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An Evaluation of the Use of Mating Marks as an Indicator of Mating Success in Male Dungeness Crabs

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AN EVALUATION OF THE USE OF MATING MARKS AS AN INDICATOR OF MATING SUCCESS IN MALE DUNGENESS CRABS

by

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A Thesis

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ABSTRACT

An Evaluation of the Use of Mating Marks as an Indicator of Mating Success in Male Dungeness Crabs

Justin C. Ainsworth

Dungeness crab (*Cancer magister*) fisheries of the eastern Pacific, from Alaska to central California, have a minimum size limit, measured as carapace width (CW), to ensure that all harvested males have had the opportunity to participate in at least one mating season as sublegal-sized crabs. Dependable indicators of female mating activity have been revealed, but mating activity of males has only been indirectly inferred through examination of so-called "mating marks", presumably created during the premating embrace between a male and female. Previous researchers found that mating marks were present on a higher percentage of sublegal-sized males than on legal-sized males, leading to implications that in a fished population large females may go unmated, and cause a reduction in overall egg production. I present results from laboratory experiments designed to discover the process of mating mark formation, along with at-sea observations documenting the occurrence of mating marks in a natural population in northern California. The experiments involved laboratory matings of male and female crabs to determine the factors that influence the formation of mating marks. Hypothetical factors that may affect mating mark formation include relative male and female sizes, mating frequency, competition, and substrate type. A total of 107 male crabs, some mated multiple times and in different conditions, were allowed to form a total of 155 premating embraces in the laboratory. No mating marks were produced in any of the controlled laboratory matings, however. Mate takeovers, when a competing male displaces the female mate of another male in a premating embrace, were observed, resulting in males forming premating embraces with each other. In addition, inter-male competitive behaviors were observed that may produce marks on the males' claws. At-sea observations revealed that as the mating season progressed, the frequency of mating mark occurrence on all male crabs increased. Throughout the 2004 mating season, sublegal crabs in northern California had a slightly higher frequency of mating marks than legal crabs on half of the collections while half of the collections yielded a slightly higher mating mark frequency on legal crabs. Reliable use of mating marks to indicate mating success would require that (1) every premating embrace, involving the smallest to largest male capable of mating, produces mating marks; (2) every premating embrace results in successful copulation and insemination; and (3) the timing and location of field samples are appropriate for estimating population-level mating activity. Data from laboratory matings and at-sea observations show that these assumptions cannot be reasonably accepted, and therefore it is misleading to use mating marks as an index of mating activity in male Dungeness crabs.

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INTRODUCTION

All Dungeness crab (*Cancer magister*) fisheries of the eastern, Pacific from Alaska to central California, have a minimum size limit, measured as carapace width (CW, excluding the tenth antero-lateral spines): minimum CW is 159 mm in the California, Oregon, and Washington fisheries, 165 mm in Alaska, and approximately 155 mm in British Columbia. Canadian and U.S. commercial fisheries prohibit the retention of females. The U.S. Dungeness crab fishery regulates landings by season (e.g., the northern California fishery is open from December through mid-July), but the commercial fishery in British Columbia historically has been conducted year-round. Annual exploitation rates can be extremely high, sometimes exceeding 90% in northern California (Gotshall 1978; Methot and Botsford 1982; Hankin 1985) and British Columbia (Smith and Jamieson 1989). The minimum size limit is intended to ensure that all harvested males have had the opportunity to participate in at least one mating season as sublegal-sized crabs, and much evidence has been presented to suggest that sublegal-sized males can and do participate in mating (Cleaver 1949; Butler 1960; Smith and Jamieson 1991; Hankin et al. 1997). Like other brachyuran crabs, mating involves the union of a hard-shelled male and a recently molted female with a premolt size smaller than her partner (Butler 1960; Hartnoll 1969). Due to extremely high exploitation rates, concern has been raised that a substantial percentage of females (especially large females) may go unmated because of a reduced abundance of large males (Smith and Jamieson 1991). However, Hankin et al. (1997) and Oh and Hankin (2004), using the presence of sperm plugs, an unequivocal indicator of female mating success, found that virtually all large females that had molted in northern California had been mated.

While sperm plug presence provides undeniable evidence of recent mating activity of females, mating activity of males has been indirectly inferred through examination of so-called "mating marks". While studying the mating behavior of Dungeness crabs, Butler (1960) observed conspicuous marks on the chelipeds of male crabs collected from the wild. He described the marks as if they "might be made from a grinding wheel," and inferred that the marks were caused by the close male-female contact during the premating embrace. Describing these "mating marks" in greater detail, Butler found that the marks "varied from a few sharp scratches to wide deeply worn bands" (pg. 641). He presumed that the amount of wear was related to the amount of time an individual crab spent in the premating embrace, and that heavy mating marks were caused by multiple mating events and the associated multiple premating embraces.

The premating embrace between hard-shelled male and female crabs begins when a male crab detects and clasps a premolt female. The premating embrace may last up to 7-10 days and involves holding the female sternum-to-sternum under the larger male (Butler 1960; Snow and Neilsen 1966). While in the premating embrace under laboratory conditions, the female may be repositioned by the male and even appear to attempt escape (Snow and Neilsen 1966; pers. observation). The male continues to hold the female in his clasp during the relatively short (approximately 1 hour) molting process. Presumably, since females are only capable of copulation just after molting, the premating embrace is a means to ensure male paternity following female molting.

Copulation, accomplished while the male and female are sternum-to-sternum, occurs immediately following the female's molt. Once molting and mating are accomplished, the male may continue to hold the female in a postmating embrace (Snow and Neilsen, 1966; Hankin et al. 1997) until the female's exoskeleton has hardened considerably. Presumably, this behavior enhances the probability of female survival after molting because soft-shelled crabs are highly vulnerable to cannibalism or predation.

While other researchers had observed polygyny in laboratory-held male Dungeness crabs (e.g. Cleaver 1949), the variable amount of abrasion in observed mating marks was only circumstantial evidence that polygamy exists in nature. This novel and convenient method of assessing an individual crab's mating activity was expanded as Butler (1960) developed a procedure to infer population-wide mating activity. This procedure involved collecting a large sample of male crabs and determining the percentage of crabs that had mating marks.

Butler (1960), and later Smith and Jamieson (1991), found that mating marks were present on a higher percentage of sublegal-sized males than on legal-sized males. Smith and Jamieson (1991) interpreted this finding as demonstrating decreased mating activity among legal-sized males. From this interpretation, they asserted that fishery removal of large males could decrease the opportunity for large females to find a mate since large, legal-sized males are relatively rare in a fished population. Because of a reduced abundance of large males, and the apparently low rate of mating activity amongst them, they speculated that the total number of fertilized eggs could decline as a consequence of fishery removal of males.

Male Dungeness crabs found in premating embraces are almost always larger than their female partner (Butler 1960; Snow and Neilsen 1966; Hankin et al. 1997). Hankin et al. (1997) collected and measured male and female crabs in premating embraces off Trinidad, California, and found that female premolt size ranged from 80.9-159.9 mm whereas male size ranged from 119.5-200.9 mm. The size ratio between a male and female in a premating embrace may affect the formation and/or severity of mating marks since the male, to begin a premating embrace, may need to position the sometimes unwilling female with his claws (Snow and Neilsen 1966).

An important component to mating mark formation and/or severity may be the time spent in the premating embrace. Cleaver (1949) described the premating embrace of Dungeness crabs as lasting several days, and the premating embrace of one premating pair of crabs observed by Snow and Nielsen (1966) lasted seven days. The maximum number of days to molting for female crabs that were collected in premating embraces in northern California and placed in seawater tanks by Hankin et al. (1997) was nine days; the mean number of days to molt was 1.66 and 3.64 days in 1992 and 1993, respectively. The Dungeness crab mating season in northern California extends from mid-February to mid-May (Hankin et al. 1997), and it is believed that male crabs are polygynous (Cleaver 1949; Butler 1960; Hankin et al. 1997).

Subsequent to Butler's introduction of mating marks, they have been used by other researchers to estimate molting probabilities (Poole 1967), evaluate molting history or status (Poole 1967; Warner 1987; Juanes and Hartwick 1990), infer size-specific mating activity (Poole 1967; Smith and Jamieson 1991), and infer molt instar-specific sexual maturity (Jamieson 1996). These uses of mating marks have all relied on an implicit assumption that mating marks are a reliable and unbiased indicator of mating activity. This thesis will attempt to determine if mating marks can be used to confidently estimate mating activity in male Dungeness crabs.

This thesis will also examine how mating marks have been used by other researchers to infer mating and/or molting activity and will present results from experiments designed to discover the process of mating mark formation. The experiments involved laboratory matings of male and female crabs to determine the factors that influence formation of mating marks. Hypothetical factors include relative male and female sizes, mating frequency, competition, and substrate type. In addition to laboratory mating experiments, mating mark frequency in the northern California Dungeness crab population was monitored throughout a mating season, allowing for a more detailed description of mating mark characteristics and frequency.

Future research using mating marks could benefit from a formal objective evaluation of this biological indicator. If population wide inferences are to be made from the frequency of mating marks in a population of Dungeness crab, it is crucial that an individual male with mating marks present can accurately be assumed to have successfully mated. Conversely, the lack of mating marks on an individual crab should indicate that it has not mated. To evaluate whether or not mating marks can be confidently used as an indicator of mating success, three conditions should be met: (1) every premating embrace, involving the smallest to largest male capable of mating, will produce mating marks; (2) every premating embrace results in successful copulation and insemination; and (3) the timing and location of field samples are appropriate for inference of population level mating activity. Data from the current research will be incorporated with previous findings from other researchers to determine if mating marks can be applied to assess mating activity of male Dungeness crabs and if previous research has met the necessary conditions to provide an accurate estimate of male mating activity.

MATERIALS AND METHODS

At-Sea Observations of Mating Mark Characteristics and Frequency

Examination of captured male crabs for mating marks

For purposes of evaluating the characteristics and frequency of natural mating marks, observations and photographs were taken from crabs captured during the 2003 spring mating season, and throughout the 2004 mating season. Crabs to be examined for mating marks were captured aboard a chartered commercial vessel using a combination of conventional (open escape port) and modified (closed escape port) crab traps near Trinidad Head, California (41° 00' N, 124° 10' W). From 10 April to 2 May 2003, a sample of 268 legal (159.0-188.6 mm) and 867 sublegal (150.0-158.0 mm) male crabs were examined for mating marks to calculate the mating mark frequency in the middle of a mating season in northern California. During April 2003, sublegal and legal male crabs were tagged as part of an associated research project on mortality and movement (Hankin, unpublished data). During the tagging operation, legal and sublegal male crabs were examined for mating marks. Observations were also conducted before, during, and after the spring 2004 mating season, from 25 January 2004 to 19 July 2004, to examine how mating mark frequency may change during the spring mating season.

A total of 1,776 legal (159.0-198.2 mm) and 1,691 sublegal (122.5-158.8mm) male crabs were examined for mating marks during the 2004 mating season (Table 1). Crabs were examined on 17 separate days from 25 January 2004 to 19 July 2004. Each

Table 1. Sample sizes for 7 mating mark examinations made during the 2003 mating season and 14 mating mark examinations during the 2004 mating season. Sublegal refers to male crabs <159.0 mm CW, legal refers to crabs ≥159.0 mm CW.

Date	Number of crabs examined	
	Sublegal	Legal
10 April 2003	70	10
17 April 2003	209	9
19 April 2003	244	45
27 April 2003	174	50
30 April 2003	6	67
01 May 2003	111	25
02 May 2003	53	62
25 January 2004	172	170
10 February 2004	80	118
28 February 2004	52	169
09 March 2004	83	173
24 March 2004	60	168
05 April 2004	51	46
07 April 2004	188	36
13 April 2004	197	76
16 April 2004	119	440
17 April 2004	214	96
23 April 2004	28	104
27 April 2004	187	47
30 April 2004	222	104
13 July 2004	38	29
Totals	2558	2044

day was considered a single observation except for four mating mark examinations in July 2004 (8 July, 12 July, 15 July, and 19 July) which were combined into one single observation due to the small catches during this time period. Therefore, data from the four at-sea examinations in July were treated as if they had been collected as a single 13 July observation.

Upon examination of the inner surface of each crab's claws, mating mark presence was recorded as "no", "slight", or "yes" (Figures 1-3). "No" crabs had no noticeable marks on either claw. "Slight" crabs had insignificant scratches or abrasion. "Yes" crabs had noticeable scratches or abrasion on one or both claws. "Slight" marks were defined as scratches or abrasion that measured <5 mm on one or both claws. "Yes" marks were obvious and prominent; "slight" marks were subtle.

