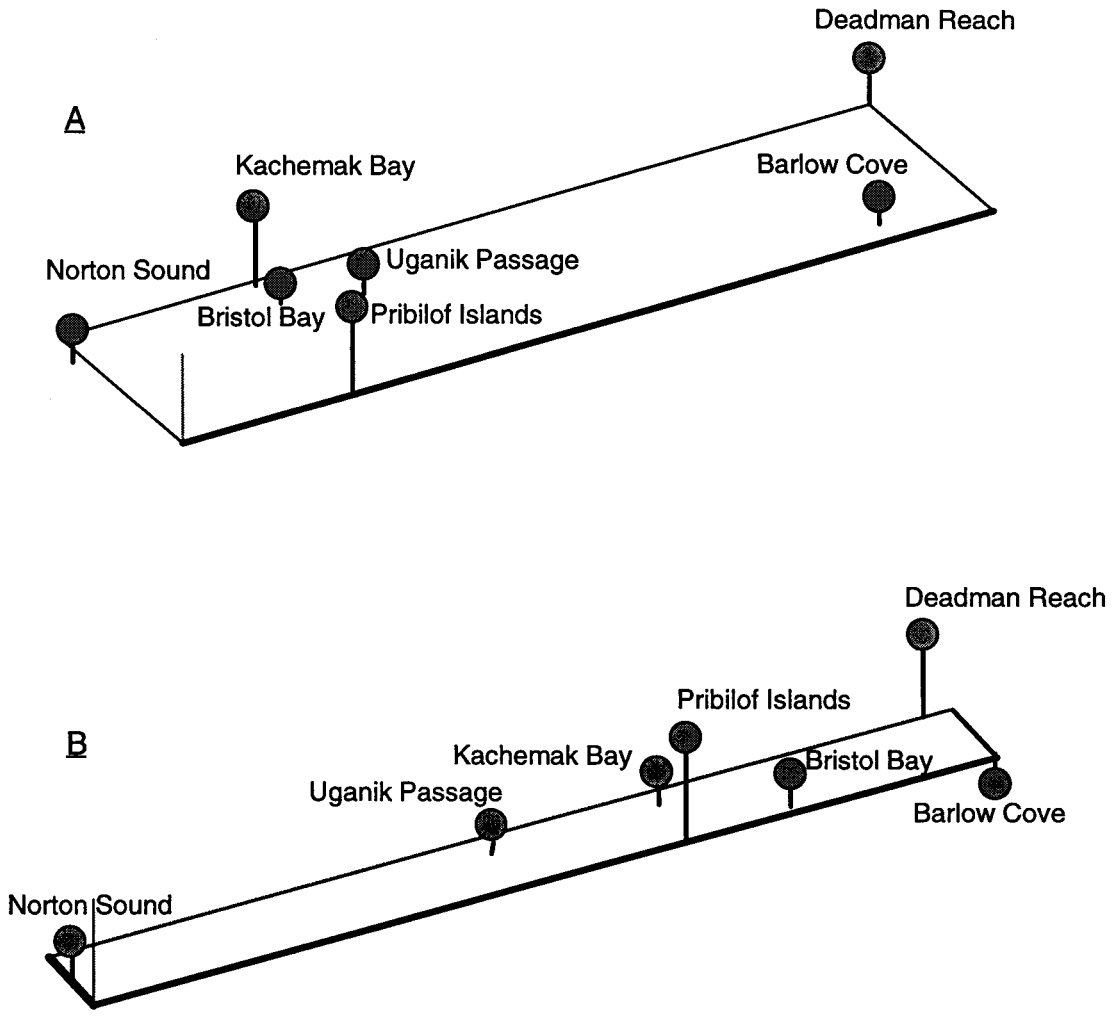

Figure 7. Multidimensional scaling plot using F_{ST} estimates from four microsatellite (A) and four allozyme loci (B).