Examination of recovered tagged male crabs for mating marks

Between 5 and 30 April 2004, 2,012 male crabs that were examined for mating marks were tagged and released in an area with an active commercial fishery near Trinidad Head, California. Crabs were tagged with either one tag (n=1,035) or two tags (n=977) along the posterior suture line. The released crabs included 946 legal and 1,046 sublegal crabs. After these crabs were released, recoveries from the commercial and sport crab fisheries allowed for an additional observation of mating marks. Examination of recovered tagged crabs allowed assessment of changes in an individual crab's mating marks between the times of release and recovery. Two hundred and seventy-one male crabs that were examined, tagged, and released during April 2004 were later recovered from the fishery and examined.

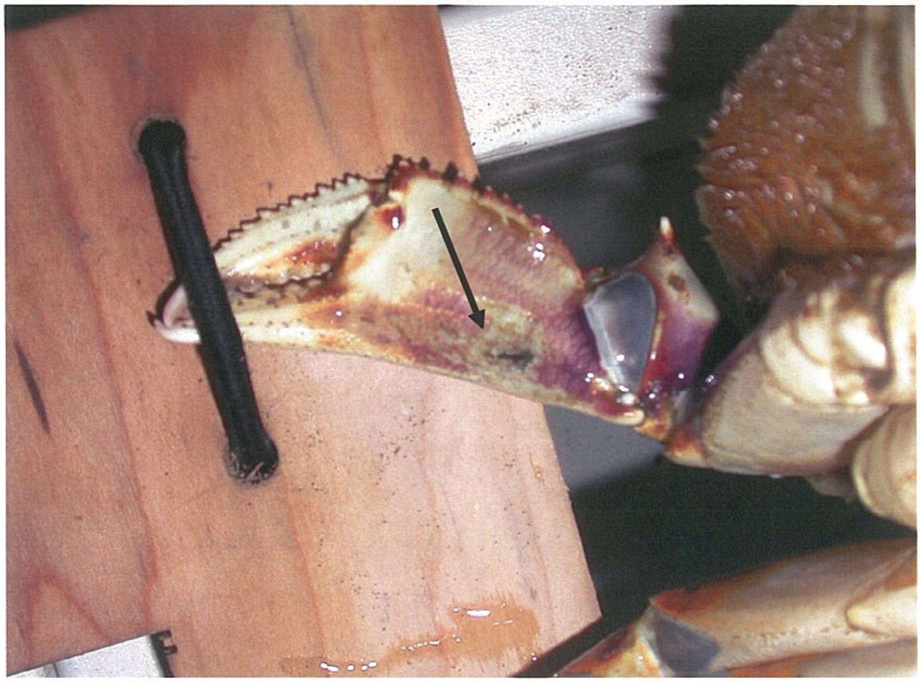

Figure 3. Claws with “yes” marks had obvious wear including scratches and/or abrasion on the inner surface.

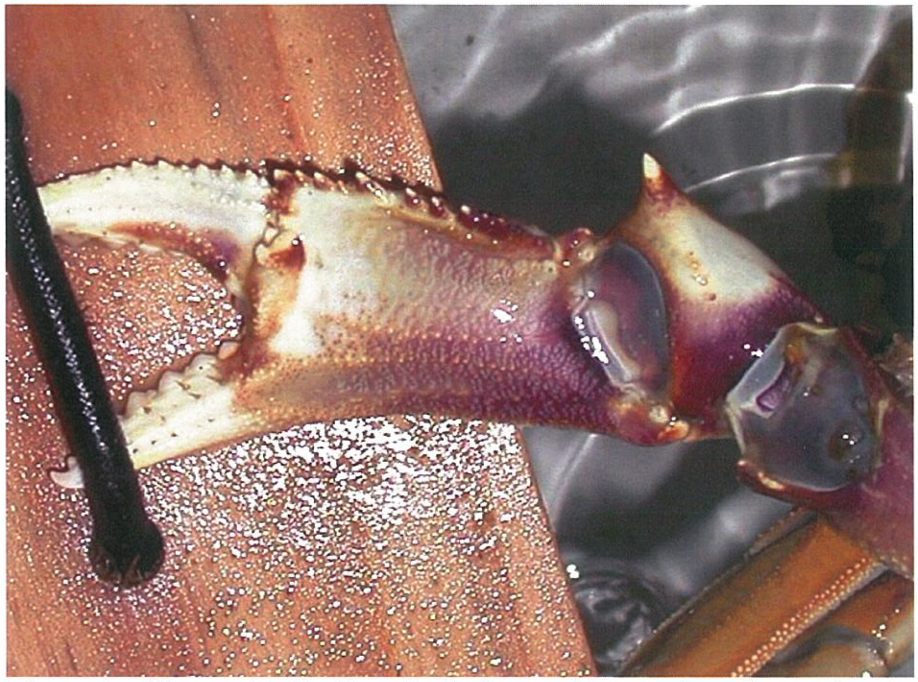

Figure 1. Claws with “no” marks had no evidence of marks or abrasions.

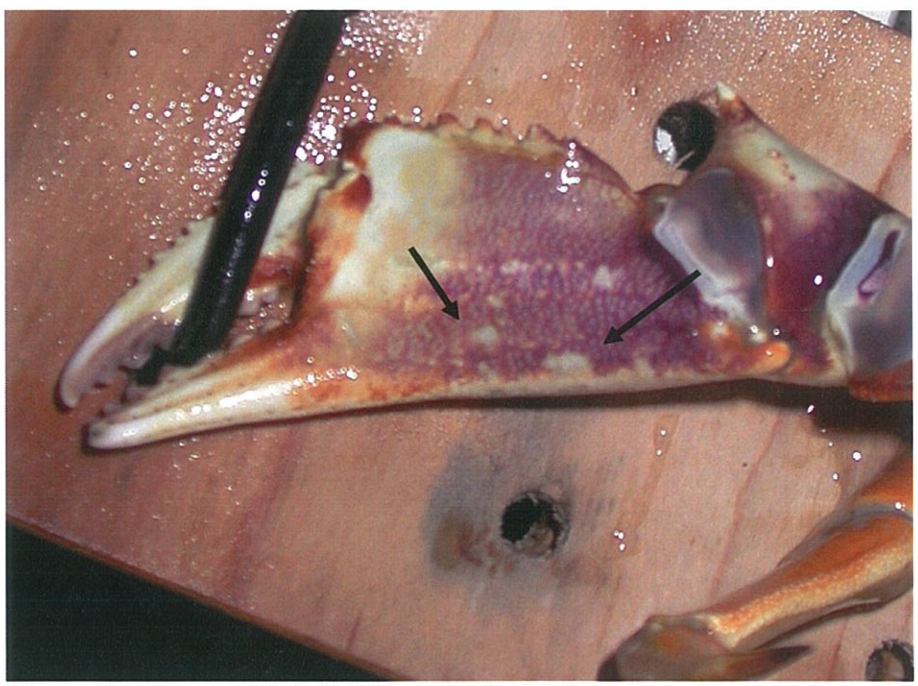

Figure 2. Claws with “slight” marks had subtle scratches or minor abrasion.

Days at large from the time of initial examination to a final examination ranged from 1 to 104 days, and averaged 28.5 days.

Classification of mating mark characteristics

A classification scheme to describe the range of observed mating mark characteristics was created from tagged crabs recovered during spring 2004. A randomly chosen sample of 200 crabs with "slight" or "yes" marks were closely examined to create a classification scheme describing mating mark characteristics. To accomplish this goal, mating marks on each claw of every individual male crab were drawn on a template. These drawings were compared and grouped into seven categories. The seven categories were created based on mating mark location on the claw, and/or the severity of the marks. Analysis of these data included calculating the mean and ranges of CW within each group and the relative occurrence of each category within the total sample. Marks were variable, but the seven types are distinguishable using their location on the inner surface of the claw as the primary criterion. A guide to the seven basic Types of mating marks (Figure 4) was used to compare observed mating marks, and each claw was assigned a mating mark Type. When crabs were found to have more than one type of mating mark (e.g. Type 1 on the left and Type 4 on the right) the crab was scored as having both Types present. Figures 6-7 are examples of three common mating mark types.

Laboratory Mating Mark Formation Tests

Experiments to verify the formation of mating marks from controlled matings of male and female Dungeness crabs were completed during the spring mating seasons of

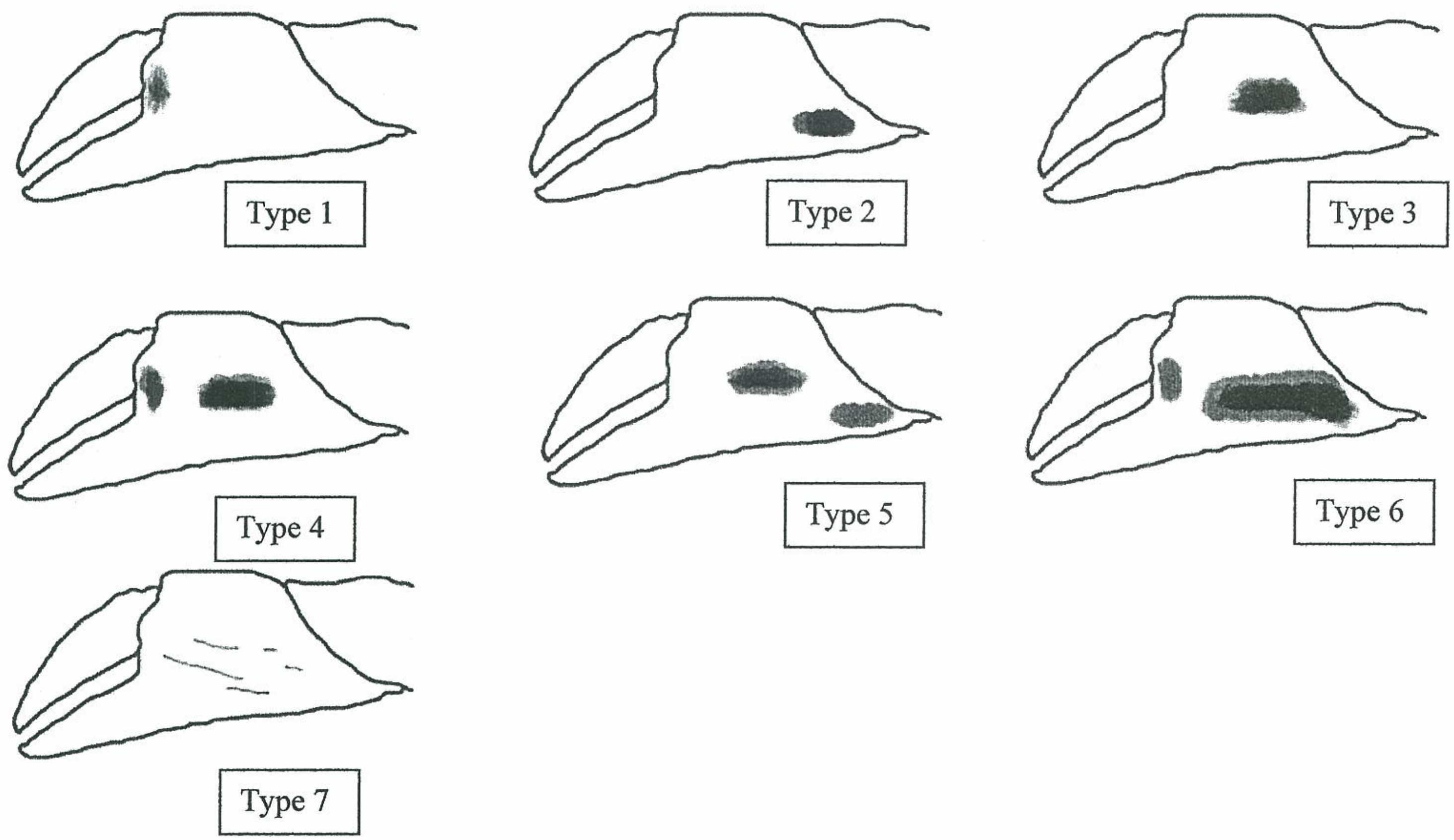

Figure 4. Guide to the seven mating mark types used to categorize mating marks observed on male Dungeness crabs collected in northern California during the 2004 spring mating season.

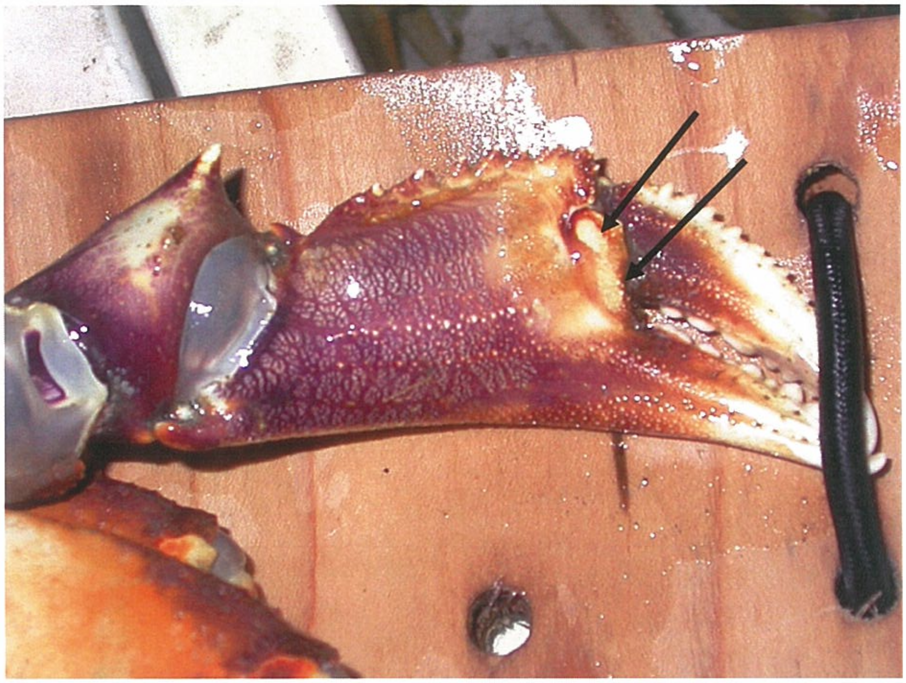

Figure 5. Type 1 mating marks are characterized by a slight abrasion on the raised protuberance located in the forward portion of the claw at the base of the pincer.

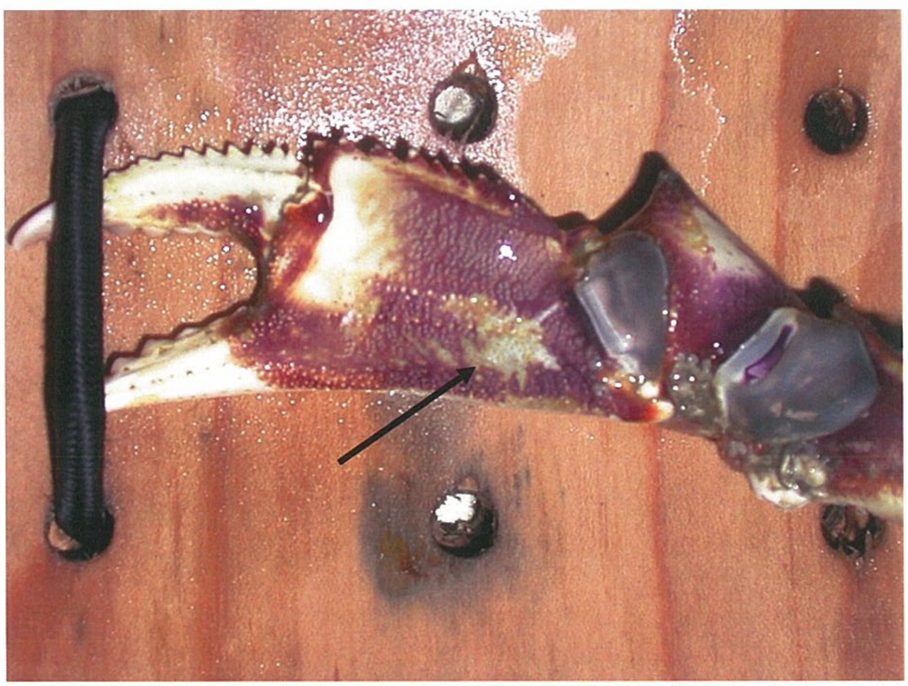

Figure 6. Type 2 mating marks are scratches located at the base of the claw.

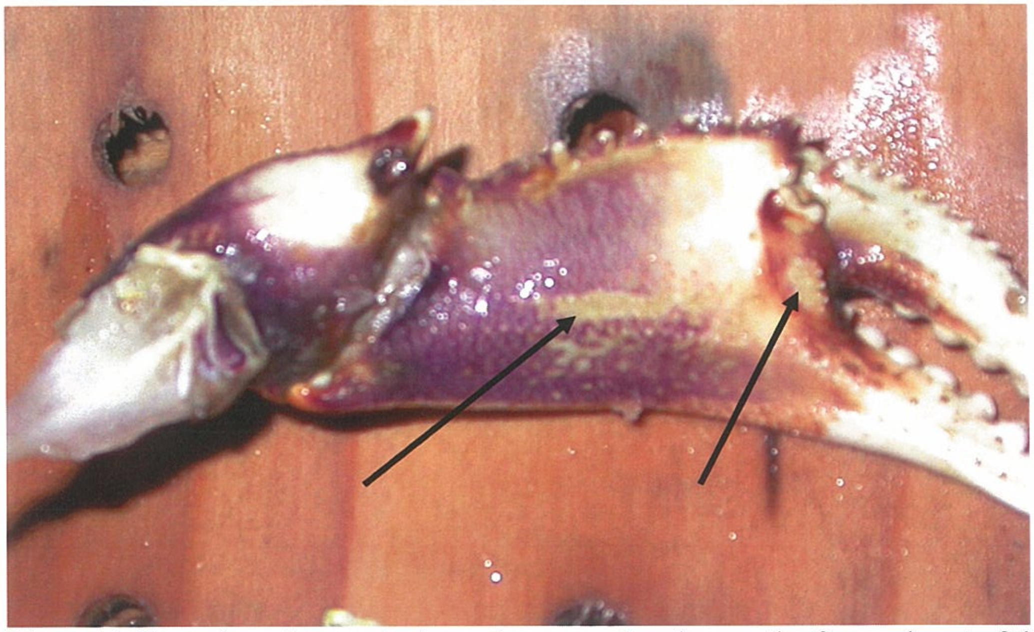

Figure 7. Type 4 mating marks have the same abrasion on the forward part of the claw as Type 1, but have additional abrasions on the middle portion of the claw.

2002, 2003, and 2004. The intention of these experiments was to examine the possible effects of male and female size, multiple male matings, male competition, and a dynamic environment on the formation of mating marks on the claws of male Dungeness crabs.

For all laboratory mating experiments, male and female crabs were collected before the mating season aboard a chartered commercial vessel using a combination of conventional (open escape port) and modified (closed escape port) crab traps near Trinidad, California. Male crabs were collected in November and held until the following mating season, or were collected just before the mating season. Only males that had no obvious abrasions or marks on the inner surfaces of their claws were used in the experiments, and care was taken to only use male crabs that were in good condition (i.e. few or no missing limbs, active, few or no exoskeletal infections, etc.). Female crabs selected for possible use in the mating experiments were assumed to have not molted during the current molting season, based upon shell condition (Hankin et al. 1989).

Male and female crabs were brought to the Humboldt State University Telonicher Marine Laboratory, Trinidad, California, and held in tanks of recirculating chilled (10° C) seawater (separated by sex and size). All crabs were fed to satiation with squid once a week, and tanks were checked daily to remove waste and dead individuals.

Sizes of male and female crabs used in laboratory mating tests ranged from 140 – 185 mm CW and from 100 – 145 mm CW, respectively. Larger female crabs were not used because annual molting probabilities for female crabs larger than 145 mm CW rapidly decrease; Mohr and Hankin (1989) estimated that the annual molting probability of a female crab with a CW of 152.5 mm to be 0.15. It was therefore not possible to collect sufficient numbers of these large females so that some would molt in the lab.

Each of the four different mating scenarios described below followed a similar protocol to select the single female used in an individual experiment. For all experiments, females that were close to molting, and therefore soon capable of copulation, were needed. Identification of such "premolt" females relied on the placement of one to three "seeker" male crabs into the tanks containing female crabs. Seeker male crabs were able to detect premolt females approximately one week before the females molted, presumably by detecting the release of a female pheromone. Premolt females located by the seeker male crabs were grasped in premating embraces. During the mating season, the all-female tanks were checked daily for premolt females in premating embraces with seeker male crabs. Once a premolt female was found, she was then placed into an experimental tank, depending on her size and the specific mating scenario being tested at the time.

Once a premolt female was selected, the male crab(s) chosen for each mating trial were measured and photographed. The inner surface of each claw of every male crab used in the laboratory mating experiments was digitally photographed before and after each mating trial. Male and female crabs were then paired in experimental cells and were monitored daily. Notes were taken of premating and mating behavior and video recordings were occasionally made to aid in monitoring. In addition, the length of time (in days) for all premating embraces was recorded, and female post-molt size was measured following mating and molting.

Test 1: size-ratio effect

This series of experiments was designed to test the hypothesis that mating mark formation and severity is a function of the male to female size ratio. The manipulation of females by males into the premating embrace involves many forceful movements (personal observation) that could cause the observed abrasions called mating marks. If the female is much smaller than her male partner, she might be easier to manipulate, resulting in little abrasion. If the female were only slightly smaller than the male, more forceful manipulation could be required, resulting in more extensive abrasions.

Male crabs used in the 2002 mating mark trials had no noticeable marks and were collected in November 2001 and March 2002. Male crabs were assigned to one of three size groups: small (142 – 149 mm CW), medium (150 – 159 mm CW), and large (160 – 185 mm CW). The large male size group contained only legal crabs, whereas the two smaller size groups contain only sublegal males. Female crabs were collected in March 2002. Females were likewise assigned to one of three size groups: small (100 – 119 mm CW), medium (120 – 129 mm CW), and large (130 – 145 mm CW). These size groups for both sexes were chosen to cover the range of sizes most often found in premating embraces in northern California (Hankin et al. 1997).

To test if mating mark formation or severity was related to the male:female size ratio, male crabs from each of the three size groups were each mated with female crabs from each of the three female size groups. With nine possible pairing combinations, the experiment was designed to be a complete block design with two factors, and each pairing combination was to be replicated as many times as possible. The two factors, male size and female size, were used to determine whether or not mating mark formation depended on the size of the male in the premating embrace, the size of the premolt female, or an interaction between the two factors. A significant interaction effect would be evident if the male:female size ratio influenced the formation of mating marks.

Thirty-four individual matings were accomplished under Test 1. Each mating trial was conducted in an experimental cell measuring 54 cm by 54 cm, created within a larger tank measuring 108 cm by 108 cm and divided into 4 cells with black plastic dividers. Tanks were supplied with flowing chilled (10° C) seawater and were aerated. All experimental cells used in this scenario had no natural substrate over the white plastic bottom of the tank, but were protected from light by a black plastic cover.

Test 2: multiple-mating effect

Male Dungeness crabs are believed to be polygynous (Butler 1960; Cleaver 1949; Hankin et al. 1997), and since a single premating embrace may be insufficient to induce formation of mating marks, male crabs were allowed to mate with multiple partners. Male and female crabs for this experiment were collected at the same time as those in Test 1. Many of the males used in Test 1 were presented with additional females in successive mating trials as part of Test 2. Tank conditions were identical to the conditions of Test 1.

Test males were observed daily during the premating and mating period with each female in order to record the total number of days spent in the premating embrace. Total days in the premating embrace began when a test male first formed a premating embrace with a female, and ended the day the female was first observed to have molted. Although female molting was occasionally observed, typically females molted (and subsequently mated) in between the daily observations. Following the female molt, females were measured and removed, and the males' claws were once again photographed. When another premolt female became available, she was placed in the appropriate test cell. The approximate cumulative total time spent in the premating embrace (in days) for each male, over the multiple matings, was recorded. Total cumulative time spent in a premating embrace by an individual male ranged from 2 to 15 days.

Eight male crabs mated with two females, nine mated with three females, and one mated with four females. Although male and female size was not of primary interest for these tests, males and females were organized into the size groups (three for each sex) previously described. The test was designed to allow for all nine pairing combinations, and mate each male with at least 3 females. However, due to an unexpectedly low molting probability of the laboratory-held females that reduced the total number of available premolt females, not every size combination and/or number of matings was able to be conducted.

Test 3: competitive behavior effect

Butler (1960) observed that two males will fight for a premolt female, with the larger of the two always the victor. A single female in a tank of multiple males will result in extremely competitive behavior among the males (personal observation). Such inter-male competition for a premating female could cause abrasion on the inner surfaces of the male's claws that fit the description of mating marks. Test 3 was designed to test the hypothesis that behavioral interactions among males competing for premating females causes formation of mating marks.

Male crabs were collected for this test in November 2002, and January and March 2003. Female crabs were collected in January 2003, and the experiments were conducted from March to May 2003. Only male crabs that had no existing claw abrasions were used in this test. Three males were placed in an experimental cell, followed by the addition of a single premolt female. Female size was not considered a factor since competition between males was of primary interest, and premolt females regardless of size were placed in the next available experimental cell.

Male size selection for each of the trials was guided by the intention that half of the competitive matings would be among three males of roughly equal size, and an equal number of the competitive matings would be among three males from each of the three size groups. To accomplish this goal, "like-sized male" competitive trials were conducted concurrently with "unlike-sized male" competitive trials. Like-size males were defined as individuals within the same size group or having carapace widths within 10 mm of another.