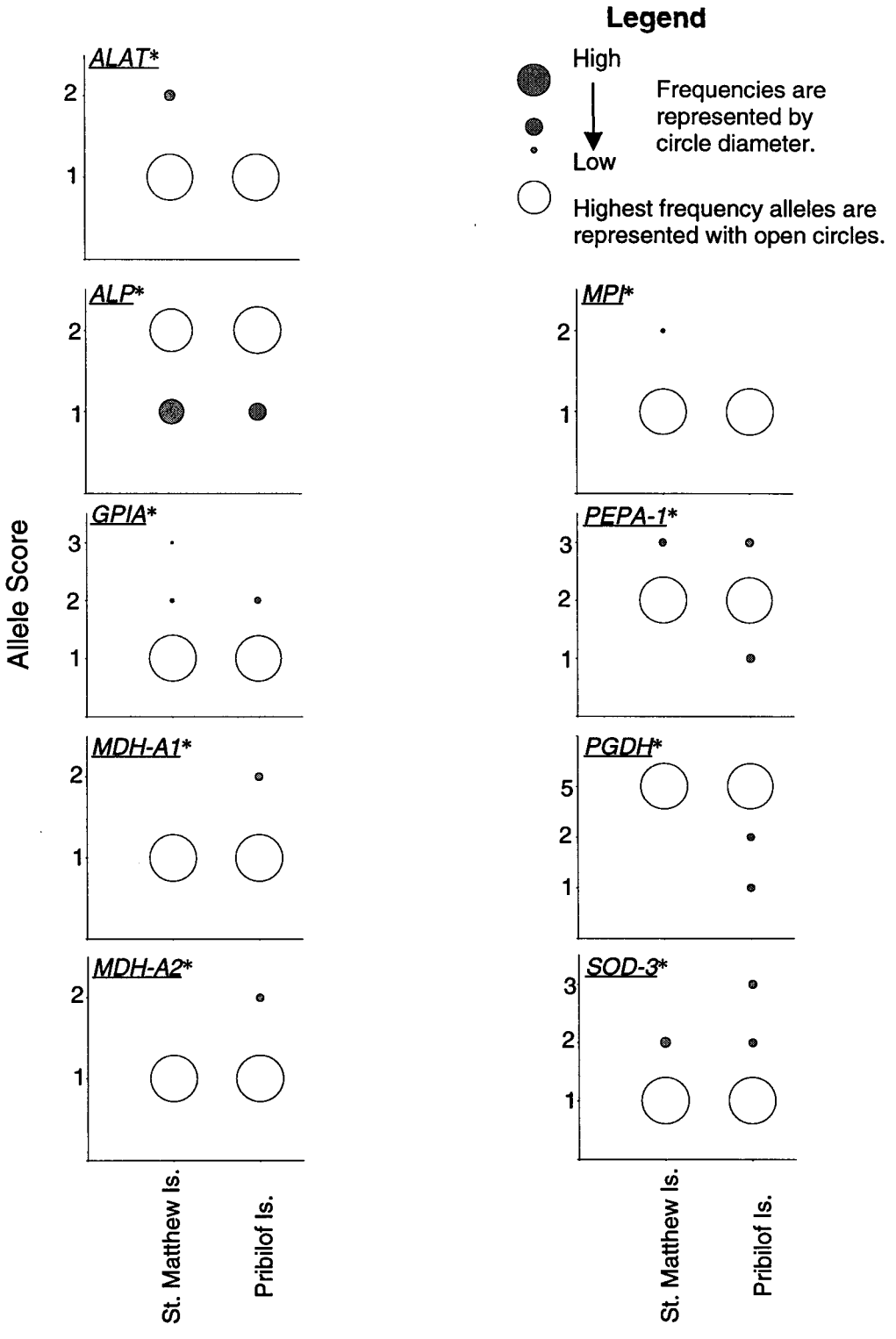

Figure 8. Allele frequency distribution at nine allozyme loci (*ALAT*, ALP*, GPIA*, MDH-A1*, MDH-A2*, MPI*,PEPA-1*, PGDH*, and SOD-3**) from two Alaskan blue king crab populations.

APPENDIX E: CRAB MANAGEMENT STRATEGIES

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Executive Summary

This project funds an ADF&G biometrician II to conduct quantitative analyses of abundance, biological, and fisheries data for crab stocks. Analyses during FY 99 focused on population estimation, optimal thresholds, harvest rates, rebuilding strategies, and stock and recruitment relationships. Length-based models and computer simulations are primary tools for these analyses. Projects completed during FY 99 include the catch-survey analysis for St. Matthew and Pribilof Islands blue king crabs in 1998, the length-based analysis to assess the Bristol Bay red king crab abundance in 1998, development of stock-recruitment relationships for Tanner crabs in Bristol Bay, and evaluation of optimal harvest strategies for eastern Bering Sea Tanner crabs. The findings were reported in five manuscripts and technical reports.

Purpose

Sound management requires precise estimates of population abundance and quantitative evaluations of alternative management strategies. In Alaska, many crab stocks are assessed annually by trawl or pot surveys, some are assessed irregularly, and some stocks lack assessments. Population estimation models are needed to make best use of multiple years of data on crab size, sex, and reproductive condition. Such models are necessary to evaluate measurement errors in annual surveys and to generate abundance estimates for stocks that are infrequently assessed.

Estimates of biological production parameters are needed to determine optimal management strategies and to calculate fishery yields for the king and Tanner crab fisheries off the coast of Alaska. For most stocks, the common biological and reference points, such as $F_{0.1}$, yield per recruit, optimum yield, and stock-recruit relationships have not been computed. The utility of fishery thresholds and alternative harvest rates have not been thoroughly evaluated either. Two main goals of this project are to improve population abundance estimation and evaluate alternative harvest strategies for crab stocks in Alaska.