Once a premolt female and three males were selected, they were added to the experimental cell and monitored for at least 10 minutes. Video recordings were made to assist with observations and daily detailed notes of male and female behavior were taken during each mating trial. Daily observation times for each mating trial depended on the behavior observed and ranged from as short as one minute to several hours. If there was no change from the previous day's observation (i.e. the same male had the female in a premating embrace), then the daily observation was brief. If, however, active competition among the males was observed, the trial was observed for a longer period, often accompanied by photo and/or video recordings. The individual crab that successfully grasped the female in a premating embrace was recorded for each trial, along with any subsequent changes resulting from successful competition from a challenging male. Eighteen mating trials were conducted under this competitive scenario. Following each mating trial (once the female had molted), all male crabs were examined for any claw abrasion and photographed.

Test 4: natural environment effect

Carefully controlled laboratory conditions may lack key natural conditions which may be related to mating mark formation. For example, burial movements or other movements of a premating pair might increase the degree of abrasion on the inner surfaces of the male's claw. Test 4 examined the possible effects that more natural laboratory conditions could have on the formation and/or severity of mating marks.

To simulate natural conditions, mating trials in 2004 were conducted in two different situations. In one situation, in the same experimental cells used in the previous experiments, a substrate of ordinary beach sand collected near Trinidad was added. Sand was of sufficient depth (15 cm) to allow for the complete burial of individual male and female crabs. In the other situation, aquarium gravel (larger than beach sand) was added as substrate to a 100 cm circular tank. In this tank, the aquarium gravel was 25 cm deep and the water inlet pipes were fully opened to create a circular current within the tank.

Under both conditions, the protocols for introducing male and female crabs to the experimental cells were consistent with the previous experiments.

In addition to the two different environmental situations, the natural substrate mating trials were conducted either non-competitively with a single male, or competitively with two males. The non-competitive matings followed protocols used in Test 1. The competitive matings, however, used protocols different from those used in Test 3. For the competitive matings of Test 4, after a single male and a premolt female formed a premating embrace, a second male was introduced. The introduced male was either larger, smaller, or roughly the same size as the original male. The resulting mate competition between the males, if any, was noted and sometimes video recorded. Eighteen competitive matings were conducted along with 19 non-competitive matings. An additional observation, embrace type, was recorded for all paired crabs in Test 4. The three types of embraces were conventional (sternum-to-sternum, anterior-to-anterior, with the male above the female; Butler 1960; Snow and Neilsen 1966), inverted, and guarding (Figures 8-10). The inverted premating embrace involves the female being held right-side up, in the same orientation as the male. A guarding embrace involves both the male and female being right side up, with the male forming a protective "cage" around the female.

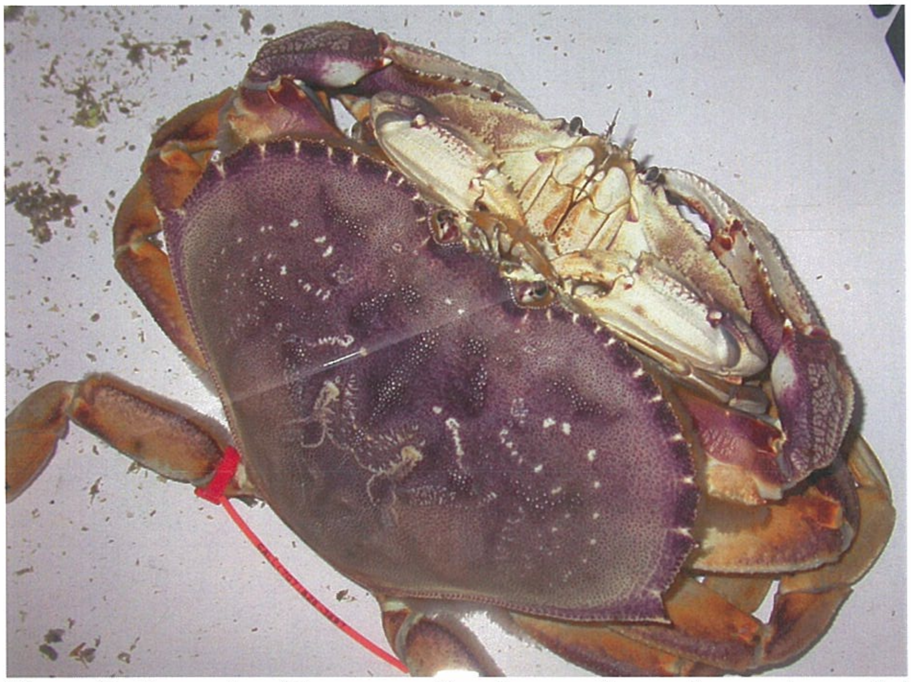

Figure 8. Example of the conventional premating embrace observed during the Test 4 experiments (in this photograph, the male is the larger of the two crabs).

Figure 9. Example of the inverted premating embrace observed during the Test 4 experiments (in this photograph, the male is the larger of the two crabs).

Figure 10. Example of the guarding embrace observed during the Test 4 experiments (in this photograph, the male is the larger of the two crabs).

RESULTS

At-Sea Observations of Mating Mark Characteristics and Frequency

Examination of captured male crabs for mating marks

During the 2003 mating season, 1,135 male Dungeness crabs were examined for mating marks. Crabs were captured on seven occasions during a brief period (22 days) between 10 April and 2 May, and were therefore analyzed as if all crabs were captured on a single day in the middle of the mating season. By doing this, the data provide a "snapshot" of mating mark prevalence on sublegal and legal male crabs in northern California during the spring mating season. Sublegal and legal crabs with "slight" marks occurred at roughly equal frequencies (Table 2). The frequency of crabs with marks (computed as the sum of those with "slight" and "yes" marks) was 50.9% for sublegal crabs and 38.8% for legal crabs.

Between 25 January and 19 July, 2004, 3,467 male crabs were examined for mating marks. Of these, 1,691 were sublegal (<159 mm), and 1,776 were legal (≥159 mm). Mating mark frequency, calculated as the percentage of crabs with "yes" mating marks, generally increased as the season progressed, reaching a peak on the final day of observation (Figure 11). This trend is also apparent among the "slight" group (Figure 12). In addition, if the "slight" group is combined with the "yes" group (Figure 13), mating mark frequency during the observation period generally increases over the mating season.

Table 2. Results of an examination of 1,135 male Dungeness crabs for mating marks between 10 April and 02 May 2003. Sublegal refers to male crabs <159.0 mm CW, legal refers to crabs ≥159.0 mm CW.

Mating mark classification	Sublegal		Legal	
	Number	Percent of sample	Number	Percent of sample
No	426	49.1	164	61.2
Slight	163	18.8	49	18.3
Yes	278	32.1	55	20.5
Total sample	867		268	

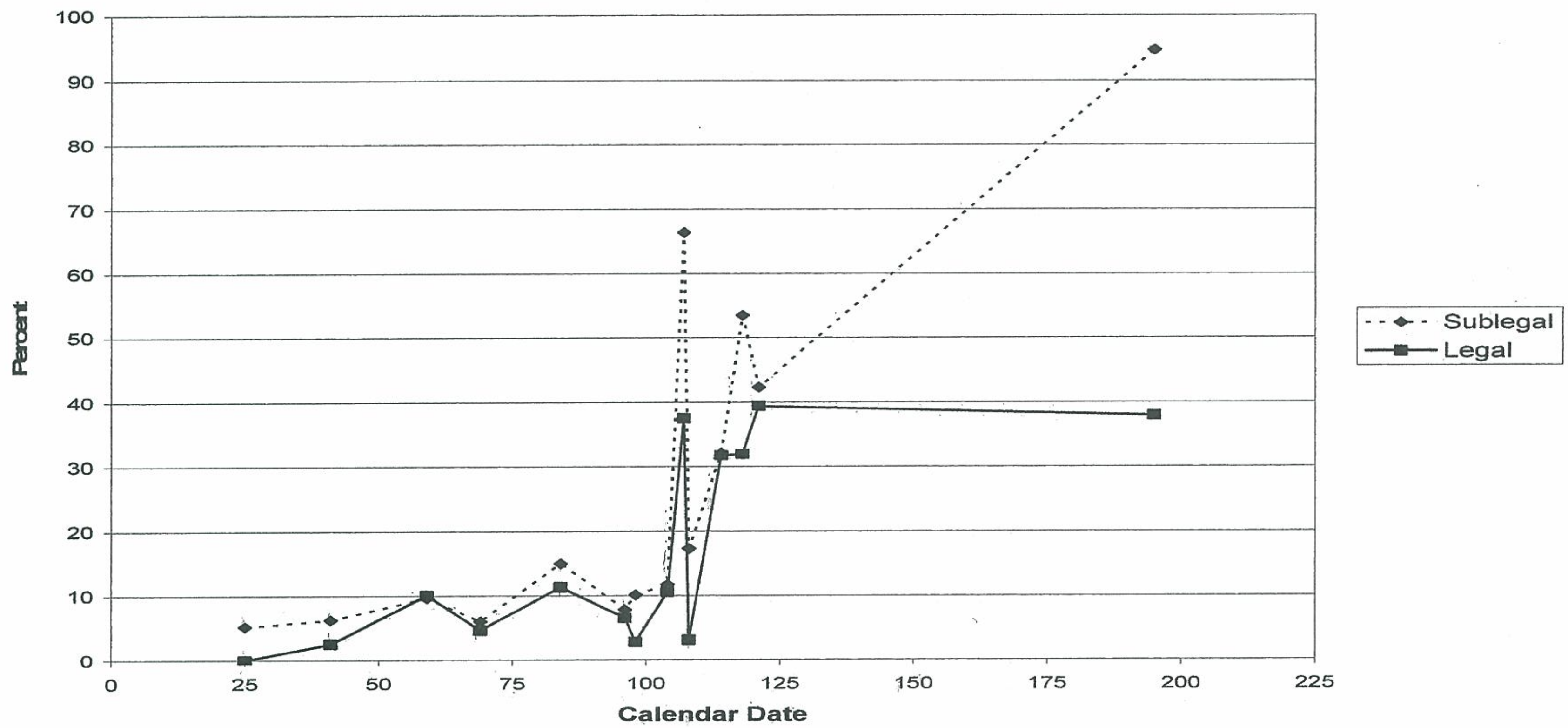

Figure 11. Percentage of crabs exhibiting "yes" marks for 14 collections of male Dungeness crabs captured in 2004 near Trinidad Head, California (1 January = Calendar Date 1).

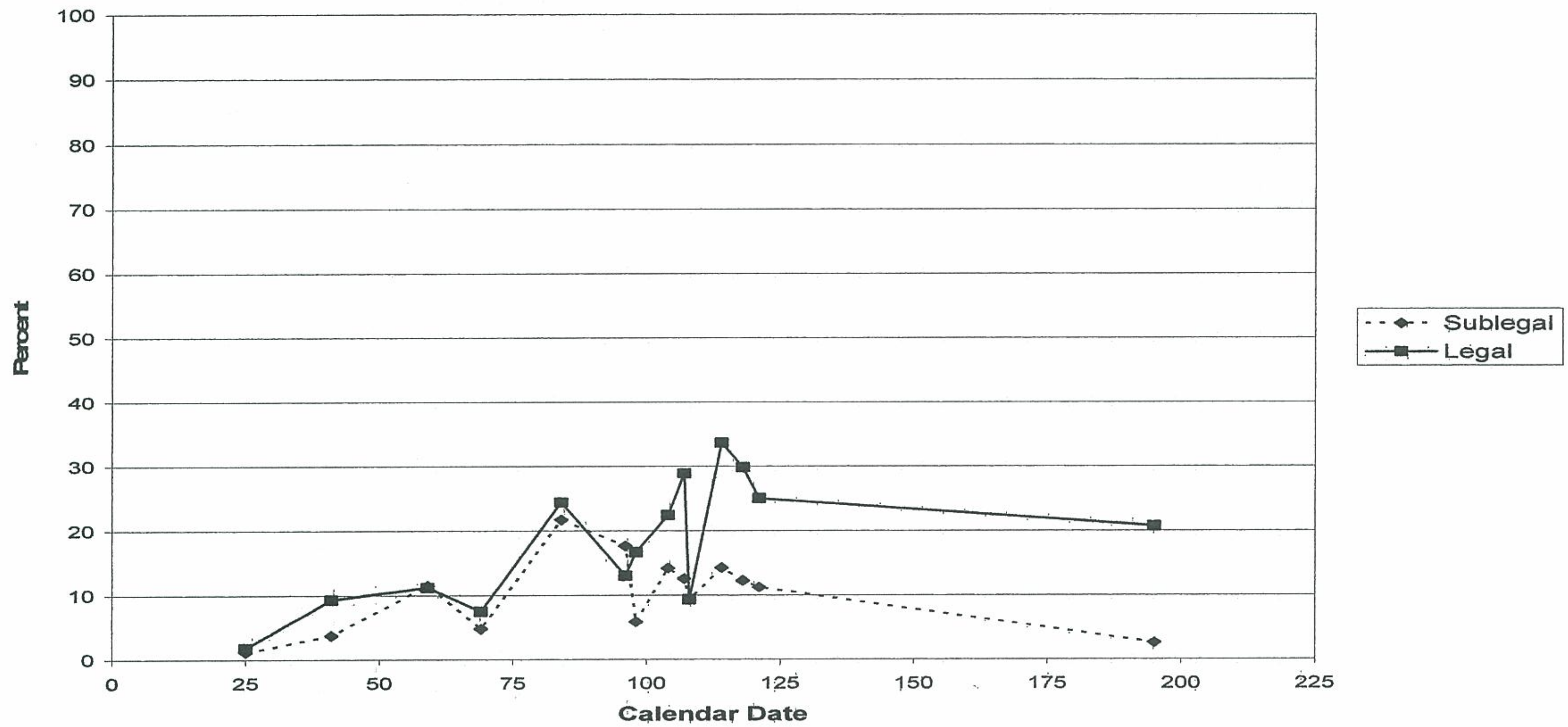

Figure 12. Percentage of crabs exhibiting "slight" marks for 14 collections of male Dungeness crabs captured in 2004 near Trinidad Head, California (1 January = Calendar Date 1).

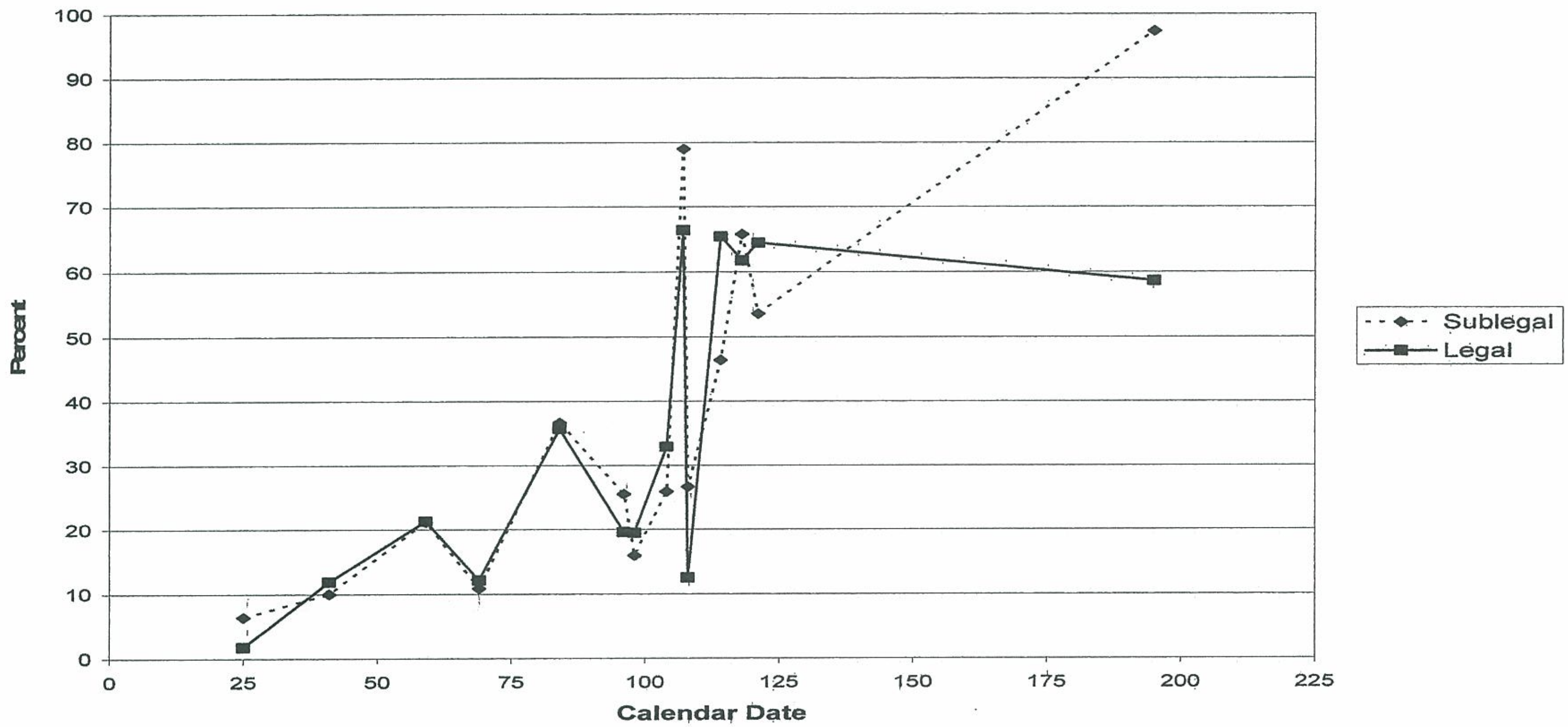

Figure 13. Percentage of crabs exhibiting "yes" and "slight" marks for 14 collections of male Dungeness crabs captured in 2004 near Trinidad Head, California (1 January = Calendar Date 1).

For all days of observation, the frequency of "yes" marks was always higher among smaller, sublegal crabs. The occurrence of "slight" marks was higher among legal crabs on all days except 5 April. "Slight" marks exceeded 20% among legal crabs on seven different observations, particularly during the April observations. Among sublegal crabs collected during the April observations, "slight" marks were found on only 10-15% of the crabs, and "slight" marks declined in frequency to <3% among the sublegal crabs collected in July. When the "yes" and "slight" marks were combined, there was little discrepancy between sublegal and legal crabs. Half of the observation datess (n=7) found a higher combined frequency of "yes" and "slight" marks on sublegal crabs, while the other half of the observations (n=7) found that legal crabs had higher combined frequency "yes" and "slight" marks. Except for the very last observation on 13 July, when the combined frequency of "yes" and "slight" marks approached 100% on sublegal crabs, the percentages of crabs with marks ("yes" and "slight" combined) were nearly identical between legal and sublegal crabs for most of the observations. During the peak of the mating season, in April, combined "yes" and "slight" mating mark frequencies were as high as 79% and 66% for sublegal and legal crabs, respectively. The combined frequency of "yes" and "slight" mating marks on legal crabs by the final observation in July, approximately 2 months after the end of the mating season, was 59% while for sublegal crabs the frequency had increased to 97%.

Old-shell crabs were encountered on all but three of the observations (24 March, 16 April, and 23 April). Old-shell crabs were identified by the presence of barnacles and/or a generally worn exoskeleton that would indicate that the shell had not been shed during the previous fall male molting season. None of the crabs captured during the at-sea observations were soft-shelled, or newly molted, crabs. Old-shell frequencies were highest among sublegal crabs. Prior to the mating season, old-shell frequency was as high as 7.5% (25 January 2004) among sublegal crabs. On the 13 July observation, old-shell crabs accounted for 21% of the 38 sublegal crabs, but only 3.4% of the 29 legal crabs captured. Of 3,467 male crabs collected between 25 January and 13 July 2004, 59 crabs were classified as old-shell. Of these, 54 were observed with "yes" marks and 2 were observed with "slight" marks for a combined mating mark presence of 95% on old-shell crabs.

Shell classification data from the associated three-year tagging project revealed a much higher percentage of sublegal-sized old-shell crabs compared to legal-sized crabs (Table 3). A total of 10,736 crabs were examined on five occasions between November 2001 and April 2004. Collections in the fall had a much higher percentage of old-shell crabs, reaching as high as 43% among sublegal crabs in November 2003.

Examination of recovered tagged male crabs for mating marks

From the 2,012 male crabs that were tagged and released after an initial mating mark examination in April 2004, a total of 271 were recovered and reexamined for mating marks. Originally, when these 271 crabs were released, 112 were observed with "no" marks, 101 with "yes", and 58 with "slight" marks (Tables 4-6). Crabs recovered ranged from 151.8 to 192.4 mm CW; 61were sublegal crabs and 210 were legal crabs.

Upon reexamination after recovery, only 45% of the crabs originally observed with "no" marks were later observed still with "no" marks. Of the crabs with "no" marks

Table 3. Sample sizes and percent of male crabs examined classified as old-shell. Old-shell refers to crabs that have not molted in the most recent male molting season (intermolt period > 1 year), determined by shell condition. Sublegal refers to male crabs <159.0 mm CW, legal refers to crabs ≥159.0 mm CW.

Dates	Sublegal		Legal	
	Total crabs examined	Percent of sample old-shell	Total crabs examined	Percent of sample old-shell
4 Nov-8 Dec 2001	521	11.9	792	1.1
26 Feb-21 Apr 2002	1198	4.0	148	6.8
4 Nov-24 Nov 2002	713	15.3	1309	0.2
5 Apr-2 May 2003	1513	1.3	451	0.4
5 Nov-23 Nov 2003	676	43.0	1397	0.4
16 Apr-30 Apr 2004	1069	2.3	949	0.1
Totals	5690		5046	

Table 4. Mating mark classification upon recovery of 112 male Dungeness with "no" marks at initial examination in April 2004. After initial examination, crabs were tagged and released into an active fishery.

Mating mark classification	Number recovered	Mean CW	Mean days at large	Minimum days at large	Maximum days at large
No	50	171.0	30	2	78
Slight	35	172.0	38	6	104
Yes	27	173.7	32	19	55

Table 5. Mating mark classification upon recovery of 58 male Dungeness with "slight" marks at initial examination in April 2004. After initial examination, crabs were tagged and released into an active fishery.

Mating mark classification	Number recovered	Mean CW	Mean days at large	Minimum days at large	Maximum days at large
No	7	177.0	24	1	51
Slight	19	176.6	17	3	43
Yes	32	176.5	28	3	75

Table 6. Mating mark classification upon recovery of 101 male Dungeness with "yes" marks at initial examination in April 2003. After initial examination, crabs were tagged and released into an active fishery.

Mating mark classification	Number recovered	Mean CW	Mean days at large	Minimum days at large	Maximum days at large
No	2	160.4	21	13	29
Slight	9	171.1	15	3	32
Yes	90	163.8	25	1	82

at release and also at recovery, the maximum number of days at large was 78. Thirty-one percent of the crabs with “no” marks were later observed with “slight” marks, and 24% were found with “yes” marks. The minimum time at large for crabs that had “no” marks which later were found to have “slight” marks was 6 days, and the minimum number of days at large for crabs that had “no” marks at release but “yes” marks upon recovery was 19. Of the crabs originally observed with “slight” marks, 55% were later observed with “yes” marks. Seven crabs with “slight” marks and two crabs with “yes” marks at the initial examination were later classified upon recovery as having “no” marks. Since the two observations were independent of each other, these crabs were erroneously classified either at release or at recovery.

Classification of mating mark characteristics

Types 1 and 2, followed by Type 4, (see Figure 4) were the most common mating mark types found in the random sample of 200 recovered tagged crabs (Table 7) with “yes” and “slight” marks in spring 2004. The data suggest mating mark type is somewhat dependent on CW, as the average size of crabs with Type 1 marks is much greater than the average size of crabs with Type 2 marks. In addition, observations and photographs support the existence of differences between the premating embrace of a larger male crab and that of a smaller male crab (Figures 14 and 15). Specifically, the claw of a large male crab will contact the back of the female’s carapace, while the claw of the small male crab will contact the female’s spines during the premating embrace. The contact location on the male claw of larger crabs is in the region of Type 1 marks, while the contact location for smaller crabs is in the region of Type 2 marks.

Table 7. The number and size of crabs for each of the seven different Types of mating marks. Number refers to the number of crabs from a sample of 200 that had at least one claw with the specified mating mark type. A guide to the seven different types is found in Figure 2.