Approach

This project funds an ADF&G biometrician II to conduct quantitative analyses of abundance, biological, and fisheries data for crab stocks. Analyses during FY 99 focused

on information germane to harvest policy: population estimation, optimal thresholds and harvest rates, rebuilding strategies, and stock and recruitment relationships. Length-based models and computer simulations are primary tools for estimating population abundance, evaluating optimal thresholds and harvest rates, and developing rebuilding strategies. The National Marine Fisheries Service trawl surveys and Alaska Department of Fish and Game trawl and pot surveys, catch sampling, and observer reports provide data for these analyses. Environmental data were obtained through several agencies.

Findings

In 1998, a length-based analysis was conducted to assess the annual abundance of Bristol Bay red king crab abundance, and a catch-survey analysis was conducted to assess Pribilof Islands and St. Matthew Island blue king crabs. To improve the assessment, we used a 3-stage model to conduct the catch-survey analysis in 1998 as opposed to a 2-stage model in prior years. A report on the status of king crab stocks in the eastern Bering Sea in 1998 was prepared as:

Zheng, J., G.H. Kruse, and M.C. Murphy. 1998. Stock status of king crabs in the Eastern Bering Sea in 1998. Alaska Department of Fish and Game, Regional Information Report 5J98-06, Juneau, Alaska.

The study on molting probabilities of mature male Tanner crabs in the eastern Bering Sea was initiated two years ago and was primarily conducted during FY 98. Our focus for this study was to examine the terminal molt hypothesis for male Tanner crabs. During FY 99, we revised the manuscript resulting from this study. Based on comments we received from some reviewers, further revision is needed before submitting it to a journal for publication. We will revise the following manuscript in the future:

Zheng, J., and G.H. Kruse. MS. Do male Tanner crab *(Chionoecetes bairdi)* undergo a terminal molt at morphometric maturity? Alaska Department of Fish and Game, Unpublished manuscript.

Work on optimal harvest strategies for eastern Bering Sea Tanner crabs has been completed. ADF&G submitted to the Alaska Board of Fisheries (BOF) a proposal to change the harvest strategy for eastern Bering Sea Tanner crabs based on this work. The BOF adopted the new harvest strategy at their meeting in March 1999. Two main parts of this study include the analysis of stock-recruitment relationships and evaluation of optimal harvest strategies. The findings are summarized in the following two papers and a report:

Zheng, J. and G.H. Kruse. 1998. A stock-recruitment relationship for Bristol Bay Tanner crab. Alaska Fishery Research Bulletin 5(2): 116-130.

Zheng, J. and G.H. Kruse. 1999. Evaluation of harvest strategies for Tanner crab stocks that exhibit periodic recruitment. Journal of Shellfish Research, *in press*.

Zheng, J. and G.H. Kruse. 1999. Overview of population dynamics and recommended harvest strategy for Tanner crabs in the eastern Bering Sea. Alaska Department of Fish and Game, Regional Information Report 5J99-04, Juneau, Alaska.

During FY 99, the principal investigator was asked to assist the North Pacific Fishery Management Council's (NPFMC's) Crab Plan Team to design a rebuilding plan for eastern Bering Sea Tanner crabs. The principal investigator conducted a computer simulation study on rebuilding schedules, as requested by the NPFMC's Scientific and Statistical Committee. This work was incorporated into a draft Environmental Assessment and Regulatory Impact Review. Work on the rebuilding plan continues as the EA/RIR goes through the Council's review process.

Finally, we examined relationships between crab recruitment success and groundfish abundance in the eastern Bering Sea. The senior author (Dr. Gordon Kruse) presented the results in the Alaska Sea Grant Symposium of Ecosystem Considerations on Fishery Management in October 1998.

All published papers from this project are routinely filed with NOAA once reprints have been received. Additional copies are available on request.

Evaluation

This project relates to the long-term research plan and crab fisheries management in three ways. First, for all major crab stocks we intend to develop estimates of population abundance by modeling available data. For crab stocks with surveys, the models provide estimates of crab abundance that are relatively insensitive to survey measurement errors in any single year. For crab stocks with only fishery performance data, catch-length models provide abundance estimates. Second, because these models embody critical biological parameters specific to a species and stock, they provide a framework within which to evaluate optimal harvest strategies. Finally, optimal harvest strategies developed under this framework can be used to optimally manage crab fisheries.

The goals and objectives for this fiscal year have been achieved with an exception of the Adak red king crab project. Because of lack of data, a catch-length analysis for the Adak red king crab population was postponed to next fiscal year (FY 00), depending on data availability. The time originally planned for the Adak red king crab study was used to develop rebuilding schedules of eastern Bering Sea Tanner crabs.

The findings from this project during this fiscal year have been disseminated to the users and public. First, four technical papers and reports documenting the findings have been

published or submitted for publication. We also distributed these manuscripts to management agencies. Second, one presentation on our findings was made in an international scientific conference (the Alaska Sea Grant Symposium of Ecosystem Considerations on Fishery Management). We also reported our findings in the ADF&G and NMFS interagency meeting in December 1998, the Board of Fisheries meeting in March 1999, and the NPFMC's Scientific and Statistical Committee meeting in April 1999. Finally, our findings were disseminated to the public through informal meetings with the public.