Mating mark Type	Number	Mean CW	Min CW	Max CW
1	80	178.8	157.3	191.2
2	52	160.6	151.8	184.8
3	23	163.2	154.6	179.3
4	29	171.8	154.8	185.0
5	9	161.3	151.8	173.0
6	10	161.9	155.0	178.2
7	11	169.6	156.9	183.2

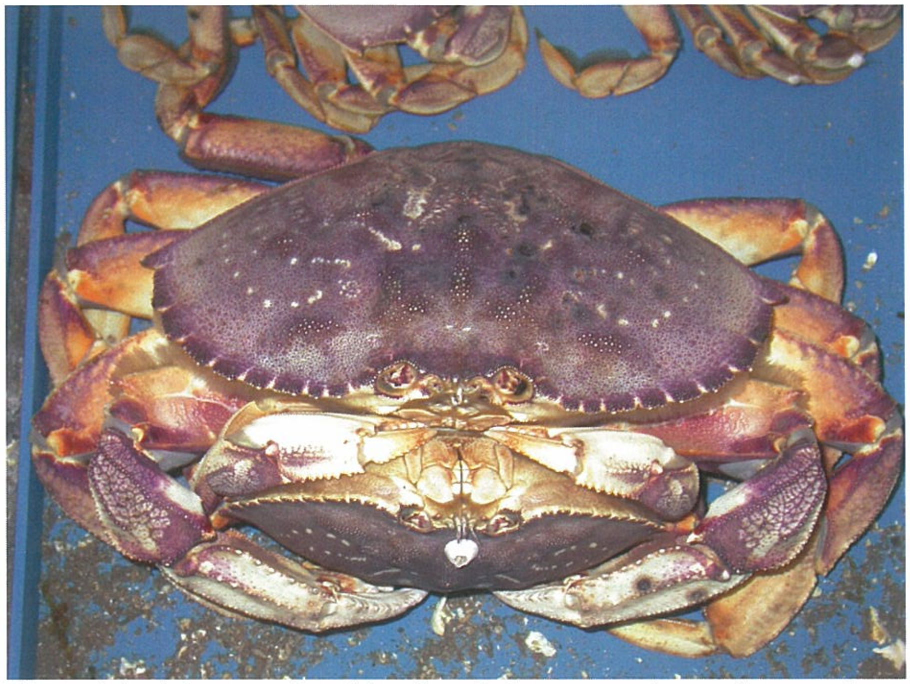

Figure 14. A large male with a female in a premating embrace, where the claws of the larger male are in contact with the carapace only, in the location of Type 1 marks (male is the upper partner).

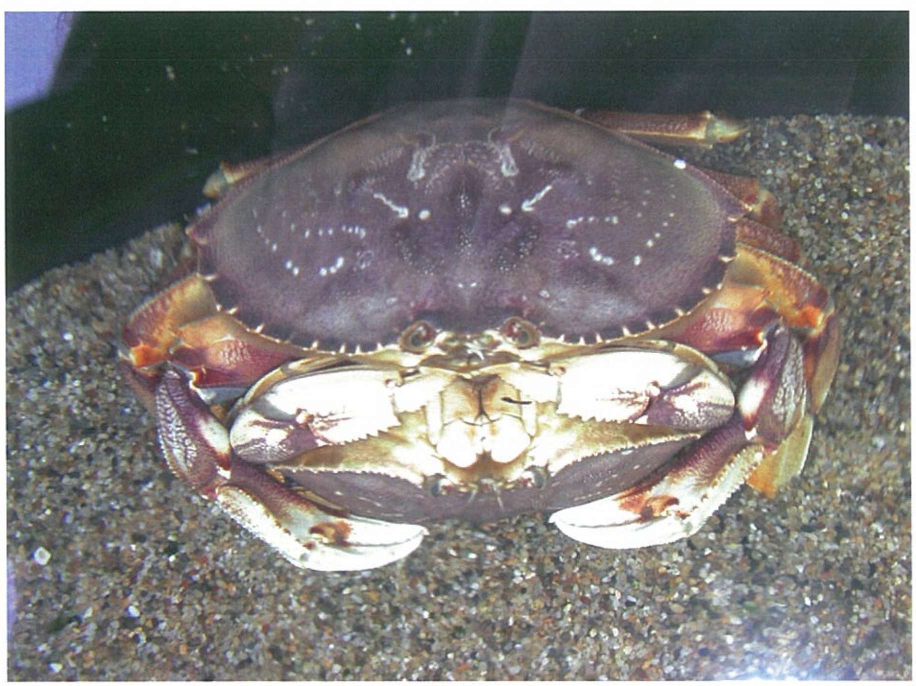

Figure 15. A small male with a female in a premating embrace, where the claws of the smaller male are in contact with the spines of the female in the region of Type 2 marks (male is the upper partner).

Laboratory Mating Mark Formation Tests

Test 1: size-ratio effect

In Test 1, 34 male crabs successfully formed a premating embrace. Male sizes in this test ranged from 142.9-183.8 mm CW. Female sizes ranged from 102.9-140.8 mm premolt CW. Time in premating embraces ranged from 1 day to 10 days. Male:female size ratio varied and ranged from 1.08 to 1.45 (Tables 8-10).

All male crabs were examined after each pairing for mating marks and digital photographs were taken of the inner claw surface. No male crabs paired under Test 1 were found to have mating marks following the premating embrace.

Test 2: multiple-mating effect

Eighteen male crabs formed 47 premating embraces in Test 2. Ten males spent over 10 days in a premating embrace, and two spent 15 days in a premating embrace (Table 11). No male crabs paired under Test 2 were found to have mating marks upon final examination after the test was concluded.

Test 3: competitive behavior effect

Eleven mating trials were conducted using three unlike-sized males competing for a single premolt female during the competitive matings in Test 3 (Table 12). In eight of the trials, the largest male formed a premating embrace with the female and the second largest male formed a premating embrace in the other three trials. Seven mating trials were conducted using three like-sized males competing for a single premolt female (Table 13).

Table 8. Small male Dungeness crabs mated with small, medium, and large female Dungeness crabs during Test 1: the size-ratio effect. Matings were conducted during the spring 2002 mating season. Days in a premating embrace (PME) refers to the number of days between the day the male first grasped the female and the day the female was observed to molt. Unknown values (un) were the result of either female mortalities following molting or if the total days in a premating embrace could not be determined.

Male size	Male CW	Female size	Female CW		Days in PME	Male CW:female premolt CW	
			premolt	postmolt		ratio	mean
Small	142.9	Small	102.9	122.4	un	1.39	
	149.5		105.9	124.0	4	1.41	1.36
	148.8		110.4	un	10	1.35	
	146.5		112.1	un	2	1.31	
	148.2	Medium	129.0	142.2	7	1.15	
	144.6		127.2	142.0	5	1.14	1.16
	146.5		124.0	139.9	9	1.18	
	142.9		121.2	137.3	5	1.18	
	148.9	Large	132.7	146.8	5	1.12	
	147.4		137.6	151.1	1	1.07	1.08
	148.4		139.0	152.1	2	1.07	
	146.8		137.7	148.5	1	1.07	

Table 9. Medium male Dungeness crabs mated with small, medium, and large female Dungeness crabs during Test 1: the size-ratio effect. Matings were conducted during the spring 2002 mating season. Days in a premating embrace (PME) refers to the number of days between the day the male first grasped the female and the day the female was observed to molt. Unknown values (un) were the result of either female mortalities following molting or if the total days in a premating embrace was not able to be determined.

Male size	Male CW	Female size	Female CW		Days in PME	Male CW:female premolt CW	
			premolt	postmolt		ratio	mean
Medium	151.3	Small	114.6	129.6	5	1.32	
	157.0		107.5	124.5	1	1.46	1.37
	155.0		115.9	134.5	1	1.34	
	150.2		109.3	un	4	1.38	
	151.6	Medium	125.7	un	un	1.21	
	150.7		122.0	134.9	3	1.24	1.22
	154.6		127.2	140.1	7	1.22	
	153.2		125.0	141.3	4	1.23	
	151.8	Large	140.8	155.2	5	1.08	1.11
	152.2		134.3	un	5	1.13	

Table 10. Large male Dungeness crabs mated with small, medium, and large female Dungeness crabs during Test 1: the size-ratio effect. Matings were conducted during the spring 2002 mating season. Days in a premating embrace (PME) refers to the number of days between the day the male first grasped the female and the day the female was observed to molt. Unknown values (un) were the result of female mortalities following molting.

Male size	Male CW	Female size	Female CW		Days in PME	Male CW:female premolt CW	
			premolt	postmolt		ratio	mean
Large	160.8	Small	109.8	122.8	2	1.46	1.42
	161.1		113.0	130.8	4	1.43	
	163.9		119.4	137.0	1	1.37	
	163.9		114.4	130.8	9	1.43	
	165.3	Medium	127.6	143.7	1	1.30	1.45
	182.3		122.6	139.9	3	1.49	
	179.4		122.1	137.6	6	1.47	
	179.7		123.1	un	2	1.46	
	183.8		120.7	un	7	1.52	
	164.0	Large	131.9	146.5	10	1.24	1.25
	176.2		134.9	147.4	8	1.30	
	162.7		136.5	147.5	6	1.19	

Table 11. Small, medium, and large Dungeness crabs mated multiple times during Test 2: multiple mating effect. Matings were conducted during the spring 2002 mating season. Days in a premating embrace (PME) refers to the number of days between the day the male first grasped the female and the day the female was observed to molt.

Male size	Male CW	Number of Matings	Female premolt CW				Total days in PME
			#1	#2	#3	#4	
Small	148.9	2	132.7	140.9			9
	147.4	2	137.6	133.3			6
	148.4	2	139.0	135.9			8
	142.9	3	102.9	128.9	114.6		2
	144.6	4	127.2	125.2	125.4	126.0	14
	146.5	3	124.0	124.7	124.4		15
	149.5	2	105.9	109.4			10
	146.8	2	137.7	134.5			5
	148.8	2	110.4	119.4			12
	142.9	3	121.2	125.0	123.5		9
Medium	151.6	3	125.7	127.5	127.4		9
	150.7	3	122.0	122.5	123.3		11
	151.3	2	114.6	112.6			13
	154.6	3	127.2	120.4	121.4		15
	153.2	3	125.0	124.0	120.0		8
Large	179.4	3	122.1	127.7	123.4		12
	179.7	3	123.1	128.7	124.0		12
	183.8	2	120.7	121.6			12

Table 12. Mating experiments conducted during the spring 2003 mating season for Test 3: competitive behavior effect among "unlike-sized" males. Premolt females were added to experimental cells containing three males; victor male was the male observed grasping the premolt female in a premating embrace. Days in a premating embrace (PME) refers to the number of days between the day the male first grasped the female and the day the female was observed to molt.

Female premolt CW	Male CW (smallest to largest)			Victor male	Days in PME
	3	2	1		
118.9	148.8	151.1	164.3	2	1
124.4	150.9	155.4	170.4	1	1
139.0	147.0	158.3	164.7	1	2
126.7	149.3	158.2	162.7	1	1
118.0	149.3	158.2	162.7	1	4
112.1	149.3	155.2	159.1	1	unknown
121.3	147.0	155.2	159.1	2	3
121.8	149.7	157.5	162.8	1	5
116.5	147.1	153.9	160.9	1	1
122.0	147.9	150.6	160.6	1	unknown
124.6	147.0	155.2	159.1	2	5

Table 13. Mating experiments conducted during the spring 2003 mating season for Test 3: competitive behavior effect among "like-sized" males. Premolt females were added to experimental cells containing three males; victor male was the male observed grasping the premolt female in a premating embrace. Days in a premating embrace (PME) refers to the number of days between the day the male first grasped the female and the day the female was observed to molt.

Female	Male CW			Victor	Days in
premolt CW	3	2	1	male	PME
114.2	162.7	164.3	165.9	2	1
124.6	147.7	151.1	157.5	1	8
129.0	148.8	153.7	158.2	2	1
128.4	157.6	158.2	158.2	un	2
143.4	149.3	158.2	158.3	1	2
118.8	157.7	158.1	168.3	1	1
123.3	157.7	158.6	159.6	3	2

None of the male crabs that participated in the competitive behavior mating trials formed mating marks. However, interesting behaviors were observed while the male crabs competed for a mate. During almost every trial, when the premolt female was added, a "battle" ensued. Battles involved pinching and probing but serious injuries were not common. Typically, the first male to find the female would quickly tuck her into a protective embrace while the other one or two males would attempt to dislodge the female. When a male would successfully form a premating embrace with a female, competitor males were observed to mount the pair "piggy-back" on top of the male or female (Figure 16). Competitor males in this position would sometimes clutch the pair for more than the 2-5 hours of daily observations. The competitor male would then attempt to remove the female from the embrace of the victor male.

Male crabs were also observed forming "competitive" embraces between themselves (Figure 17). These embraces had the same appearance as a premating embrace, but instead involved two males. The competitive embrace was only observed in cells containing a premolt female.

Test 4: natural environment effect

Nineteen premating embraces were formed during the non-competitive matings, resulting in males spending between 1 and 10 days in a premating embrace (Table 14). Conventional premating embraces were observed in 15 of the 19 trials, seven involved guarding, and 3 formed inverted premating embraces. Five males were observed embracing the premolt female using more than one embrace type on different daily

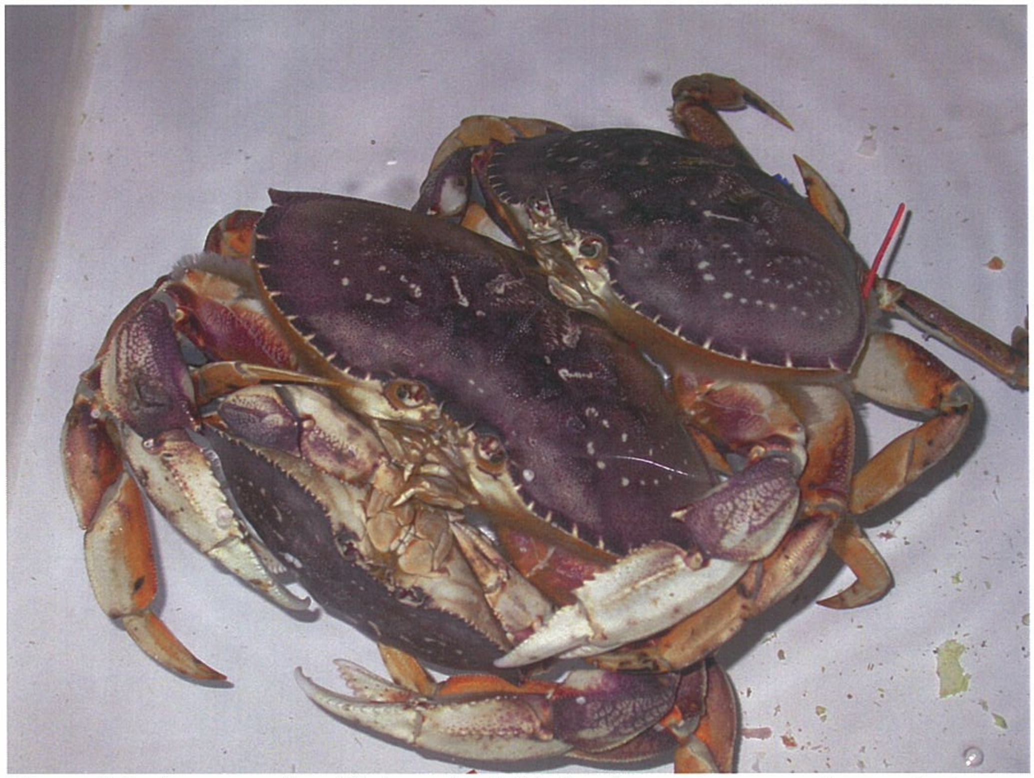

Figure 16. An example of “piggy-back” behavior as the competing male (upper right in the photograph) is attempting to dislodge the female (lower left) in a conventional premating embrace with another male.

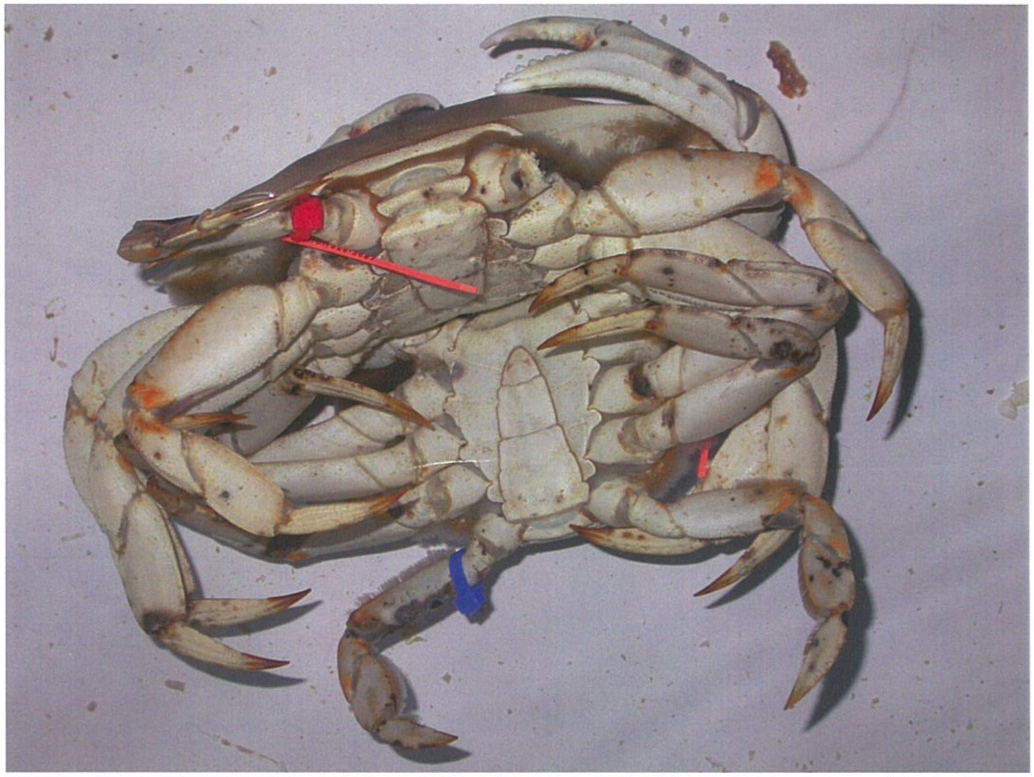

Figure 17. An example of two males in a “competitive” embrace. This behavior was only observed when a premolt female was present in the same tank. As each male held on to each other, neither was able to grasp the premolt female in a premating embrace.

Table 14. Non-competitive mating experiments conducted during the spring 2004 mating season for Test 4: the natural environment effect. Matings were conducted on a sand substrate or on a gravel substrate with a swift current. Embrace type refers to: G=guarding; I=inverted premating embrace; P=conventional premating embrace. Days in a premating embrace (PME) refers to the number of days between the day the male first grasped the female and the day the female was observed to molt. Unknown values were the result of either female mortalities following molting or if the total days in a premating embrace could not be determined.

Male CW	Female CW		Substrate	Current	Embrace type	Days in PME
	premolt	postmolt				
158.1	137.8	150.2	sand	no	G	3
154.6	134.9	147.2	sand	no	I/P	2
158.0	113.6	132.4	sand	no	P	10
158.2	128.7	136.6	sand	no	P	2
154.9	132.2	un	gravel	yes	G/I/P	3
174.2	126.6	138.7	sand	no	G	unknown
143.4	134.5	147.9	gravel	yes	G/P	2
145.0	107.6	125.4	sand	no	G/P	3
154.6	130.2	141.9	sand	no	P	1
152.8	131.9	un	gravel	yes	P	1
154.6	125.1	137.5	sand	no	G	1
161.3	125.7	137.7	sand	no	G/P	1
158.1	132.6	146.4	sand	no	P	2
169.7	117.1	129.5	sand	no	P	2
145.0	129.1	137.3	sand	no	P	2
158.2	127.7	138.8	sand	no	P	2
157.2	120.4	132.3	sand	no	P	7
152.8	127.2	un	sand	no	P	2
158.2	127.7	142.8	sand	no	I	5

observations. Male and female crabs were observed on many occasions to partially or completely bury themselves in the sand or gravel substrate. When a guarding embrace was observed, it typically involved a mostly buried female with the male hovering protectively above her. Paired crabs in a premating embrace were observed partially buried in the sand substrate.

Of the 18 competitive mating trials conducted under Test 4, the competitor male (added after the initial male was allowed to embrace the premolt female) was larger than the embracing male in 14 of the trials and smaller in the remaining 4 (Table 15). When the competitor male was larger, the female was taken away on 9 occasions, was retained by the original smaller male on 2 occasions, and was indeterminate (female molted before a victor male was observed) on 2 others. When the competitor male was smaller, and a victor male could be determined, the competitor was always unsuccessful at taking away the female.

Male crabs grasped their mate using three different types of premating embraces (see Figures 8-10, Tables 14-15). Eleven of the pairings were observed on different occasions to utilize two or all three of the embrace types. Conventional premating embraces were observed in 27 of the 37 pairings, inverted embraces were observed in eight pairings, and guarding embraces were observed in 13 of the pairings.

A total of 37 matings were conducted on a natural substrate, both competitively and non-competitively, during Test 4. None of the males involved in these mating trials were found to have formed mating marks.

Table 15. Competitive mating experiments conducted during the spring 2004 mating season for Test 4: the dynamic environment effect. Male #1 was allowed to guard or grasp the premolt female before male #2 was introduced. Victor male refers to the male observed guarding or grasping the female following the second male's introduction. Victor males were unknown (un) when the female molted before a victor could be observed. Matings were conducted on a sand substrate or on a gravel substrate with a swift current. Embrace type refers to: G=guarding; I=inverted premating embrace; P=conventional premating embrace. Days in a premating embrace (PME) refers to the number of days between the day the male first grasped the female and the day the female was observed to molt. Unknown values were the result of either female mortalities following molting or if the total days in a premating embrace was not able to be determined.

Male #1 CW	Male #2 CW	Female CW		Victor male	Substrate	Current	Embrace type	Days in PME
		premolt	postmolt					
158.1	177.1	116.4	unknown	(1)	Sand	no	I/P	2
154.6	165.3	120.9	132.8	(2)	Sand	no	G/P	1
154.4	168.0	119.9	134.9	(2)	Sand	no	P	3
157.2	174.2	138.8	152.1	(2)	Sand	no	P	3
149.6	166.8	134.1	148.6	(2)	Gravel	yes	P	3
154.6	160.4	127.0	unknown	(2)	Sand	no	I/P	unknown
158.2	175.7	130.0	144.4	(2)	Sand	no	G/I	2
158.1	168.0	118.7	130.3	(1)	Sand	no	G	2
152.8	168.2	122.9	136.1	(1)	Gravel	yes	G	1
145.0	157.6	122.2	136.6	(2)	Sand	no	G/I	1
165.0	155.2	125.8	140.3	(1)	Sand	no	P	2
164.5	158.9	125.5	140.8	(1)	Sand	no	P	3
154.6	156.9	136.1	148.3	(2)	Sand	no	P	1
145.0	174.1	122.4	131.3	un	Sand	no	unknow	3
169.7	157.3	138.3	147.6	un	Sand	no	P	1
168.2	152.8	132.5	unknown	(1)	Gravel	yes	P	1
158.9	164.5	120.2	130.9	(2)	Sand	no	P	2
157.3	169.7	126.5	unknown	(2)	Sand	no	G/I	3

DISCUSSION

This study was motivated by the need to validate a method of inferring male Dungeness crab mating activity that has been used for over forty years. When first introduced, the novel approach of examining male crabs for mating marks to infer mating success must have been promising. Yet Butler, after first describing the phenomenon of mating marks in 1960, never again mentioned or used mating marks in his later published research.

Butler (1960) found that the frequency of mating marks among sublegal crabs was roughly twice that among legal crabs sampled in British Columbia. Similar observations of much higher frequencies among smaller crabs were noted later by other researchers working in British Columbia (Smith 1988, Smith and Jamieson 1991). Although their identification of mating marks may have been reliable, their interpretation of these observations can be seriously questioned. Through generation of data from controlled laboratory matings, at-sea examinations of male Dungeness crabs in northern California, and a critical review of published literature, I believe that mating mark presence cannot be used as a reliable indicator of mating activity for male Dungeness crabs.

In this study, male Dungeness crabs from northern California were captured in a manner that was very similar to the methods used by previous researchers, and were examined for mating mark presence throughout the spring mating season of 2004. As the mating season progressed, the frequency of mating mark occurrence on all male crabs increased. In contrast to research conducted previously on male crabs in British

Columbia, there was not a striking difference between the percentages of smaller versus larger crabs with mating marks. Throughout the 2004 mating season, sublegal crabs in northern California had a slightly higher frequency of mating marks than legal crabs on half of the collections while half of the collections yielded a slightly higher mating mark presence on legal crabs.

The coincident increase of mating mark prevalence during the spring mating season may serve as strong evidence that mating marks result from mating activity of male Dungeness crabs. However, for several reasons explained below, it may be safe to conclude that the percentage of crabs with mating marks does not accurately predict the percentage of crabs that have actually mated. In this study, 107 male crabs, some mated multiple times and in different conditions, were allowed to form a total of 155 premating embraces. It was surprising that no mating marks were produced in these controlled laboratory matings. Several behaviors were observed that could have conceivably produced marks on the males' claws, but none of the crabs had any marks similar to the marks observed on crabs captured during the spring mating season. If mating marks are the result of male claw abrasion that occurs during the premating embrace, then one might conclude that the controlled laboratory matings did not sufficiently reproduce the conditions in which crabs actually mate. One must also consider the possibility, however, that not every natural mating event produces mating marks, or that mating marks are only produced on male crabs that spend considerably longer cumulative time in a premating embrace than was tested during the laboratory experiments. Hankin et al. (1997) calculated that an individual male may possibly mate with up to 6-12 females in a given mating season, but the maximum number of successive premating embraces formed in the laboratory setting of this study was four.

Assumptions for Valid Use of Mating Marks as an Indicator of Successful Mating

Previous researchers who have used mating marks have not carefully stated the assumptions that must be met for the presence of mating marks to be a reliable indicator of male mating activity. Butler (1960) declared that the premating embrace between a male and female crab causes the formation of mating marks on males. Therefore, an individual male crab with mating marks must have been in a premating embrace since the previous molt, since superficial exoskeletal scratches like mating marks are lost following the molt. To infer successful mating from mating mark presence, then one must also assume that every premating embrace results in a successful copulation.

If one were trying to estimate the percentage of crabs at a given time that had mated in the current year using mating marks, there exists a possibility of false positive inference. This possibility exists because a small but significant portion of crabs do not molt annually (Warner 1987, Poole 1967). Warner (1987), examining male crabs in November 1975 (after the male molting season), found that 26% of male crabs were old-shelled. Observations of sublegal crabs in November 2001, November 2002, and November 2003 in this study found a 12%, 15%, and 43% occurrence of old-shell crabs, respectively. On old-shell crabs, observed mating mark occurrence was extremely high (95%), and Warner (1987) even used mating marks as a definite indicator of a male crabs' lack of molting in the previous molting season. Therefore, a portion of crabs may show mating marks from the previous mating season, but may have not mated in the most recent mating season. For example, in at-sea observations of mating marks made on 25 January 2004, 9 of the 172 sublegal crabs examined had mating marks. This is a surprising finding considering that substantial mating activity was nearly a month away. However, 8 of the 9 crabs with mating marks were classified as old-shell, and their mating marks were presumably created during the previous mating season (2003) rather than in the current season (2004).

Failure to adequately account for the molting status of male crabs can also produce a false negative inference concerning recent mating activity. This is especially likely in geographic regions such as British Columbia where there is temporal overlap between the mating season and the male molting season (Butler 1960; Butler 1961). There, the male molting season begins in the spring and extends into the summer mating season. The two seasons (male molting and mating) overlap and therefore newly molted male crabs will be present during the mating season. A crab examined for mating marks during the time that the two seasons overlap may have mated and formed mating marks in the current year, but then molted and lost it's mating marks. Therefore, one would conclude that the crab had not mated in the current season when it actually had.

The possibility of false positive (from old-shell males showing mating marks from the previous season) and false negative (from newly-molted males that had lost mating marks) inferences to occur when estimating the mating history of an individual crab must be carefully considered, especially when mating mark presence is used to infer population-wide mating activity from a sample of male crabs. A formal estimation algorithm for this purpose has never been developed or considered. Instead, mating marks have been assumed, without validation, to be a reliable indicator of mating activity.

Reliable use of mating mark presence as an indication of mating success would require that (1) every premating embrace, involving the smallest to largest male capable of mating, will produce mating marks; (2) every premating embrace results in successful copulation and insemination; and (3) the timing, location, and sampling effort are appropriate for estimating the population-level mating activity. The validity of the three assumptions will now be assessed on the basis of data collected in this study and reported elsewhere.

Assumption 1: the premating embrace produces mating marks

Results of laboratory experiments did not suggest that the size of the male in a premating embrace affects the formation or severity of mating marks, but these experiments did not disprove the possibility that large males in a premating embrace are less likely to form mating marks. Since mating marks are the presumed result of abrasion between the male and female in a premating embrace, small males that are paired with a large female partner could conceivably experience more abrasion and large males mating with small females might experience less abrasion. The experiments failed to resolve this possibility, and the question remains open.

In this study, when a small male embraced a female in a conventional premating embrace, the spines of his female partner were found to be in contact with the inner surface of the male's claws. In contrast, when a large male grasps a female in a conventional premating embrace, the claws rest on the carapace of the female and the spines are not in contact with the male's claws (see Figure 8). As a result, the claws of large male crabs may receive much less abrasion, since their claws do not receive the scoring action of the female's spines. This appears to be supported by at-sea observations, since the frequency of "slight" marks was higher among larger crabs. In addition, the mating mark Type found to be associated with the largest mean CW (Type 1, mean=178.8 mm CW), is much less severe than the Type with the smallest mean CW (Type 2, mean=160.6 mm CW). Type 1 marks were often very faint and hard to distinguish whereas Type 2 marks were prominent and obvious. The effect that this could have on biasing mating inferences from mating mark examinations is clear: larger male crabs with faint Type 1 marks could be erroneously classified as lacking mating marks.

If mating marks are more likely to be produced on smaller males than on larger males, their value as a diagnostic tool should be questioned. Jamieson (1996) admits that "not all male Dungeness crabs that have mated have mating marks," which leads one to wonder why mating marks were so confidently used when doubts existed as to their function as a mating indicator. Knuckey (1996) used mating "scars" to determine functional maturity in male mud crabs *Scylla serrata*, and also found that not every crab that had mated had evidence of mating scars. Why some premating embraces result in mating marks while others do not may have been answered by Butler (1960), who believed the degree of abrasion in mating marks is caused by the prolonged time spent in the premating embrace by some male crabs. The results of Test 4 show that male

Dungeness crabs, however, will employ different types of premating embraces, and the degree of possible abrasion may be different between the three observed types of premating embraces.

The premating embrace, also known as precopulatory guarding or precopula, is not uncommon in crustaceans and has received much attention as an adaptive strategy (Jormalainen 1998). The underlying assumption for the evolution of this behavior is paternity assurance, and diverse mate guarding behaviors have been classified (Christy 1987) and modeled (Grafen and Ridley 1983). Dungeness crabs exhibit female-centered competition, classified as "search and defend" (Christy 1987), and the duration of the precopulatory period for crabs in this mating association may be dependent on the costs to the female of being guarded (females in precopula may not feed) balanced with the gains of being guarded by a high quality male at the time of her molting.

Grafen and Ridley (1983), modeling a theoretical crustacean mating system where mate competition is high, predicted that larger male crabs would spend less time mate guarding than small males. The evolutionary stable mating behavior predicted in their model is a result of their assumption that in a competitive mating system, larger males could outcompete smaller males for mates (a phenomenon that was observed for Dungeness crabs during the competitive matings in this study). This effectively increases the number of available females in the population for larger males, increasing their potential mating success, and they would therefore choose females that are nearer to their molt. By choosing females nearer to the molt, less time is spent mate guarding by larger males, increasing the probability of finding additional mates. The time spent guarding by

Dungeness crabs in the wild has never been estimated, but the findings of Grafen and Ridley (1983) predict that smaller males should spend more time guarding their mates than large males.

Assumption 2: every premating embrace results in successful mating

The premating embrace, the presumed source of mating marks, is a precursor activity to mating in Dungeness crabs and many other crustaceans. To infer that mating marks therefore indicate successful mating, every premating embrace must result in successful mating. This assumption, however, contradicts evidence from this and other studies of Dungeness crabs because mate takeover likely occurs in nature.

Some males in a premating embrace lost their female partner to a competing male during Test 4. Males were extremely aggressive in competitive interactions, suggesting that in the wild mate competition could be intense. Butler (1960) observed males competing for females during "field observations"and stated that "invariably the larger male is successful." Large male *Cancer pagurus*, a species closely related to Dungeness crab, have been observed displacing females from the premating embrace of smaller males (Edwards 1966). Knuckey (1996) recognized the limitations of using mating scars in mud crabs as an indicator of actual mating; overestimations of mating success could result "if males embrace females long enough to develop scars without succeeding in copulation." Thus, in a species such as Dungeness crab, where some males can outcompete others for mates, some crabs may receive mating marks from the premating embrace without successful copulation.

Assumption 3: appropriate sampling effort

Three additional factors affecting observations of mating marks include the timing, location, and size of the sample used to infer mating activity. These factors must be carefully considered to determine whether or not the sample is representative of the population being studied. Without considering these biological, geographical, and temporal factors, invalid inferences of mating activity could result.

Time of field observations with respect to the male molting season is especially important, since sexually mature male Dungeness crabs will lose existing marks when they molt. The ideal time to sample for mating marks should be immediately following the annual mating season, but prior to the annual male molting season. Observations of mating marks in northern California in 2004 reached a maximum in July after all mating was essentially completed. Examinations for mating marks should therefore occur (1) after the mating season and (2) before the next male molting season. In northern California, where females molt prior to males, it is possible to achieve this timing, but in British Columbia, due to the overlap of molting seasons for males and females, it is not.

The mating season in British Columbia typically occurs from May to September (Butler 1960) while the male molting season (for sexually mature males) also occurs during the summer (Butler 1961). A sample of crabs taken during the summer would occur during the mating season, not after, and would also be during the active male molting season. Some fraction of the resulting sample would therefore consist of newly molted males from which mating history could not be validly estimated.

The samples of crabs examined for mating marks by Butler (1960) were in fact taken during the summer, and he noted that "most of the large males had molted recently." The sample was therefore potentially full of false negatives with so many newly molted crabs, biasing downward his estimate of mating activity among large males. The data used by Smith and Jamieson (1991), and later Jamieson (1996) and Jamieson et al. (1998), was first collected by Smith (1988), and contained many examinations of mating marks during the summer male molting season.

The effect of an active fishery compounds the problem of examining mating marks during the male molting season. The fishery at the time of Butler's (1960) and Smith's (1988) collection was active during the summer, reducing the amount of hard-shelled crabs. As a result, in this area where high exploitation removes most hard-shelled large males, a survey of legal-sized males during the summer would have a greater percentage of soft-shelled individuals than would occur in an unexploited population. Sublegal crabs during the summer are also actively molting to legal size and become vulnerable to harvest, but none of the hard-shelled sublegal crabs would be removed due to size restrictions. Samples taken during the summer in British Columbia would include both newly molted males that had mated but showed no mating mark evidence, and also newly molted males that may mate later in the season.

Also critically important to any sampling scheme is the adequacy of the sample to accurately represent the parameter being estimated. Considering the abundance of male Dungeness crabs, samples must be sufficiently large. The population for which parameters such as mating activity are estimated must also be strictly defined. When the differences between crab populations in British Columbia and other populations to the north and south are considered, what may be true in one place may not necessarily be true in other locations. Mating and male molting seasons vary within the calendar year across the range of Dungeness populations and must affect prevalence of mating marks.

Smith and Jamieson (1991) reported mating mark presence from a large sample of crabs (n=20,106) between 122.5 mm CW and 197.5 mm CW, collected in British Columbia. From this sample, they contended that few crabs greater than the minimum legal size (154 mm CW) had mated since mating mark presence was roughly 10-15% in crabs this size. Roughly 30-40% of sublegal crabs they examined, however, had mating marks. The large sample of crabs had been an accumulation of many samples taken over an approximate 18-month period. Shell-condition data provided by Smith (1988, see his Figure 4.10.2) for these same data revealed that samples collected between April and July 1985 contained 40-55% soft-shelled individuals; samples collected between February and June 1986 contained approximately 50% soft-shelled individuals. Coinciding with the samples containing high levels of soft-shelled crab was a very low percentage (near 0%) of legal crabs with mating marks. During periods that hard-shelled crabs made up nearly 100% of the sample (September-December 1985), the percentage of legal males with mating marks was much higher and approached 40% in November. Although a very large number of crabs were examined (20,106), observations were conducted during two male molting periods (with high occurrence of soft-shelled crabs) and only one winter (the intermolt period when most crabs are hard-shelled).

Alternative Explanations for Mating Mark Formation

Since the initial presumption over 40 years ago that mating marks are the result of the close contact between a male and female Dungeness crab in a premating embrace, Dungeness crab researchers have not presented any firm evidence to support this. While the at-sea observations in the current study did show an increasing occurrence of mating marks on all male crabs roughly concurrent with the spring mating season, their frequency in the population may be the result of competitive behavior only associated with mating. The failure to produce mating marks from 155 premating embraces formed during Tests 1-4 in this study would suggest that other explanations for the phenomenon of mating marks should be considered. While observing the competitive matings in Test 3, male crabs exhibited two behaviors that have not been described before that may cast doubt on the assumption that mating marks are only formed from premating embraces. The two specific behaviors, "piggy-back" mate competition and male-male "competitive embrace", did not produce any noticeable scratches or abrasion, but have similarities to the true premating embrace between a male and female crab. The "piggy-back" involved a competing male attempting to disurpt the premating embrace with another male. This behavior is similar to a premating embrace since the competing male will embrace the male or female from behind in an attempt to dislodge the female. A male-male "competitive embrace" was only observed when a premolt female was present. The two males would embrace each other in a manner very similar to the conventional premating embrace. If these two behaviors observed in the lab occur with regularity in the wild, it is possible that some “mating marks” may be formed via agonistic male-male behaviors associated with mating.

The interpretation of previous research, and the careful suggestions for future research, is the primary force of this thesis. Butler (1960), and later Smith and Jamieson (1991), believed that large male crabs had a much lower mating activity than sublegal crabs because of a lower occurrence of mating marks among larger crabs. They had both assumed a direct correlation between mating marks and mating activity existed. Subjecting their data to a set of simple assumptions to test if this belief was acceptable found that it can be misleading to use mating marks as an index of mating activity in male Dungeness crabs.

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